

Bugweed Biocontrol: New insights into the
biological control agents of *Solanum mauritianum*,
Gargaphia decoris and *Anthonomus santacruzi*



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DECLARATION

I declare that this Dissertation is my own work. It is being submitted for the Degree of Master of Science at the University of the Witwatersrand, Johannesburg. It has not been submitted by me before for any other degree, diploma or examination at any other University or tertiary institution.



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4th day of November 2016

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DEDICATION

“What makes life valuable is that it doesn’t last forever, what makes it precious is that it ends” – Emma Stone

This dissertation is dedicated to both my Grandfather, William Sneddon, and my Brother, David Douglas Cowie, who were both major influences in my life, but will sadly never get to see this accomplishment.

To honour a promise made to you both... And now you shall live on through me... I’ll carry a piece of you into everything that I do next...

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ABSTRACT

Solanum mauritianum Scopoli (Solanaceae) is a perennial tree or shrub native to South America, which has become a prominent and widespread invader in numerous sub-tropical countries around the world. In South Africa, *S. mauritianum* is listed as one of the country's worst ecological weeds, having been targeted for biological control efforts since 1984. Despite some constraints, biocontrol efforts have seen the successful release of two promising biocontrol agents.

The first of these biocontrol agents, released against *S. mauritianum*, was the sap-sucking lace bug, *Gargaphia decoris* Drake (Hemiptera: Tingidae). Sap-feeding by *G. decoris* metabolically impaired the leaves, resulting in a reduction to their photosynthesis, with a greater effect on plants growing in full-sun compared to plants growing in the shade. This difference was attributed to higher leaf temperatures experienced in the sun. Herbivory reduced transpiration rates by more than 50%, resulting in a reduction in evaporative cooling of the leaf. The increased physiological damage experienced by full-sun plants may be a combination of stresses, particularly the direct effect of chlorophyll removal via herbivory and the indirect effect of accumulated heat–light stress.

The flowerbud-feeding weevil, *Anthonomus santacruzi* Hustache (Coleoptera: Curculionidae), was released in 2008 as a biological control agent against *S. mauritianum*. The hypothesis that climate, particularly low temperature and low relative humidity, restricts the survival and establishment of *A. santacruzi* in South Africa was tested. Thermal assessments on *A. santacruzi* adults calculated the CT_{min} and LT_{50} as 4.1 ± 0.2 °C ($n = 20$) and 4.2 ± 0.3 °C ($n = 90$) respectively. The LH_{50} of *A. santacruzi* adults was calculated as 46.9%. The establishment of *A. santacruzi* at only the warm and humid release sites in South

Africa advocates for the consideration of low temperature and low humidity as factors impeding the agents' establishment and spread, particularly on the cooler and drier Highveld.

Furthermore, the impact of *A. santacruzi*'s florivory on the reproductive output of *S. mauritianum*, as well as the potential of the agent to act as an indirect pollinator was assessed. Overall direct floral damage caused by *A. santacruzi* was trivial, with only ~5% of the anther and ~2% of the petal area being removed. However, the consequent effects of *A. santacruzi* were considerably more damaging, with 25% and 66% reductions in flowering and fruiting respectively. Additionally, fruits produced from inflorescences exposed to *A. santacruzi* were smaller in size, with fewer, less viable seeds. The feeding and presence of *A. santacruzi* also maintains the potential for indirect effects on the pollination of *S. mauritianum*. This suggests that in areas with well-established *A. santacruzi* populations, the weevils may simultaneously facilitate the self-pollination and potential inbreeding of *S. mauritianum*.

Keywords: Agent impacts and effects; biological control; Bugweed; climatic unsuitability; ecophysiology; indirect effects; post-release evaluation.

GLOSSARY OF TERMS & ABBREVIATIONS

Autogamy – ‘Autogamous pollination’, self-fertilisation of plants, particularly flowers which have been self-pollinated.

Biotic release – Term linked to the ERH, that suggests the success of invading species, in novel areas, is attributed to the fact they are “freed” from their native/specialised predators, allowing them to invest resources into growth and reproduction.

Chlorosis – Loss of green colouration (chlorophyll pigment) in plant leaves, frequently caused by insect sap-feeding, nutrient deficiencies or aging of the leaf.

CLIMEX – A climate modelling software that allows the user to predict/model the potential geographic distribution of organisms based on global/local meteorological data.

Climate matching – A modelling technique used to compare geographic localities in terms of their climatic similarity (see CLIMEX).

CT_{min} – ‘Critical thermal minima’, temperature at which insects lose locomotory function.

ERH – ‘Enemy Release Hypothesis’ (see Biotic release).

Florivory – Organisms, particularly arthropods, which feed primarily on the flowers and associated floral structures (buds, anthers, stamens) of plants (angiosperms).

F_v/F_m – A chlorophyll fluorescence measure indicating the relative “health” of a leaf’s photosystems (*PSII*), often used as a plant stress indicator.

Highveld – An elevated southern African region(s) typically between 1200 – 1800m a.s.l which experience warm rainy summers and cool dry winters.

LT₅₀ / LH₅₀ – ‘Lethal Temperature/Humidity 50’, a controlled exposure to conditions in which 50 % of the test population survive and 50 % die.

Mucivory – Organisms, namely arthropods that feed solely on plant fluids, predominately sap.

NPQ – ‘Non-photochemical quenching’, a protective process in plants, which dissipates excess light energy as heat.

Peduncle – A stalk which is composed of an inflorescence of many flowers, which subsequently develop into a fruit bearing cluster (infructescence).

SPAD – ‘Single Photon Avalanche Diode’, a device used to measure plant leaf “greenness”, commonly used to estimate the chlorophyll content of leaves.

Xanthophyll cycling – a protective process initiated by plants to prevent/minimise damage from heat and excessive light (UV) stresses.

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CHAPTER ONE:

GENERAL INTRODUCTION

Biological invasions

Biological invasions currently pose one of the greatest threats to global biodiversity and ecosystem services, being second only to habitat loss and destruction (Walker and Steffen, 1997; Chornesky and Randall, 2003). Invasive plants in particular, are major contributors towards the ongoing and increasing problem of biological invasions (Allendorf and Lundquist, 2003; Pyšek et al., 2008; Downey and Richardson, 2016). Although biological invasions are arguably a natural occurrence, their frequency and rate of introduction has significantly increased and accelerated due to various human globalisation activities, particularly international trade (Allendorf and Lundquist, 2003; Pimentel, 2011). The deliberate and accidental introduction of non-native plants has seen numerous species established worldwide (Lowe et al., 2004). Global estimates suggest that as many as 26 000 plants have the potential to be invasive, with 10 000 being problematic invaders and of these only around 4000 have been introduced so far (Rapaport, 1991; Reichard and White, 2001).

Invasions by non-indigenous plants have been linked to the displacement and arguably the extinction of countless native species (Walker and Steffan, 1997; Gurevitch and Padilla, 2004; Downey and Richardson, 2016). Invasive plants maintain the potential for serious ecological damage and severe detriment to the agricultural and socio-economic sectors (Olson, 2006). There is no shortage of examples highlighting the spread and detrimental effects of weed invasions, including *Eichhornia crassipes*, *Lantana camara*, *Bromus tectorum*, *Opuntia spp.* and *Solanum mauritianum* (Holm et al., 1997; Lowe et al. 2004).

Invasive alien plants are relatively well researched and increasingly documented, but the fundamental questions as to what makes a species invasive and how can they be adequately controlled remains an ongoing problem (Kolar and Lodge, 2001; Hurley et al., 2016).

Numerous hypotheses have been put forward as to what makes an alien species invasive, with one of the most central and widely accepted theories being the Enemy Release Hypothesis (ERH: 'Biotic release') (Keane and Crawley, 2002; Colautii et al., 2004). The ERH predicts that invading species are freed from their natural (co-evolved native) predators, permitting them resource investment towards growth and reproduction (Cronk and Fuller, 2001; Keane and Crawley, 2002). This results in the invading species gaining an improved competitive ability over native species, and may be an explanatory factor behind the rapid recruitment, spread and establishment of weed species (Maron and Vila`, 2001; Colautii et al., 2004). The ERH is largely based on three prevailing conditions in the introduced range: i) co-evolved predators are absent, ii) specialist predators found on closely related native species are unlikely to 'switch-over' to the invasive species and iii) generalist predators are less damaging to the invaders than to the native species (Keane and Crawley, 2002; Liu and Stiling, 2006). Although some of these conditions remain debated, the ERH is still accepted and is linked to many other ecological theories and practices (Maron and Vila`, 2001). One of these practices, arising from the ERH and its underlying predator-prey dynamics, is biological control (Liu and Stiling, 2006), one of the few cost-effective, sustainable and permanent solutions to controlling invasive species (van Wilgen et al., 2002; van Wilgen et al., 2004; De Lange and van Wilgen, 2010).

Biological control of invasive plants

Biological control, commonly termed biocontrol, is a method which aims to reduce and control invasive species, commonly weeds, using host-specific natural enemies, namely predators, parasitoids and pathogens (Harris, 1985; Olckers and Hulley, 1995). These weeds are often referred to as targets, whilst the natural enemies used in their control are termed agents (Julien, 1987). Arthropod, mainly insect, agents are frequently used, given their typically host-specific nature, but other agents may include mites, fungi, nematodes and bacteria (Stiling and Cornelissen, 2005; Hajek et al., 2007; Goldson et al., 2014). In countries with problematic weed invasions, agents are released into infested areas in the hopes of diminishing and controlling invasive plant populations (McFadyen, 1998; Moran et al., 2005). Although three types of biological control strategies exist: classical, augmentative and conservative (Bellows and Fisher, 1999), classical biological control is favoured. Classical biological control involves the importation of co-evolved and host-specific natural enemies from the weed's country of origin, which are then mass-reared and subsequently released into infested areas (McFadyen, 1998; Bellows and Fisher, 1999). Under ideal conditions, these released agents establish and spread, attacking and decreasing weed populations as they proliferate (McFadyen, 1998). These agent-weed populations ultimately become self-regulating, reducing and maintaining weed populations at an acceptable level, ideally a predetermined ecological or economic threshold (Muniappan et al., 2009).

Classical biological control programs frequently involve the importation and release of more than one agent species (Morin et al., 2009; Goldson et al., 2014). Two theoretical models have arisen concerning biological control utilising multiple agents, the first is the 'Cumulative model', in which success is simply due to an accumulation of agents and their resulting damage (Denoth et al., 2002; Stephens et al., 2013). However, the successes of multiple agent species remains debated, with studies suggesting that a single agent is largely

responsible for the success (Denoth et al., 2002; Stephens et al., 2013). Furthermore, multi-agent biocontrol is also suggested to reduce the efficacy of biocontrol through agent competition (Stiling and Cornelissen, 2005; April et al. 2011; Buccellato et al., 2012). The second model is the ‘Lottery model’, in which a single agent is considered responsible for the majority of the biological control success and the selection of which is by ‘chance’ (Myers, 1985; McEvoy and Coombs, 1999; Sheppard, 2003). However, ongoing pressure to identify safe and promising agents has seen an increase in pre-release assessments aimed at identifying and importing fewer, more effective, agents (McClay and Balciunas, 2005; van Klinken and Raghu, 2006). Regardless of the validity of these models, the question still remains as to what the individual agent impacts and interactions will be as well as their resulting effect on the biocontrol success.

Overall, numerous local and global successes have been attained through the practice of biological control. South Africa in particular, has achieved remarkable biological control success (61% success rate) since its inception in 1913 (Zimmermann et al., 2004), having brought more than 30 invasive species under complete or substantial control (SAPIA, 2011). Recent studies by De Lange and van Wilgen (2010) have estimated the savings of biological control to be as high as R 31.5 billion. Biocontrol is now understood to be one of the most cost-effective practices for the management of invasive species (De Lange and van Wilgen et al., 2010). Despite the ongoing promise of biological control, these programmes may suffer many ecological, scientific and socio-economic constraints (Muniappan et al., 2009).

Constraints to biological control

Although the benefits of biological control frequently outweigh the costs, many programmes often suffer various limitations. Initially these may involve difficulties in

securing adequate research funding, a challenge often experienced in developing countries, which may be exacerbated by the need for quarantine and mass-rearing facilities (Wajnberg et al., 2001). The nomination, selection and importation of promising and suitable agents requires relevant scientific experience, and if executed poorly is costly, incurring unwanted expenditure and risks (Muniappan et al., 2009). In countries where biological control programmes exist the decision to release agents is cleared by relevant regulatory bodies (Wajnberg et al., 2001). Although beneficial, these applications for release may be tedious processes, easily confounded by ambiguous host-specificity results of agents, with suggestions that in certain cases the release of safe and potentially effective agents have been erroneously rejected (Fowler et al., 2012; Downey and Paterson, 2016).

However, the majority of difficulties in biocontrol programmes are experienced during and after agent releases (Carson et al., 2008; Morin et al., 2009). The establishment of biocontrol agents can be an intensive process, requiring numerous releases, which are affected by a variety of abiotic and biotic factors including release numbers, release sites, seasonality, climate and predation (Broughton and Pemberton, 2008). Compounding these issues is the ongoing requirement of continuous or periodic post-release assessments, evaluating the performance, spread and efficacy of the agents in the field (Morin et al., 2009).

In South Africa, *Solanum mauritianum* is a notorious invasive tree which has suffered a troubled biocontrol history (Hakizimana, 2011; Olckers, 2011). Constraints surrounding the biological control of *Solanum mauritianum* in South Africa have included a temporary suspension to the programme, host-specificity concerns, poor agent establishment and performance, as well as a limited availability of agent research subsequent to their release.

Assessing biological control (agents)

Post-release evaluations are an integral, but often lacking, component of biocontrol programmes and typically consist of establishment surveys, agent incidence ratings and damage assessments (Morin et al., 2009). These post-release evaluations are performed subsequent to the successful establishment of biological control agents, and seek to assess agents in terms of their impact on target weeds (Thomas and Willis, 1998). This rating system is divided into five categories, dependent upon the agent's impact on the target weed (Klein 2011). The categories are (i) Extensive (ii) Considerable, (iii) Moderate, (iv) Trivial and (v) Unknown (see Klein, 2011). Subsequent to these evaluations, the status of the target weed itself, in terms of priority and required level of control should be identified (Carson et al., 2008). Despite recent amendments to these post-release evaluations, biocontrol assessments are still criticised in terms of their seemingly visual and often subjective measures (Morin et al., 2009).

Increasing pressure to accurately quantify agent performance, particularly damage, to problematic weed populations, has seen assessments mainly focus on monitoring agent incidences and weed densities (Briese, 2004; Carson et al., 2008). Although these parameters are quantifiable, permitting a basic inference into agent efficacy, they lack the ability to assess more crucial ecological interactions (Morin et al., 2009). Suggestions surrounding weed biocontrol have sought to shift the focus from a linear perspective towards an ecological one, particularly by incorporating the response of the weeds and the agent-weed interactions (Holt, 1991; Sheppard, 2003; Briese, 2004). The incorporation of ecophysiology, particularly gas exchange (photosynthesis, stomatal conductance, water-use efficiency), has permitted great insights into weed biology and behaviour at leaf, plant and community levels (Long and Bernacchi, 2003; Kant et al., 2015). Despite the recent integration of biocontrol

studies incorporating weed ecophysiology, the use of these studies remains exceedingly limited in the literature (see Marlin et al., 2013; Venter et al., 2013).

Climatic unsuitability

The effects of climate are known to be highly influential on the distribution and establishment of many insect biological control agents (Roberston et al., 2008). Despite consisting of numerous variables, biocontrol-climate studies have historically focused on the effects of low temperature (critical thermal minima) (King, 2011). Many studies have highlighted that poorly matched climatic variables maintain the potential to reduce the efficacy of biological control programmes (Byrne et al., 2004). In areas where the establishment of newly introduced biocontrol agents is inadequate or unsuccessful, a mismatch of one or more climatic variables is frequently the cause (Byrne et al., 2002; Byrne et al., 2004). McEvoy and Combs (2000) estimate that 44% of all insect biocontrol agents fail to establish due to climatic unsuitability. In many aspects the effects of climate on the establishment and efficacy of biological control agents can be complex, requiring a systematic and ecological approach to identify them.

Recent advancements in biocontrol have seen the incorporation of ‘climate’ through climatic modelling, particularly using CLIMEX (Sutherst and Maywald, 1985). CLIMEX models are based on long-term meteorological data from various localities which can be used to generate a potential distribution for a particular species (Sutherst and Maywald, 1985; Kriticos et al., 2015). These models permit inference into species’ potential distribution or establishment in novel regions or countries, in relation to their climate (Kriticos et al., 2015). This modelling approach has proved highly beneficial in biocontrol programmes providing numerous insights and predictions. These predictions include i) the feasibility and likelihood of agent establishment and spread, ii) identification of preferential release and collection sites

and iii) areas in which agents are likely to experience climatic stresses (e.g. Byrne et al., 2002; Senarthe et al., 2006; Roberston et al., 2008). Additionally, studies by Rott and Ponsonby (2000) and Dhileepan et al. (2005) documented that biocontrol agents introduced into areas exhibiting similar climates to their native range are often more effective.

Indirect effects of biological control

Assessments focused on the potential indirect effects of agents is an increasing trend in post-release evaluations, despite being one of the most challenging aspects of biocontrol studies (Louda et al., 2005; Morin et al., 2009). The indirect effects of biocontrol cover a broad range of ecological risks or implications associated with the release and establishment of biocontrol agents (Follett and Duan, 2000; Louda et al., 2005). There are a variety of indirect effects which include the risk of non-target damage, implications on the local ecosystem functioning, interactions with native flora and fauna, as well as the occurrence of unintended agent interactions with the target plants (Cory and Myers, 2000; Wajnberg et al., 2001).

In many cases the assessment of indirect agent-weed interactions is an interesting but contentious issue often overlooked but frequently debated (Wills and Memmot, 2005). However, the stimulus for these debates may not entirely be based on circumstantial evidence, but rather a reflection of the difficulty in outlining and assessing these indirect effects and interactions (Follett and Duan, 2000; Thomas et al., 2004). The underlying assumption that damage to target weeds by biocontrol agents is purely inhibitory is fundamentally flawed (Louda et al., 2005). Ecologically speaking plant species will respond to attack through a variety of compensatory mechanisms (Zvereva et al., 2010). The ecological and dynamic nature of biocontrol thus suggests that agent-weed interactions could yield unexpected and potentially unwanted results (Pearson and Callaway, 2005). Studies

highlighting these indirect weed-agent interactions remain under-represented in the biocontrol literature. Additionally, agent species that establish but fail to adequately reduce target weed populations are suggested to facilitate these indirect ecological effects and interactions (Pearson and Callaway, 2003, 2005). One of South Africa's more challenging biological control programmes, encompassing a variety of the aforementioned constraints and hindrances, has been that against the notorious invader *Solanum mauritianum* (Olckers, 2009).

Solanum mauritianum

Solanum mauritianum Scopoli (syn. *Solanum auriculatum* Aiton) (Solanaceae), commonly referred to as Bugweed, Wild tobacco bush (Australia) or Woolly Nightshade (New Zealand), is a severe environmental weed of global significance (Olckers and Hulley, 1989; Van Den Bosch et al., 2004; ISSG, 2006). Originating from South America (Argentina, Brazil, Paraguay and Uruguay), *S. mauritianum* has rapidly become a widespread invasive tree having naturalised in many countries; including India, Kenya, Mauritius, Madagascar, Swaziland, Australia, Papua New Guinea, New Zealand and South Africa (Olckers and Zimmermann, 1991; Van Den Bosch et al., 2004; Barboza et al., 2009).

The earliest reports of *S. mauritianum* in South Africa were as an ornamental species in KwaZulu-Natal during the 1860s (Roe, 1972; Olckers, 2009). This fast growing and highly reproductive tree has invaded many of the eastern, higher rainfall regions of the country, becoming problematic in the Western and Eastern Cape, KwaZulu-Natal, Gauteng, Mpumalanga and Limpopo (Olckers, 2003, 2009, 2011; Fig. 1.1). The weed is known to grow in a wide variety of habitats including riparian zones, degraded grasslands, conservation reserves, forest margins, commercial plantations and agricultural areas; being particularly

problematic and costly in the forestry and agricultural sectors (Olckers, 1999; Barboza et al., 2009).

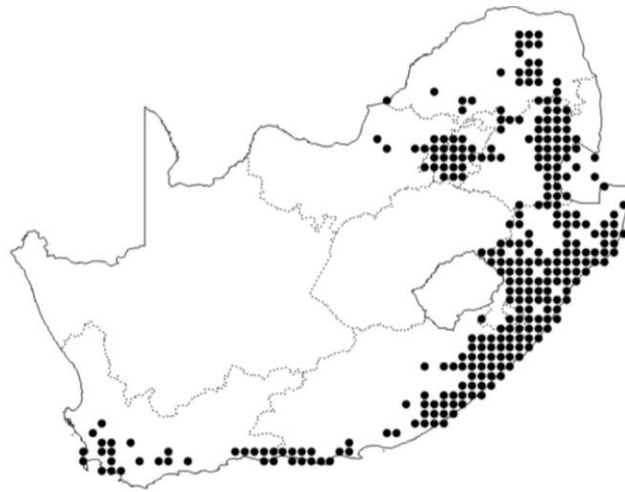


Fig. 1.1. *Solanum mauritianum* distribution in South Africa (taken from Henderson, 2001).

Solanum mauritianum is a small broad-leafed, perennial tree typically reaching approximately four meters in height (Fig. 1.2A) and is often covered in fine felt-like hairs, which are a dermal and respiratory irritant (Henderson, 2001; Maroyi, 2012). The plant is capable of self-pollination and can regenerate rapidly subsequent to being felled (Witkowski and Garner, 2008; Rambuda and Johnson, 2004). *Solanum mauritianum* may flower and fruit (Fig. 1.2B-C) as early as six months after germination, and may continue to flower and fruit indefinitely (Olckers, 2009). These fruits contain copious numbers of seeds (± 150) which are *S. mauritianum*'s primary reproductive avenue (Witkowski and Garner, 2008; Olckers, 2011). The seeds are key to the weed's rapid invasion, with average viability ranging between 76-98% as well as seedbank dormancy periods of up to two years (Campbell and van Staden, 1994; Witkowski and Garner, 2008). These seeds are primarily dispersed by frugivorous bird species, in particular the African Olive (Rameron) pigeon (*Columba arquatrix*), Dark-capped Bulbul (*Pycnonotus tricolor*) and Red-winged Starling (*Onychognathus morio*), which have all shown preferences to fruits of *S. mauritianum* (Jordaan et al., 2011; Mokotjomela et al.,

2009, 2013). Notably the African olive pigeon has been linked to high seedling recruitment, particularly in commercial plantations and natural forests (Oatley, 1984).

The invasive success of *Solanum mauritianum* is largely linked to the plant's ecology and life history traits (Olckers and Zimmermann 1991). The proliferation of *S. mauritianum* and its widespread invasion across South Africa is likely an example of the Enemy Release Hypothesis (Keane and Crawley, 2002; Colautti et al., 2004). The rapid growth, continuous flowering and excessive fruiting of *S. mauritianum* in South Africa is likely due to the tree having undergone 'biotic release'. South Africa's climate also provides similar conditions to those experienced by the plant in its native sub-tropical, South American range, further accommodating the species' invasion and spread throughout most of the country (Pedrosa-Macedo et al., 2003). Early surveys have estimated that *S. mauritianum* has invaded as much as 18% (1.8 million ha) of South Africa's land (Versfeld et al., 1998).

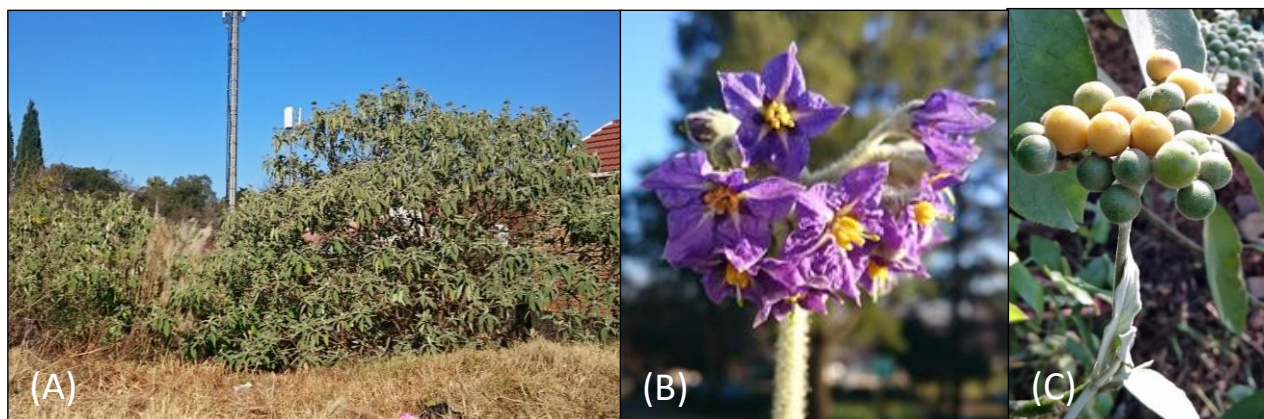


Fig. 1.2. A mature *Solanum mauritianum* tree (A), flowering inflorescence (B), and an infructescence with developing 'green' and mature 'yellow' fruits (C) (Photos: BW Cowie).

The invasion and establishment of *S. mauritianum* in certain areas often results in the recruitment and formation of dense stands, which outcompete and shade-out indigenous vegetation, often preventing the growth of native flora (Henderson, 2001; ISSG, 2006). Furthermore, suggestions of potential allelopathic effects inhibiting the growth and

recruitment of surrounding vegetation have been studied, although this remains preliminary and debated (Van Den Bosch et al., 2004; Thelen et al., 2005). Given the ability of *S. mauritianum* to invade natural areas, resulting in dense, uniform stands, which alter the landscape, it has been listed as a transformer species (Henderson, 2001). The foliage and particularly the unripe fruits are high in alkaloids making the plant toxic. The alkaloid Solasodine is primarily responsible for the toxicity of the fruits and leaves (Watt and Breyer-Brandwijk, 1962; Campbell and van Staden, 1990). The leaves and stems are also covered in pubescent, felt-like hairs which are highly unpalatable, deterring wild game or domestic herbivores (Henderson, 2001; Olckers, 2009). The entire plant is also toxic to both humans and animals, excluding the frugivorous bird species that are unaffected by the alkaloids, feeding on the fruits with impunity (Everist, 1981). *Solanum mauritianum*, in particular its fruits, may also act as a pest reservoir providing an additional host for several fruit fly (Tephritidae) species during winter months (Copeland and Wharton, 2006).

Biological control of *Solanum mauritianum*

Given the severe ecological and socio-economic detriment of *S. mauritianum*, South Africa was the first country to target the tree for biological control (Olckers, 1999). Biocontrol of *S. mauritianum* in South Africa, was first initiated during 1984, when conventional controls, namely slashing, herbicides and mechanical removal, were deemed ineffective and ultimately unsustainable (Olckers, 1999, 2009). Overall the sole use of mechanical/chemical clearing efforts is undesirable as the cost of clearing programmes frequently outweigh the estimated value of the cleared land (Olckers, 1998) and in certain cases may increase the densities of *S. mauritianum* (Witkowski and Garner 2008). Furthermore, van Wilgen et al. (2004) predicted that only 3% of the total area invaded by *S.*

mauritanum had been cleared, and that complete clearing of the invasion would take 23 years.

A total of 17 potential agents were imported and assessed for the biological control of *S. mauritanum* (Olckers and Zimmermann, 1991; Olckers, 2000). Many of these candidates were promising but rejected or shelved over host-specificity concerns surrounding both native and cultivated *Solanum* species, particularly eggplant, *Solanum melongena* (Olckers, 2000; Olckers et al., 2002; Withers et al., 2002; Klein, 2011). Only two biocontrol agents have been released against *S. mauritanum* in South Africa, a sap-sucking lace bug, *Gargaphia decoris* Drake (Hemiptera: Tingidae) and a flower-bud feeding weevil, *Anthonomus santacruzi* Hustache (Coleoptera: Curculionidae) (Klein, 2011; Olckers, 2011).

Gargaphia decoris

The lace-bug, *Gargaphia decoris* (Fig. 1.3A), is classified as an outbreak species, with females laying large egg batches (± 160 eggs), predominately on the abaxial leaf surface (Lotter, 2004). Nymphs hatched from eggs (± 2 weeks) feed gregariously on leaf sap, resulting in chlorotic specks (Olckers and Borea 2009, Fig. 1.3B-C). Extensive feeding by *G. decoris* results in the chlorosis of leaves which typically causes their premature abscission (Olckers, 2000). Laboratory trials highlighted that *G. decoris* was host-specific, showing promise as a biological control agent for *S. mauritanum* (Olckers, 2000). During no-choice tests *G. decoris* displayed oviposition and feeding on eggplant, *S. melongena*, albeit at low levels (Olckers, 2000; Withers et al., 2002). *Gargaphia decoris* showed strong preference for *S. mauritanum* during choice tests, which included eggplant, indicating non-target feeding would be highly unlikely (Olckers and Lotter, 2004; Hope and Olckers, 2011). Withers et al. (2002) further suggested that should any non-target feeding occur the damage would be negligible.



Fig. 1.3. *Gargaphia decoris* final instar nymphs (A), newly moulted adults and nymphs (B) and mature adult (C) (Photos: BW Cowie).

Initial releases of *G. decoris*, were carried out at several sites within KwaZulu-Natal during 1999 and later in the Limpopo and Mpumalanga provinces in 2001 (Klein, 2011; Olckers, 2011). To date, *G. decoris* has been classified as an inefficient agent in the field, with overall damage being listed as trivial (Klein, 2011). The seemingly poor performance of *G. decoris* has largely been due to the inadequate and often sporadic establishment of the agent (Witt, 2007; Olckers and Borea, 2009). Originally cold winter temperatures were blamed for the low densities at which *G. decoris* established, which saw the importation of *G. decoris* stocks from cooler regions of Brazil (Hope and Olckers, 2011). However, assessments by Barker and Byrne (2005) found that both the original population and stocks imported from Brazil could adequately tolerate low temperatures, particularly the cold South African winters.

Preliminary studies suggested that egg, nymph and adult predation by generalist predators, such as mirids, coccinellids and formicids, may be responsible for the low densities at which *G. decoris* establish (Lotter, 2004; Hakizimana and Olckers, 2013b). More recently studies have suggested that shading influences both the persistence of *G. decoris* populations in the field (Patrick and Olckers, 2014) and more crucially, the susceptibility of *S.*

mauritianum plants to herbivory (Ghebremariam et al., 2014). Despite its importance, the effects of shading on the performance (herbivory) of *G. decoris* and response of *S. mauritianum*, remains untested. Despite these effects, erratic outbreaks of *G. decoris* which have been extensively damaging to *S. mauritianum* in the field are documented, but remain localised and insufficient in controlling the weed (Klein, 2011; Olckers, 2011).

Anthonomus santacruzi

Largely regarded as the most promising biocontrol agent against *S. mauritianum*, the flower-bud weevil, *Anthonomus santacruzi* (Fig. 1.4A-D), was released in KwaZulu-Natal during 2008 (Olckers, 2011) and more recently in Mpumalanga in 2014 (A. Sasa pers. comm.). Releases of *A. santacruzi* were intended to reduce the flowering, high seed production and therefore dispersal and recruitment of *S. mauritianum* (Olckers 2008). Adults feed on the flowers and shoot tips, with females ovipositing into the new buds (Olckers, 2003; Barboza et al., 2009). The larvae hatch, then feed within the buds, destroying them as they develop (Olckers, 2003; Gillespie and Besaans, 2014). These buds are unable to recover, thus reducing the fruiting capability of the plant. However, the effectiveness and interactions of *A. santacruzi*'s florivory (flower-feeding) on the reproductive output and success of *S. mauritianum* remains debated (Hakizimana, 2011).

Laboratory assessments that highlighted the agent's promise as a fruit-reducing agent also sparked concerns surrounding the weevil's host-specificity (Olckers, 2009, 2011). Initial concerns surrounded ambiguous oviposition and survival by *A. santacruzi* on cultivated and native *Solanum* species during no-choice tests (Hakizimana and Olckers, 2013a). However, *A. santacruzi* displayed a consistent preference for feeding and oviposition on *S. mauritianum* during choice-tests (Olckers, 2003; Hakizimana, 2011). The preference of *A. santacruzi* for *S. mauritianum* highlighted an artificially expanded host range experienced in the laboratory,

and one which was unlikely to be utilised by the weevil in the field (Harris, 1985; Olckers, 2003). Concerns were then shifted towards *A. santacruzi*'s relationship to the Boll Weevil (*Anthonomus grandis*), a major pest of cotton (Clarke and Burke, 1996; Olckers, 2008). However, studies by Olckers (2008) concluded that *A. santacruzi* posed no threat to the South African cotton industry.

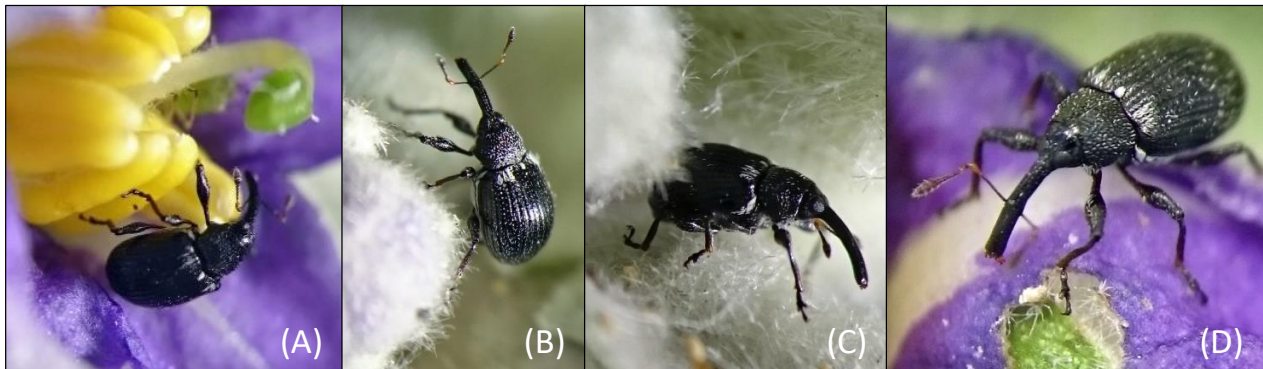


Fig. 1.4. *Anthonomus santacruzi* adult weevils present on *S. mauritanum* anthers (A), developing buds (B), inflorescence stalks (C) and flower petals (D) (Photos: BW Cowie).

Post-release evaluation of *A. santacruzi* is ongoing but remains minimal, with the weevil's damage status and resultant efficacy currently classified as unknown (Klein, 2011). Much like *G. decoris*, native predators and climate were both suggested to impact the establishment and survival of *A. santacruzi* in South Africa (Hakizimana and Olckers, 2013b). In particular, inflorescence inhabiting predators, including crab spiders (Thomisidae), were predicted to impede *A. santacruzi*'s survival and reproduction (Hakizimana and Olckers, 2013c). Further studies however, concluded that such predators, particularly spiders, would pose little threat to *A. santacruzi* populations (Hakizimana and Olckers, 2013c). Additionally, suggestions that climate, namely temperature, was influential in the distribution and establishment of *A. santacruzi* are emphasised but remained largely understudied (Olckers, 2003, Hakizimana, 2011). Hakizimana (2011) suggested that *A. santacruzi* populations were likely constrained by low temperatures in South Africa, after establishment

studies at warmer coastal and cooler inland release sites. However, the effects of low humidities on *A. santacruzi* may be an influential factor limiting the agent's establishment and spread but lacked any formal scientific assessment and investigation (Hakizimana, 2011).

Rationale

The ongoing spread and detrimental impacts of invasive species will undoubtedly see the demand for biological control increase (Wajnberg et al., 2001; Pimentel, 2011). Although biological control efforts against *S. mauritianum* were initiated 32 years ago, they have not yet attained the desired levels of control (Klein, 2011; Olckers, 2011). The evaluation of biological control programmes is a vital and often overlooked component, which is used to justify ongoing project expenditure (McFadyen, 1998; King, 2011). The severe ecological and economic effects caused by *S. mauritianum*, particularly in South Africa, requires that biological control research be continued. The close taxonomic relation of *S. mauritianum* to various native and cultivated *Solanum spp.* suggests that South Africa may be unlikely to assess any additional new agents at present. This advocates that further research into the released agents, *Gargaphia decoris* and *Anthonomus santacruzi*, is imperative, in order to optimise and improve the biocontrol efforts against *S. mauritianum*. Biocontrol research efforts should seek to investigate, identify and address many of the factors constraining the agents' establishment and performance, so that informed decisions can be made regarding the development or abandonment of these agents. Few previous studies have evaluated the performance and efficacy of the biocontrol agents, *G. decoris* and *A. santacruzi*. Lastly this research has the potential to contribute towards ongoing research efforts into *S. mauritianum* biocontrol in other countries, particularly New Zealand.

Study Aim and Objectives

This study aims to investigate the impacts of *Anthonomus santacruzi* and *Gargaphia decoris* on *Solanum mauritianum* as well as formally contribute towards the scientific research on the biocontrol of *S. mauritianum* in South Africa. The work addresses some of the current knowledge gaps surrounding the biological control agents *Gargaphia decoris* and *Anthonomus santacruzi*. The research objectives were as follows:

- Objective 1.** Assess the feeding performance and ecophysiological, namely photosynthetic, interactions of *Gargaphia decoris* with *Solanum mauritianum* plants, under full-sun and shaded conditions.
- Objective 2.** Assess the possible constraints posed by climate, particularly low temperature and low relative humidity, on the survival of *Anthonomus santacruzi* adults, and consequent implications for the species' establishment and spread in South Africa.
- Objective 3.** Assess the impacts of *Anthonomus santacruzi* feeding on *Solanum mauritianum* inflorescences, particularly reproductive output (fruit and seed production), as well as the weevil's potential contributions as a pollinator of the weed.

Dissertation outline

The first chapter of this dissertation consists of a general introduction, including a brief review of the literature on the current biological control efforts against *S. mauritianum* in South Africa. Chapter Two is largely an ecophysiological paper which sought to assess the feeding performance of *G. decoris* and response of *S. mauritianum* plants under both full-sun and shade conditions (Objective 1). In Chapter Three, the possible climatic constraints to the establishment, survival and spread of *A. santacruzi* were investigated (Objective 2). Temperature, humidity and climatic matching analyses (CLIMEX modelling), were carried out and integrated to suggest the potential climatic factors that will reduce the survival of *A. santacruzi* adults and consequently their longevity, establishment and spread. Chapter Four largely focuses on the interactions of *A. santacruzi* with the inflorescences of *S. mauritianum*, particularly the effects of feeding on fruit/seed set, and the potential of *A. santacruzi* to act as a pollinator of the weed (Objective 3). The final chapter is an overview of the current and future prospects for *S. mauritianum* biocontrol in South Africa, including the implications for *G. decoris* and *A. santacruzi*, the revival of previously shelved agents and the potential incorporation of biocontrol with integrated management actions. This chapter also provides recommendations for future research toward improving *S. mauritianum* biocontrol efforts.

This dissertation has been prepared and written in the format of scientific papers. The repetition of information given this format is unfortunately unavoidable but has been minimised as far as possible.

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2. Exacerbation of photosynthetic damage through increased heat–light stress resulting from *Gargaphia decoris* sap-feeding ☆



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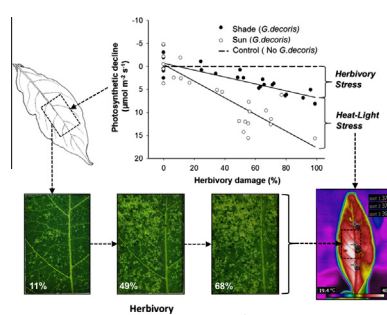
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HIGHLIGHTS

- *Gargaphia decoris* herbivory reduced photosynthesis of *Solanum mauritianum* in both shaded and full-sun conditions.
- Photosynthesis declined due to chlorophyll removal and, indirectly, through physiological impairment.
- Herbivory rates were greater in the shade but feeding was more damaging in sun plants.
- Given the agent's potential effectiveness for biological control, factors constraining its performance need to be addressed.

GRAPHICAL ABSTRACT



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ABSTRACT

Solanum mauritianum (Bugweed) is one of the world's worst ecological weeds, being targeted for biological control in South Africa since 1984. One of two promising biocontrol agents released against *S. mauritianum* is a sap-sucking lace bug, *Gargaphia decoris* (Hemiptera: Tingidae). There are few studies on physiological changes induced by feeding damage of biological control agents on their target weeds. Chlorophyll removal by *G. decoris* feeding caused metabolic impairment which resulted in a reduction of photosynthetic rates of *S. mauritianum* leaves, with a greater effect on plants growing in full sun compared to plants growing in shade. This difference was related to higher leaf temperatures in the sun. Herbivory caused a 52% reduction in transpiration rates, resulting in a reduction in evaporative cooling of the leaf. Although *G. decoris*'s feeding rates were greater on the shade plants, feeding per unit area was significantly more damaging to plants continually exposed to sunlight. The increased physiological damage experienced by full-sun plants may be a combination of stresses, particularly the direct effect of chlorophyll removal via herbivory and the indirect effect of accumulated heat–light stress. Given *G. decoris*'s effectiveness for biological control, factors constraining its performance in the field need to be identified and addressed.

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☆ BWC, MJB, ETFW and NV conceived and designed the experiments. BWC and NV performed the experiments. BWC, MJB, ETFW and NV analyzed the data. MJB and ETFW provided critical input into data and statistical analyses. BWC and NV wrote the manuscript.

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1. Introduction

Solanum mauritianum Scop. (Solanaceae) is a small woody shrub or tree native to the tropics and sub-tropics of South America (Olckers, 2011). Over the last century it has become an emergent invader in various countries worldwide (Olckers, 2009). *Solanum*

mauritianum's invasion has been prolific in South Africa, particularly in the eastern, higher rainfall regions of the country (Olckers, 2003, 2011). The weed forms dense monospecific stands, outcompeting indigenous vegetation in natural habitats, and is detrimental in commercial forestry plantations (Olckers, 2009; Atkinson et al., 2014) and riparian areas (Witkowski and Garner, 2008). South African biological control efforts against *S. mauritianum* have seen two agents released, a sap-sucking lace bug, *Gargaphia decoris* Drake (Hemiptera: Tingidae) in 1999 and a flower-bud feeding weevil, *Anthonomus santacruzi* Hustache (Coleoptera: Curculionidae) in 2008 (Klein, 2011).

Gargaphia decoris was first released at several sites in KwaZulu-Natal during 1999 and later in Limpopo and Mpumalanga provinces in 2001 (Olckers, 2011). The adults and nymphs feed gregariously on leaf sap, resulting in chlorotic spots (Olckers and Borea, 2009). Continual feeding results in complete leaf chlorosis, accompanied by premature abscission (Olckers, 2000). To date, *G. decoris* is considered ineffective as a biological control agent, with overall damage being classed as trivial (Klein, 2011). Its poor performance has largely been attributed to populations remaining at low levels (Olckers and Lotter, 2004). Egg, nymph and adult predation by generalist predators, including Formicidae, Miridae and Coccinellidae has been implicated as the reason for population numbers not proliferating (Lotter, 2004). Decreased abundance of *G. decoris* in winter is seemingly linked to the lowered availability and the quality of *S. mauritianum* leaves (Winston et al., 2014). Despite these adverse effects, erratic outbreaks of *G. decoris* which are extensively damaging to *S. mauritianum* have been documented (Olckers, 2011).

Mucivory (feeding on plant fluids, commonly sap) as shown by lace-bugs, is thought to impose greater localised physiological change, particularly photosynthetic stresses, to plants than mechanical leaf feeding (Meyer, 1993). The removal of vital leaf compounds including photosynthates, sugars, chlorophyll and water, are costly for the plant to replace in response to mucivorous feeding (Gonda-King et al., 2014). Numerous studies on sap-feeding insects are frequently linked to reductions in plant chlorophyll content and photosynthetic rates, with this form of chlorophyll removal being largely accepted as a cause of reduced photosynthetic rates (Cabrera et al., 1994; Zvereva et al., 2010). Insect herbivory can also cause general plant responses which have indirect consequences on photosynthesis, namely disruptions to leaf vasculature, altered resource reserves and induced down-regulation of photosynthesis (Nabity et al., 2009).

Plant responses elicited by insect herbivores are widely documented but differ depending on the mode of herbivory experienced (Nykänen and Koricheva, 2004; Zvereva et al., 2010). In the case of sap-feeding insects, plant responses are mostly physiological rather than structural or chemical (Dungan et al., 2007). However, these physiological responses, such as stomatal closure, are frequently inadequate to compensate for sap-removal and may be restricted by plant available resources and the current environmental conditions, particularly light intensity and duration (Roberts and Paul, 2006; Dungan et al., 2007). Light, which can vary significantly under natural conditions, maintains a direct influence on both the photosynthetic capabilities of the plant as well as plant-insect interactions (Muth et al., 2008; Ruban, 2009). High light environments often experience greater temperatures, given their direct exposure to solar radiation, but may benefit plants in terms of improved photosynthesis, growth and ability to compensate for herbivory (Roberts and Paul, 2006; Ruban, 2009). Whereas shaded as opposed to full sunlight habitats, tend to maintain cooler and more buffered conditions creating microhabitats preferable to certain insect herbivores (Muth et al., 2008; Diaz et al., 2011; Patrick and Olckers, 2014). These differing light environments also

influence insect behaviours, such as feeding, reproduction and migration (Patrick and Olckers, 2014; Uyi et al., 2015). Overall light conditions have variable effects on insect herbivores, plant physiology and their insect-plant interactions (Roberts and Paul, 2006).

Few studies have evaluated the physiological responses of invasive plants in relation to feeding damage by their biological control agents (Marlin et al., 2013; Venter et al., 2013). This study aimed to quantify the photosynthetic response of *S. mauritianum* to *G. decoris* feeding, under two natural light conditions. To interpret photosynthetic changes, the underlying metabolic mechanisms responsible were investigated. We tested the hypothesis that plants exposed to full-sun would maintain greater photosynthetic rates compared to the shaded plants when exposed to *G. decoris* herbivory. *Gargaphia decoris*'s behaviour was assessed in relation to leaf surface temperature and position (adaxial or abaxial surfaces) under both light conditions, to ascertain if differences in photosynthetic rates under these light conditions were caused by differential *G. decoris* feeding.

2. Methods and materials

2.1. *Gargaphia decoris* herbivory and leaf greenness

Solanum mauritianum plants were grown from seed, in a greenhouse, to ~1 m (height) and then randomly divided into full-sun and shade environments in the glasshouse section of the insectary at the University of the Witwatersrand, Johannesburg, where they were acclimated for four weeks. This area in which plants were housed received only natural light for the duration of the experiment. Maximum daily photosynthetic photon flux density (PPFD) in the glasshouse, were 1800 and 45 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in the sun and shade environments respectively, and moderate air flow (0.6–0.83 m s^{-1}) was maintained via a fan. The sun and shade PPFD values were matched to that of field conditions, of canopy understories and open areas respectively. Minimum/maximum temperatures were 15 °C and 26 °C respectively, with the daytime range between 20.5 °C and 26 °C.

Within both the sun and shade conditions, six plants were randomly assigned as controls (no *G. decoris*) and six as treatments (with *G. decoris*). The second or third youngest unfolded leaf below the apical bud on each plant was enclosed in a mesh bag with 10 *G. decoris* adults. Control plants leaves were bagged but did not include any *G. decoris*. *Gargaphia decoris* was obtained from cultures reared in the same facility. Bagged treatment leaves were inspected twice daily for insect mortality in order to maintain 10 adults per leaf for the duration of the study.

Leaf greenness was measured using a single photon avalanche diode (SPAD) 502 Chlorophyll Meter (Minolta, Osaka 542, Japan) on the same area used for gas exchange. Numerous authors have reported strong relationships of SPAD values to leaf chlorophyll content, in a number of families and genera, including *Solanum* (Netto et al., 2005; Uddling et al., 2007). The experiment was concluded on the 18th day when the treatment leaves were senescing.

2.2. *Gargaphia decoris* feeding site selection

The number of *G. decoris* on either the adaxial (upper) or abaxial (lower) leaf surface was recorded on full-sun and shade leaves on clear-sunny days only, from 09:30 until 15:30, for a period of five days. Given the sedate nature of *G. decoris*, leaf position assessments were taken at 30 min intervals. Individuals located perpendicularly on leaf margins were recorded as adaxial dwelling.

2.3. Gas exchange and leaf damage area

Net leaf CO₂ and H₂O exchange on the second or third youngest unfolded leaf below the apical bud was measured using a LI-6400XT portable open-path infrared gas analyser (LI-COR Biosciences, Lincoln, NE, USA). PPFD within the cuvette was set at 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (saturating intensity determined from A-PPFD response curves) using a blue–red LED light source (6400-02B). During measurements, leaf temperatures were maintained at 25 °C, leaf to air vapour pressure deficit (VPD) was kept below 1.5 kPa and CO₂ concentration was supplied at 400 $\mu\text{mol mol}^{-1}$. All measurements were made between the hours of 11:00 and 15:00. The same 6 cm² area of leaf (control and treatments) was inserted into the cuvette on each sampling day as this allowed us to monitor gas exchange on an area of increasing herbivory damage (treatments). Net photosynthesis (A), stomatal conductance (g_{st}), transpiration (E) and intercellular CO₂ concentration (C_i) values were calculated according to the equations of von Caemmerer and Farquhar (1981). When A and g_{st} showed less than $\pm 2\%$ change over a 3 min period, data were recorded. Leaf damage was quantified by photographing the portion of leaf used in gas exchange, and chlorotic damage was analysed using ImageJ imaging software (Ver. 1.48, Wayne Rasband, National Institute of Health, USA).

2.4. Photosynthesis to temperature curve

The photosynthetic response to temperature (A-temp) curves was constructed on sun control plants to determine the thermal threshold where impairment of photosynthetic metabolism was evident. Net leaf CO₂ and H₂O exchange was measured on sun control leaves. PPFD within the cuvette was set at 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ using a blue–red LED light source. Stomatal conductance (g_{st}) was shown to decrease with increases in temperature, thereby imposing a limitation to CO₂ diffusion across the epidermal layer, which altered the C_i . To avoid this effect, C_i was maintained at 300 $\mu\text{mol mol}^{-1}$ by adjusting the reference CO₂ concentrations at each of the following target leaf temperatures: 23, 25, 27, 29, 31, 33, 35 and 37 °C. Data were recorded at the target temperatures when A and g_{st} showed less than $\pm 2\%$ change and C_i less than $\pm 1\%$ change over a 3 min period.

2.5. Chlorophyll fluorescence

Directly following gas exchange measurements, all plants were placed in the shade and dark adapted overnight (clip placed in a random position within the 6 cm² area where gas exchange and herbivory damage was measured). Initial fluorescence (F_o) and maximum fluorescence (F_m) were measured and the maximum quantum yield of photosystem II (PSII) ($F_v/F_m = F_m - F_o/F_m$) was calculated using an OS1p Modulated Chlorophyll Fluorometer (OptiSciences, Inc. NH 03051 USA). F_o was measured using a PPFD of 0.11 $\mu\text{mol m}^{-2} \text{s}^{-1}$ which was the lowest intensity to elicit a minimal fluorescence signal from the reaction centres without assuming that electron transport through PSII was induced (Pheophytin remaining in the oxidised state). The maximum saturation intensity pulse used to measure F_m was 11000 $\mu\text{mol m}^{-2} \text{s}^{-1}$, which was assumed to completely reduce the PSII reaction centres (primary acceptor – Q_A reduced) (Murchie and Lawson, 2013).

2.6. Leaf surface temperature

Leaf surface temperatures were measured on control and treatment plants (mesh bags removed directly before measurements) from the shade and sun positions between 12:00 and 13:00 on day 14 (>50% herbivory damage) using a FLIR E60 infrared camera

(FLIR systems Inc. Wilsonville, Oregon, USA). Thermal images were taken by moving plants quickly out of direct sunlight to prevent solar reflectance influencing the results. Thermal images of the adaxial and abaxial leaf surfaces were analysed (FLIR Tools+ 5.2.1) to calculate the mean and maximum leaf surface temperatures. Maximum leaf surface temperatures were calculated using the highest thermal value that was present over at least 5% of the leaf's surface.

2.7. Statistical analysis

Gas exchange, chlorophyll fluorescence and SPAD data measured over time were compared between treatments using one-way repeated measures (time) analysis of variance (ANOVA) with a Tukey HSD post hoc (StatSoft, 2014). Linear regressions were fitted to photosynthetic rates against herbivory damage for both sun and shade leaves. A Standardised Major Axis (β_{SMA}) test with herbivory damage set as a continuous predictor was used to detect heterogeneity amongst fitted slopes for the sun and shade treatments (Warton et al., 2006). A full-cross General Linear Model (GLM) with a Tukey HSD post hoc, was used for multiple comparisons amongst and within treatment and control, as well as between adaxial, abaxial and maximum leaf surface temperatures. Lastly *G. decoris* density on the adaxial leaf surfaces between full-sun and shaded plants, during diurnal periods, was assessed with a Students *t*-test (assuming unequal variance; $\alpha = 0.05$).

3. Results

3.1. Gargaphia decoris leaf herbivory and feeding site selection

Chlorosis due to *G. decoris* feeding was significantly greater on leaves in the shade throughout the course of the experiment (Fig. 1a). Decreases in SPAD values occurred with increasing herbivory damage, indicating the removal and/or damage of chlorophyll through feeding (Fig. 1b). Significantly lower densities of *G. decoris* ($t_{31} = 24.2$; $P = 0.0001$) were documented on the adaxial surface of sun plants when compared to the same leaf surface of plants in the shade (Fig. 2). In both sun and shade plants *G. decoris* only experienced 2% mortality throughout the study.

3.2. Gas exchange

In response to *G. decoris* herbivory overall photosynthesis (A) was significantly reduced by 11 and 6.6 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for sun and shade leaves respectively (Fig. 3a, b). Herbivory resulted in a significant reduction in A from day 11 onwards in the sun plants, whereas shaded plants were only significantly affected by day 18. The sun control leaves' A peaked at day 11 as they matured then showed a decline. Stomatal conductance (g_{st}) for treatment plants showed a gradual decline over time and the effect of herbivory became apparent by day 11 in both the sun and the shade plants (Fig. 3c, d). Intercellular CO₂ concentrations (C_i) in response to herbivory in both sun and shade plants relative to their controls were statistically greater from day 11 onwards, showing increases of 37 and 32 $\mu\text{mol mol}^{-1}$ respectively (Fig. 3e, f). Transpiration (E) for both sun and shade treatments displayed similar trends to g_{st} (Fig. 3g, h). Photosynthetic decline was calculated as the A treatment values recorded on a day subtracted from the A mean of the control on the same day for each light condition, and plotted against leaf feeding damage. Photosynthetic decline showed that treatment leaves in the sun declined significantly faster, and even at a herbivory damage of 50%, the effect of *G. decoris* feeding on sun plants was significantly more severe than that of shade plants ($\beta_{SMA} = 8.9$; $P = 0.003$) (Fig. 4).

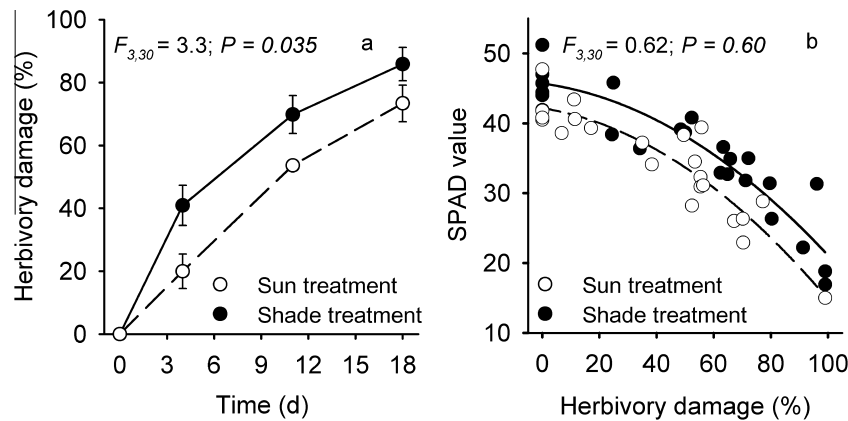


Fig. 1. (a) Leaf herbivory damage (%) over time for *Solanum mauritianum* plants in shade and sun. Damage was defined as chlorotic areas resulting from *Gargaphia decoris* feeding, $n = 6$ plants for each data point (mean $\pm$ SE). (b) Leaf greenness measured by SPAD value in relation to leaf herbivory damage (%), Shade $r^2 = 0.86$, Sun $r^2 = 0.85$. (F_{stat} and P -value indicate overall difference between sun and shade treatment leaves using repeated measures ANOVA).

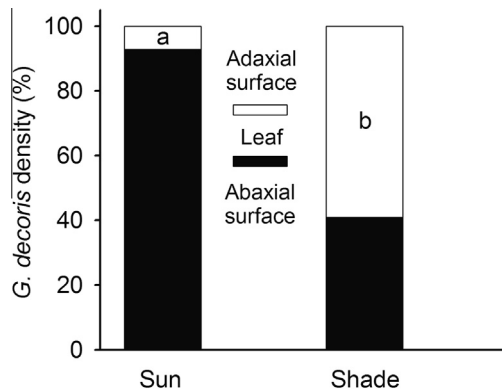


Fig. 2. Relative density (%) of *Gargaphia decoris* on the adaxial (upper) and abaxial (lower) leaf surface of *Solanum mauritianum* plants in the sun and shade. Different lowercase letters indicate significant differences between *G. decoris* densities on the adaxial surfaces of sun and shade plants during diurnal periods ($P < 0.05$).

3.3. Chlorophyll fluorescence and SPAD values

Maximum *PSII* quantum efficiency (F_v/F_m) in the sun treatment plants declined significantly over time compared to their controls, while the shade treatment plants showed no change compared to their controls (Fig. 5a, b). SPAD values in the treatment plants also declined over time, with the sun plants showing a significant decline from day 11 onwards, while shade plants only became significantly different upon day 18 (Fig. 5c, d).

3.4. Leaf temperatures

Sun leaves exposed to *G. decoris* feeding displayed significantly higher mean surface temperatures relative to their controls on day 14 (Fig. 6). The mean adaxial leaf surface temperature of sun leaves were 8 °C higher than the controls and the mean maximum temperatures for sun leaves were 40 °C which was significantly higher than the control (30 °C). Sun control leaves maintained the same mean surface temperature as the shade control leaves, suggesting that herbivory adversely interfered with leaf temperature control. The photosynthesis to leaf temperature curve (photosynthetic efficiency at a given temperature), showed an optimal photosynthetic range between 25 and 30 °C, and above 31 °C declined rapidly, indicating a thermal threshold and signs of temperature induced inhibition of photosynthesis (Fig. 7).

4. Discussion

Gargaphia decoris's herbivory significantly reduced overall *S. mauritianum* photosynthesis in both sun and shade plants. Sap-feeding siphons water and other vital leaf compounds, particularly chlorophyll and photosynthates, which are required in the plant's physiological pathways, consequently limiting the photosynthetic rate of damaged leaves (Smith and Schowalter, 2001; Nagaraj et al., 2002) and ultimately plant growth. Feeding by *G. decoris* resulted in the formation and coalescence of chlorotic regions, also reported by Olckers (2000), ultimately reducing the photosynthetic function of damaged leaves. Sap-feeding is known to elicit further physiological responses, notably the reduction of stomatal conductance (Larson, 1998), which may be a trade-off to conserve water at the expense of photosynthesis. Decreased stomatal conductance may aid the plant in initially retaining water (and nutrients) in targeted leaves, but with time this alteration to water and nutrient transport reduces overall photosynthetic output (Schaffer and Mason, 1990; Larson, 1998). Furthermore, woody plant species, such as *S. mauritianum*, seemingly have a poor capacity to compensate for resource losses due to sap-feeding (Zvereva et al., 2010).

Contrary to expectations expressed in the main hypothesis, sun plants showed a greater decline in photosynthetic rate relative to plants in the shade. Chlorophyll removal in both sun and shade treatment plants, resulted in metabolic and not stomatal limitations to photosynthesis, indicated by increases in intercellular CO_2 over the final week. This difference in intercellular CO_2 between the sun treatment and their control plants suggests an increase in metabolic impairment. However, this impairment cannot be explained solely by chlorophyll removal, as both sun and shade plants had similar reductions in SPAD values ($\sim$ chlorophyll concentration). Herbivory significantly lowered leaf stomatal conductance in sun plants (no stomatal limitations to photosynthesis), in turn lowering their transpiration rates. Decreases in transpiration rates impede the leaves' ability to maintain evaporative cooling (Ball et al., 1988). Consequently, this reduction in transpiration caused a 23% increase in sun leaves' surface temperature compared to control leaves. These increases in temperature make the leaves susceptible to overheating, likely compounding the effects of heat–light stress and resulting in further metabolic impairment (Schymanski et al., 2013).

Solanum mauritianum's photosynthetic metabolism was shown to be sensitive to temperatures above 30 °C which has been documented in a variety of C_3 species (Wise et al., 2004; Sage and Kubien, 2007; Greer and Weedon, 2012). Heat stresses at ambient CO_2 results in a number of physiological impairments, which inhibit

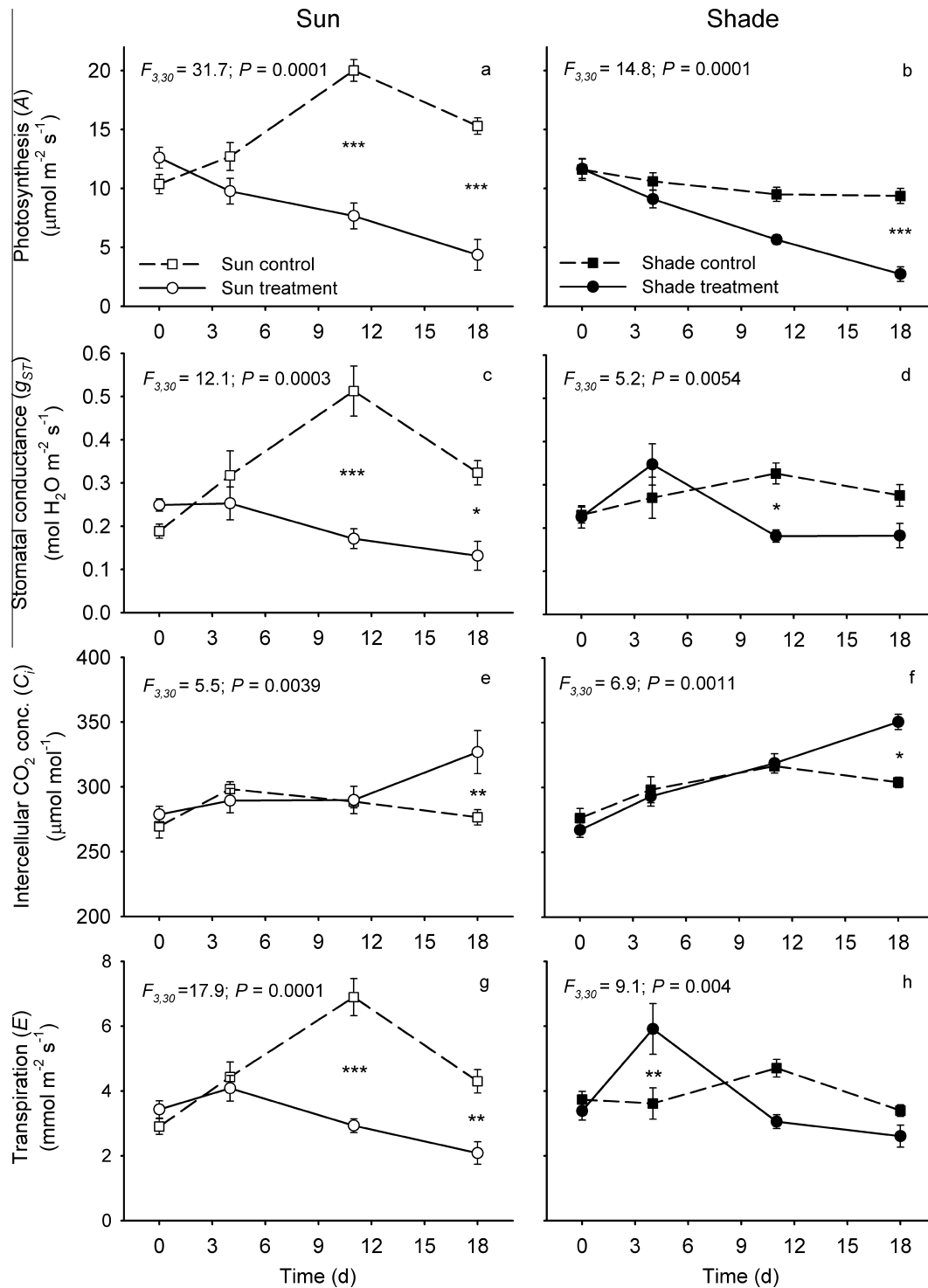


Fig. 3. (a, b) Photosynthesis (A), (c, d) stomatal conductance (g_{ST}), (e, f) intercellular CO_2 concentration (C_i) and (g, h) transpiration rate (E) for *Solanum mauritianum* plants in the sun and shade, with and without *Gargaphia decoris* herbivory over time. (F_{stat} and P -value indicate overall difference between treatment and control leaves using repeated measures ANOVA). $n = 6$ plants for each data point (mean $\pm$ SE). Asterisks (* = $P < 0.05$, ** = $P < 0.01$ and *** = $P < 0.001$) indicate significant differences between treatment and control leaves on corresponding days.

photosynthetic metabolism (Sage and Kubien, 2007). The enzymatic activity of Rubisco (fixing of CO_2 during photosynthesis) is altered with increasing temperature as its affinity for O_2 increases, thereby increasing oxygenase (photorespiration) activity (Sage and Kubien, 2007). Temperatures exceeding $38^\circ C$ are likely to result in the deactivation of Rubisco (Sharkey, 2005). However, returning the leaf to optimal temperature should mitigate the

effects of photorespiration, but Rubisco deactivation may be permanent (Sharkey et al., 2001; Haques et al., 2014), which could be exacerbated by continual *G. decoris* feeding. Furthermore, prolonged exposure to heat stress episodes are known to damage the structure of thylakoid membranes, subsequently reducing photosynthetic electron transport and function (Wise et al., 2004; Sharkey, 2005).

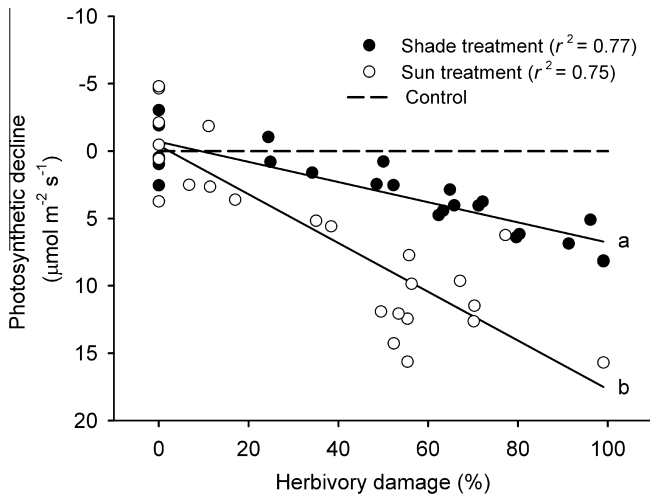


Fig. 4. Photosynthetic decline (control minus treatment for each light condition) plotted against herbivory damage by *Gargaphia decoris* to *Solanum mauritianum* plants in the sun and shade. Regressions for both sun and shade plants ($n = 24$; $P = 0.0001$). Different lowercase letters indicate significant differences between treatment slopes ($\beta_{SMA} = 27.87$; $P = 0.001$).

Maximum *PSII* quantum efficiency (F_v/F_m) is also thought to be negatively affected by temperatures above a plants' thermal optimum (Sage and Kubien, 2007). In the case of *S. mauritianum* these are likely to be temperatures above 30 °C. The F_v/F_m decrease in treatment sun plants (<0.68) may then be due to the initiation of non-photochemical quenching (NPQ) processes (Murchie and

Lawson, 2013). Although occurrences of NPQ processes are documented in this study, as to whether these processes were photo-protective and/or photo-damaging remains unclear. Given that plants were dark-adapted overnight, the damage experienced was likely to have occurred from processes that relax over longer time scales (hours +) (Maxwell and Johnson, 2000). This long term damage could be either the photo-inactivation of *PSII* reaction centre (damage to the D1 protein) and/or the activation of xanthophyll cycling (photo-protective) (Demmig-Adams and Adams, 1992; Murchie and Lawson, 2013). Although photo-protective mechanisms of *PSII* exposed to direct solar radiation (and associated thermal increases) are documented through xanthophyll cycling, this requires a stable supply of leaf compounds (carotenoids) and other resources (Cazzonelli, 2011). The continual loss of many of these resources, through *G. decoris*'s feeding would be expected to result in increasingly poorer reserves, subsequently weakening the leaf's resistance to stresses, including heat, light and herbivory.

Herbivory rates were greater in the shade as opposed to full-sun plants. Shaded plants such as *S. mauritianum* found in the forest understories (Atkinson et al., 2014), experience buffered eco-climatic conditions, compared to plants in open and exposed environments (Danks, 2002; Uyi et al., 2015). These buffered environments may provide favourable conditions for lace-bugs, promoting increased, potentially optimal, feeding. This was illustrated by *G. decoris* predominantly dwelling on the underside of the sun leaves during diurnal periods, and is likely a display of thermoregulatory behaviour to avoid excessive leaf surface temperatures (Clissold et al., 2013). Although a recent study by Patrick and Olckers (2014) highlighted the potential for partially shaded conditions to improve *G. decoris* performance, this study showed

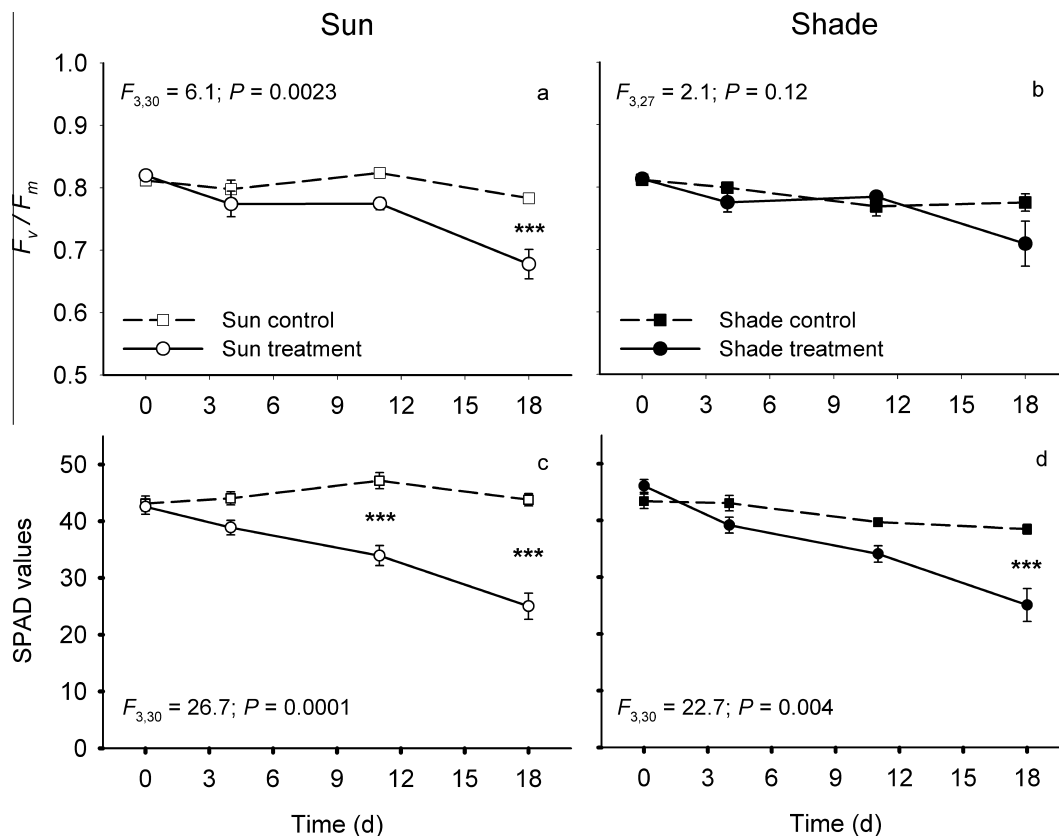


Fig. 5. (a, b) Maximum *PSII* quantum efficiency (F_v/F_m) and (c, d) SPAD values for *Solanum mauritianum* plants in the sun and shade, with and without *Gargaphia decoris* herbivory over time. $n = 6$ plants for each data point (mean $\pm$ SE). (F_{stat} and P -value indicate overall difference between treatment and control leaves using repeated measures ANOVA). Asterisks symbols (*= $P < 0.05$, **= $P < 0.01$ and ***= $P < 0.001$) indicate significant differences between treatment and control on corresponding days.

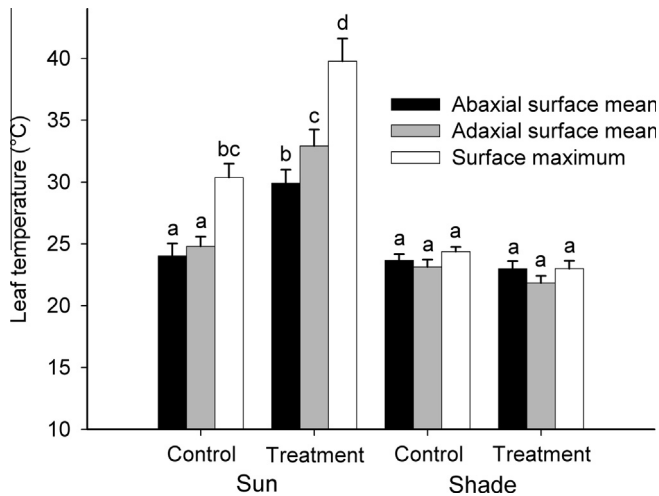


Fig. 6. Mean leaf temperatures for adaxial (upper) and abaxial (lower) leaf surfaces, and maximum leaf surface temperatures for *Solanum mauritianum* plants in the sun and shade, with and without *Gargaphia decoris* herbivory after 14 days. $n = 6$ plants for each data point (mean $\pm$ SE). Different lowercase letters indicate significant differences ($P < 0.05$).

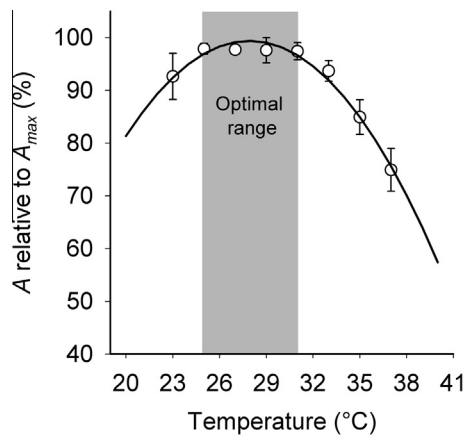


Fig. 7. Photosynthesis to temperature curve (A -temp) showing *Solanum mauritianum* photosynthesis (A) relative to photosynthetic maximum (A_{max}) of control leaves in the sun in relation to leaf temperature ($C_i = 300 \mu\text{mol mol}^{-1}$). A_{max} was taken as the highest rate of photosynthesis observed under the set conditions. $n = 3$ plants for each data point (mean $\pm$ SE). Fitted quadratic function ($y = -124.95 + 16.1x - 0.29x^2$; $r^2 = 0.78$).

that under controlled conditions *G. decoris* displayed similar efficacy in both sun and shaded conditions with regard to feeding rates and physiological damage.

In conclusion, *G. decoris* was documented to significantly reduce the photosynthetic output of *S. mauritianum* plants, causing the greatest overall effect in sun conditions. The increased photosynthetic inhibition experienced by treated plants in the sun may be a combination of direct chlorophyll removal as well as the indirect accumulation of photosynthetic and PSII damage caused by continued heat–light stress. Furthermore, *G. decoris* feeding does not seem to directly damage leaf photosystems but rather impedes their photosynthetic performance through leaf resource removal and metabolic impairment. Herbivory rates displayed by *G. decoris* were greater in the shade, however, feeding per unit area was significantly more damaging to leaves in direct sunlight. Ultimately this has implications for the biological control of *S. mauritianum* using *G. decoris*, requiring the evaluation of factors currently constraining the agent's establishment in the field, given the agent's proven effectiveness demonstrated in this study.

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CHAPTER THREE:



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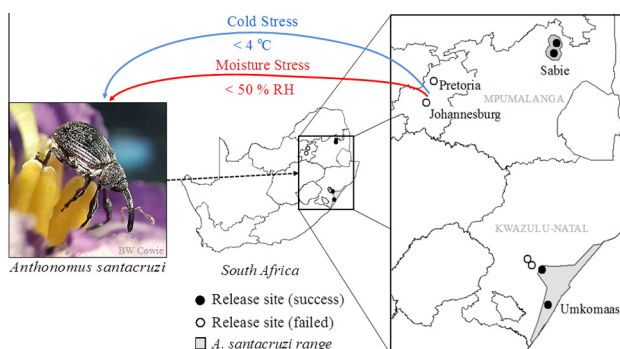
3. Does climate constrain the spread of *Anthonomus santacruzi*, a biological control agent of *Solanum mauritianum*, in South Africa?

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HIGHLIGHTS

- Climate analyses revealed that Highveld sites were poorly suited to *A. santacruzi* establishment.
- Cold stress to *A. santacruzi* adults is likely to occur at temperatures below 4.1 °C.
- Prolonged humidities below ~47% will hinder the longevity of *A. santacruzi* adults.
- This study highlights the importance of climate assessments in biological control research.

GRAPHICAL ABSTRACT



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ABSTRACT

The flowerbud-feeding weevil, *Anthonomus santacruzi* Hustache (Coleoptera: Curculionidae), was released in 2008 as a biological control agent against the South American invasive tree *Solanum mauritianum* Scop. (Solanaceae), in South Africa. The weevil has since established in two localities, namely along the KwaZulu-Natal South Coast and around the Sabie region of Mpumalanga. Recent releases on the Highveld region (Johannesburg and Pretoria) have been unsuccessful to date. This study aimed to test the hypothesis that climate, particularly low temperature and low relative humidity, restricts the survival and establishment of *A. santacruzi* in South Africa. The survival of *A. santacruzi* adults in relation to low temperatures and relative humidities was assessed in the laboratory. Climate analyses revealed that matches of <50% were associated with failure to establish and that minimum temperature and relative humidity displayed the greatest discrepancies between South American collection and South African release sites. Thermal assessments on *A. santacruzi* adults calculated the CT_{min} and LT_{50} as 4.1 ± 0.2 °C ($n = 20$) and -4.2 ± 0.3 °C ($n = 90$) respectively. The LH_{50} of *A. santacruzi* adults was calculated as 46.9%. The establishment of *A. santacruzi* at only the warm and humid release sites in South Africa advocates for the consideration of low temperature and low humidity as factors impeding the agents' establishment, particularly on the cooler and drier Highveld.

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1. Introduction

Solanum mauritianum Scop. (Solanaceae) is a small broad-leaved perennial shrub or tree native to the South American countries of Brazil, Uruguay and Argentina (Olickers, 1998). *Solanum*

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mauritanum has become an environmental weed in various tropical and sub-tropical countries across the globe, one of which is South Africa (Olckers, 2009). The earliest reports of the weed in South Africa were in the KwaZulu-Natal Province (KZN) during the 1860s (Olckers and Zimmermann, 1991). This fast growing and highly reproductive tree has become prevalent in many of South Africa's eastern higher rainfall regions, particularly the Western and Eastern Cape, KZN, Gauteng, Mpumalanga and Limpopo Provinces (Olckers, 2003, 2011). *Solanum mauritanum* is capable of flowering year round and is known to grow in a variety of habitats, including riparian areas, degraded and disturbed lands, forest margins and commercial plantations, being particularly problematic in the forestry and agricultural sectors (Witkowski and Garner, 2008; Olckers, 2009). South Africa has targeted *S. mauritanum* for biological control since 1984, when conventional control methods were deemed ineffective and unsustainable (Olckers, 1999). Despite testing numerous biological control candidates, only a sap-suckling lace-bug *Gargaphia decoris* and most recently, a flower-bud feeding weevil, *Anthonomus santacruzi* Hustache (Coleoptera: Curculionidae), have been released against *S. mauritanum* in South Africa (Olckers, 2009; Klein, 2011).

Anthonomus santacruzi was released in 2008 in an attempt to reduce *S. mauritanum*'s flowering capacity, excessive seed production and dispersal (Olckers, 2008; Klein, 2011). Adult weevils feed primarily on flowers throughout the year, but are known to feed on young rolled-leaves and newly developing shoots (Olckers, 2003; Barboza et al., 2009). As adults these weevils typically live for three to six months, with females producing ~16–60 larvae (Hakizimana, 2011). Females oviposit eggs directly into newly developing buds, in which the larvae hatch and feed voraciously, destroying the buds as they develop (Olckers, 2003). Larval development and pupation occur entirely within the buds of *S. mauritanum*, with the entire life-cycle requiring approximately one to two months from egg to adult emergence (Olckers, 2003). The reproductive traits and longevity of *A. santacruzi* predisposes the species to overlapping generations due to the weevils' relatively short developmental time (Olckers, 2003). Overall, these traits of *A. santacruzi* suggest a strong potential for establishment in South Africa (Hakizimana, 2011).

Initial releases of *A. santacruzi* occurred at several sites in KZN during 2008–2011 with successful establishment (Hakizimana, 2011; Olckers, 2011). More recently, in 2012–2014, *A. santacruzi* was released at sites within the Sabie region of Mpumalanga, with confirmed establishment (A. Sasa, pers. comm.). Releases of *A. santacruzi* during 2013 and 2014, in the high altitude regions (Highveld) of Pretoria and Johannesburg have failed to establish. The South African Highveld, which is characterised by its elevation between ~1500 m and 2000 m, experiences warm rainy summers and cold dry winters with an absence of rainfall (Schulze et al., 1997). Despite high densities of *S. mauritanum* at numerous release sites, the agent's establishment has been confirmed only along the southern coastline of KZN (Olckers, 2011; Winston et al., 2014) and within the Sabie region of Mpumalanga. Native predators, in particular those inhabiting inflorescences such as crab spiders (Thomisidae), were predicted to reduce the survival and reproduction of *A. santacruzi* (Olckers, 2011; Hakizimana and Olckers, 2013a,b). However, Hakizimana and Olckers (2013b) later concluded that predators inhabiting inflorescences posed little, if any threat to the establishment of *A. santacruzi*. Instead climatic unsuitability is suspected of causing establishment failure of *A. santacruzi* within the inland regions of the country (Hakizimana, 2011). Parental stocks of *A. santacruzi* were imported from the warm and humid, sub-tropical regions of Corrientes and Misiones in north-eastern Argentina (Olckers, 2011). Olckers (2011) and Hakizimana (2011) suggested that the establishment of *A. santacruzi* may be constrained by the South African climate,

particularly low temperatures, after preliminary findings on failed releases at cooler inland areas of the country.

Climatic unsuitability or incompatibility refers to a mismatch of abiotic variables which restrict a species spread, proliferation and ultimately survival (Byrne et al., 2004). Insect abundance and distributions are known to be strongly influenced by numerous climatic factors. Although both temperature and relative humidity are understood to be highly influential factors in insect survival, dispersal and establishment (Byrne et al., 2002), humidity itself is frequently overlooked, and currently lacks any formal climatic assessment with regard to *A. santacruzi*. Humidity can influence insect habitat selection particularly in tropical and sub-tropical species, including the Curculionidae (Weissling and Giblin-Davis, 1993). Directly, humidity affects an insects' physiology, often dictating behaviour, ecology and subsequently its distribution (Chown and Nicolson, 2004; Norhisham et al., 2013). Smaller insects, such as *A. santacruzi*, also display a heightened susceptibility to desiccation at low humidities and high temperatures, given their relatively large surface area to volume ratio (Chown and Nicolson, 2004).

Given the constraints posed by climate, particularly temperature and humidity, on the establishment of insect species used as biological control agents, it is imperative that these aspects be investigated and better understood. In the case of *A. santacruzi*, climate matching, thermal-physiology and humidity assessments are absent from the literature. This study aimed to assess the potential constraints posed by climate, namely low temperature and low relative humidity, on the survival and establishment of the biological control agent *A. santacruzi*, with particular focus on the failed releases in the Highveld regions of South Africa.

2. Material and methods

Anthonomus santacruzi adults were collected from numerous field sites along the KZN South Coast. Collection sites included, Amanzimtoti (S 30° 2' 03.80" E 30° 53' 46.25"), Cannonbrae (S 30° 12' 0.46" E 30° 77' 44.62"), Scottburgh (S 30° 16' 42.97" E 30° 45' 20.70"), Umkomaas (S 30° 13' 11.32" E 30° 47' 45.00"), and Margate (S 30° 51' 30.66" E 30° 22' 04.14"). Adult weevils were kept at the University of the Witwatersrand, Johannesburg (S 26° 11' 20.97" E 28° 01' 55.37") and sustained on a mixture of fresh flowers, buds and leaves for a week prior to experiments.

2.1. Climatic suitability

CLIMEX (Maywald et al., 2015), a climate prediction-modelling program, was used to compare the climate of the Argentinean collection range, of *A. santacruzi*, with known release sites in South Africa. CLIMEX comparisons between collection and release sites were based on mean annual rainfall, rainfall seasonal pattern, minimum and maximum temperatures, relative humidity and soil moisture.

South African release sites were compared to the Argentinian collection range, specifically in terms of mean minimum temperature and mean humidity. Climatic data were generated by averaging monthly data for 2014 using two nearby localities per site: Argentinian (Corrientes, Posadas), KZN South Coast (Umkomaas, Port Shepstone), Sabie Region (Sabie, Graskop) and the Highveld (Johannesburg, Pretoria).

2.2. Critical thermal minima and low lethal temperatures

The Critical thermal minima (CT_{min}) for adult *A. santacruzi* were assessed using a double jacket system connected to a programmable water bath (Julabo F32-ME, Seelbach, Germany).

Weevils, sealed individually in test tubes, were submerged in a water bath set at 25 °C and given a 10 min thermal equilibration period. The water was cooled at a rate of 0.25 °C per minute. CT_{min} was recorded as the temperature at which *A. santacruzi* was unable to right itself and failed to respond to a stimulus (using a fine brush). Individuals that failed to self-right were removed and their response to the stimulus was tested immediately; individuals that responded coherently to the stimulus were removed and no data recorded.

Lower lethal temperature (LT_{50}) of *A. santacruzi* was assessed following the same water bath procedure as the CT_{min} . Ten adult weevils, were subjected to a target temperature for two hours, then removed and allowed 24 h to recover in a petri dish with *S. mauritanum* inflorescences at 25 °C. The experiment was repeated, with different individuals, at temperatures of 1, 0, –1, –2, –3, –4, –5, –6 and –7 °C (until which point 0% *A. santacruzi* survival was observed).

2.3. *Anthonomus santacruzi* humidity assessments

The survival of *A. santacruzi* adults with respect to relative humidity (RH) was examined using two-litre humidity chambers maintained at 25 ± 1.5 °C. Eight chambers were used, maintaining RH's of ~80%, ~70%, ~60%, ~50%, ~40%, ~30%, ~20% and ~10%. The RH conditions within each chamber were attained using salt solutions, adapted from Winston and Bates (1960), namely 260 g/L NaCl (RH_{80%}), 200 g/L KOH (RH_{70%}), 320 g/L NaOH (RH_{60%}), 350 g/L KOH (RH_{50%}), 920 g/L NaI (RH_{40%}), 500 g/L NaOH (RH_{30%}), 820 g/L NaOH (RH_{20%}) and 100 g KOH dry pellets (RH_{10%}).

In total 480 adult *A. santacruzi* individuals were randomly assigned to one of the eight humidity chambers, each containing 60 individuals. Within each chamber, individuals were randomly placed into one of six housing jars, with 10 individuals per jar. Of the six jars, three contained inflorescences and three contained whole leaf material. Survival counts of *A. santacruzi* within each of the housing jars were conducted daily for two weeks. Inflorescences and young leaves used for feeding were replaced on alternating days having first been surface sterilised using a 2% sodium hypochlorite solution and rinsed with sterile distilled water.

2.4. Data analysis

Climatic suitability data for collection and release sites were predicted using the *Match Climates* feature in CLIMEX and interpreted according to their generated composite match indices (Kriticos et al., 2015). Mean minimum temperature and humidity data for the South African release sites were each compared to Argentinian collection range (Corrientes, Posadas) by means of a one-way analysis of variance (ANOVA) with repeated measures. Survivorship of *A. santacruzi* sustained on inflorescences and leaves at relative humidities of 10, 20, 30, 40, 50, 60, 70 and 80% were each compared using repeated measures ANOVA and assessed for statistical significance using a Tukey HSD post hoc (Statsoft, 2014). Lethal temperature (LT_{50}) and lethal humidity (LH₅₀), at which 50% of the *A. santacruzi* population was predicted to perish, were calculated by means of probit analyses as per Mitchell et al. (1993).

3. Results

3.1. Climatic suitability

Overall, the South African release sites' (Fig. 1) climatic parameters (Table 1) had a mean match of 58% to the Argentinian

collection range (Table 2). The Umkomaas and Sabie release sites displayed overall matches of 72% and 66% when compared to the native collection sites. A clear difference was found between the Argentinian collection range and the Highveld release sites, Johannesburg and Pretoria, which provided the lowest overall matches of 46% and 48% respectively. Climatic matching indicated that relative humidity and minimum temperature had the greatest discrepancies of the climate variables between the collection and release sites, particularly in Johannesburg and Pretoria (Table 2).

Compared to the Argentinian range, the Highveld's mean low temperature was significantly colder than the Argentinian collection range as well as the remainder of the South African release sites ($F_{3,4} = 35.2$; $P < 0.01$) (Fig. 2A). Mean relative humidity of the KZN South Coast was significantly greater than the Argentinian range, whereas the Highveld was significantly less humid ($F_{3,4} = 84.4$; $P < 0.01$). The Highveld was also significantly less humid (Fig. 2B) and drier (Table 1) than any of the other South African release sites.

3.2. Critical thermal minima and low lethal temperatures

Temperatures at which adult individuals of *A. santacruzi* lost the ability to self-right and respond to stimuli ranged between 2.9 and 5.9 °C. The mean critical thermal minima (CT_{min}) of adult weevils was 4.1 ± 0.2 °C ($n = 20$). The lethal temperature (LT_{50}) calculated for *A. santacruzi* adults was -4.2 ± 0.3 °C ($n = 10$ per temperature class) (Fig. 3).

3.3. *Anthonomus santacruzi* humidity assessments

Survivorship displayed by *A. santacruzi* was highest at RHs of 50–80% on inflorescences ($F_{7,16} = 61.5$; $P < 0.01$), and at RHs of 60–80% on leaves ($F_{7,16} = 62.6$; $P < 0.01$). *Anthonomus santacruzi* kept at RHs of 30% and below displayed complete mortality on both inflorescences and leaves (Fig. 4). The lethal humidity (LH₅₀) calculated for *A. santacruzi* adults sustained on inflorescences was 46.9% ($n = 240$) (Fig. 4).

4. Discussion

The current widespread establishment of *A. santacruzi* along the KZN South Coast (Gillespie and Besaans, 2014) and its localised establishment around the Sabie region of Mpumalanga, are attributed to the sites' climatic similarity to that of the weevil's native range. The failure of *A. santacruzi* to establish at the Highveld sites are likely due to the region's lower temperatures and relative humidities experienced throughout the year, particularly during the dry winter season (May–September). Numerous studies highlight successful establishment of biological control agents at sites that display similar climates to those of the agents' native range (Grevstad, 1999; Byrne et al., 2004). The current establishment of *A. santacruzi* at only the warmer and more humid release sites suggests that climate is a major factor influencing the persistence of this species.

Given the importance of climate on insect survival, particularly temperature and humidity, mismatches of these variables will be detrimental (Byrne et al., 2002). As suggested by Olckers (2011), low temperatures may restrict the establishment of *A. santacruzi*. However, the relatively low CT_{min} (4.1 °C) of *A. santacruzi* adults suggests that the species displays a degree of cold tolerance. This cold tolerance, although surprising for a sub-tropical insect, is not uncommon in Anthonomine species including *A. pomorum*, *A. eugenii* and *A. grandis*, all of which display diapause at sub-zero temperatures (Kostál and Simek, 1996; Riley, 2008; Showler, 2009). Insects typically begin accumulating cold stress at

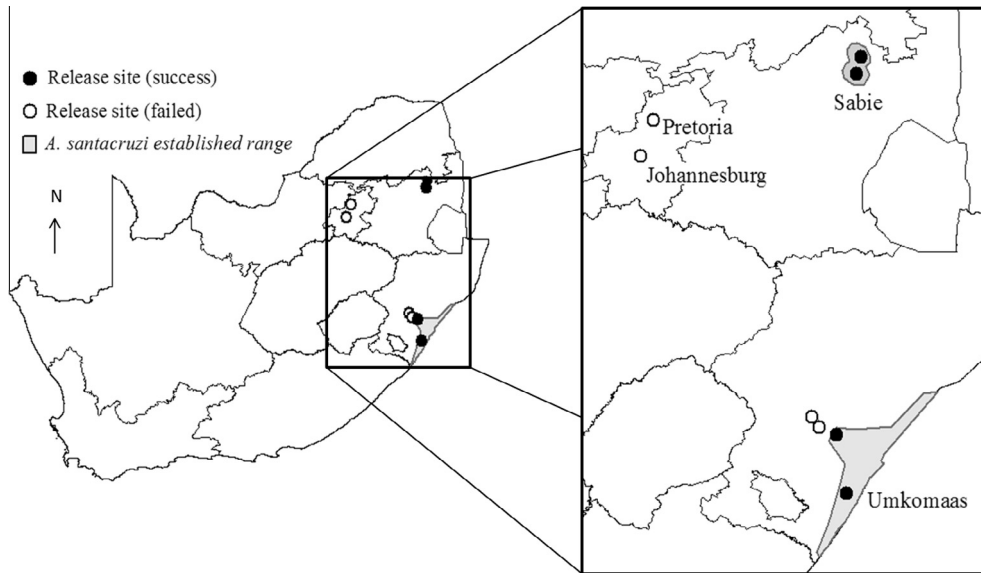


Fig. 1. Distribution of the biological control agent *Anthonomus santacruzi* in South Africa (Gillespie and Besaans, 2014), including known release sites, both successful and failed (Hakizimana, 2011; Olckers, 2011; A. Sasa, pers. comm.).

Table 1

Comparison of the elevation and climatic parameters of South African release sites and Argentinian collection sites of *Anthonomus santacruzi*.^a

Location	Altitude (m a.s.l.)	Mean annual rainfall (mm)	Mean annual temperature (minimum maximum)	Mean annual relative humidity (minimum maximum)	Establishment
Argentina Collection sites					
Corrientes	54	1179	22.1 °C (16.5 27.7)	72.5% (65.9 79.0)	Native
Posadas	133	1649	21.1 °C (15.1 27.1)	69.6% (57.1 82.1)	Native
South Africa Release sites					
Umkomaas	17	1056	20.2 °C (16.4 23.6)	78.9% (78.1 79.5)	Success (widespread)
Sabie	998	1071	19.7 °C (13.4 26.3)	65.2% (55.3 75.1)	Success (localised)
Pretoria	1369	785	17.2 °C (10.3 24.2)	52.2% (37.5 66.0)	Failed
Johannesburg	1692	713	16.1 °C (9.9 22.3)	55.1% (40.4 69.9)	Failed

^a Climatic data generated from weekly averages taken from CLIMEX (Maywald et al., 2015).

Table 2

CLIMEX comparison of the South African release sites of *Anthonomus santacruzi* to the collection range in Argentina, South America.

CLIMEX match indices (%) to Argentinian collection region							
South African Release sites	Overall match	Annual rainfall	Rainfall seasonality	Maximum temperature	Minimum temperature	Relative humidity	Soil moisture
Umkomaas	72	75	80	59	84	29	68
Sabie	66	69	72	74	70	28	67
Pretoria	48	57	65	62	43	10	46
Johannesburg	46	55	62	56	42	13	44

temperatures equal to or below their CT_{min} (Chown and Nicolson, 2004; Lalouette et al., 2011). In the case of *A. santacruzi* adults, cold stress is likely to accumulate at temperatures of ~ 4 °C and below. On the Highveld, mean low temperatures may drop below 4 °C for as many as four to five weeks consecutively during winter (June–July) (Maywald et al., 2015). The calculated CT_{min} and LT_{50} of *A. santacruzi* suggest that low temperatures may not be a limiting factor in certain regions of the country. However, it should be noted that winter temperatures near the CT_{min} may restrict or halt insect development (Byrne et al., 2002), and in the case of *A. santacruzi*, may likely extend to both the egg and larval stages.

Cold stress accumulation may not be the only factor affecting the establishment of *A. santacruzi* in South Africa. The exceedingly poor survival of *A. santacruzi* at relative humidities below 50% may

further account for the weevil's failed establishment on the Highveld. Humidity itself has various effects on insect physiology, with low humidities frequently retarding development, reducing longevity and slowing oviposition (Chown and Nicolson, 2004; Norhisham et al., 2013). Prolonged periods of low humidity (<47%), given the absence of winter rainfall on the Highveld (Schulze et al., 1997), will seriously affect the longevity and survival of adult weevils. The Highveld region typically experiences in excess of 100 days of RH below 50% during winter, with weekly minimum RH frequently dropping below 30% (Schulze et al., 1997; Maywald et al., 2015). These climatic variables predict that the Highveld's temperature and humidity during winter will directly reduce the longevity, survival and establishment of *A. santacruzi* adults.

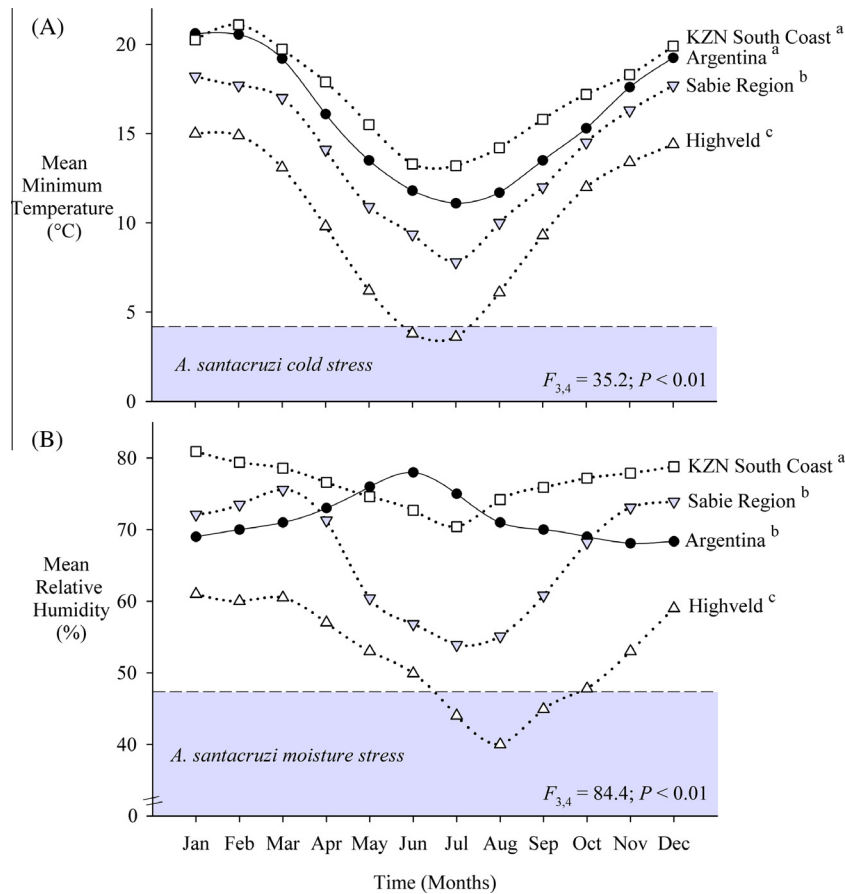


Fig. 2. Mean minimum temperature (A) and mean relative humidity (B) for the Argentinian collection range and South African release sites (Highveld, KZN South Coast and Sabie Region) of *Anthonomus santacruzi*. Climatic data generated from CLIMEX (Maywald et al., 2015). Lowercase letters indicate significant differences ($p < 0.05$) in minimum temperature (A) and mean relative humidity (B) between sites. F_{stat} and P -value indicate overall difference in site's mean minimum temperature (A) and mean relative humidity (B) using repeated measures ANOVA.

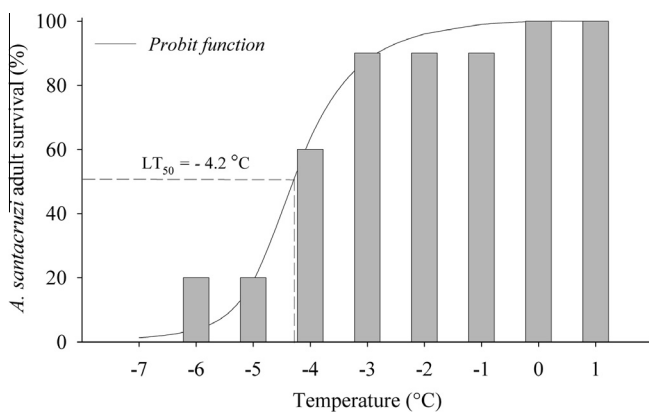


Fig. 3. *Anthonomus santacruzi* adult survival (%) after a two hour exposure to temperatures of 1, 0, -1, -2, -3, -4, -5, -6 and -7 °C ($n = 10$; per temperature class) and 24 h recovery period. Survival data were fitted with a probit function to determine lethal temperature (LT_{50}).

The susceptibility of *A. santacruzi* adults to low temperatures and relative humidities may not be a simple relationship, as insects often exploit a variety of strategies to compensate for these climatic constraints (Danks, 2002). Primarily, insects may increase energy or

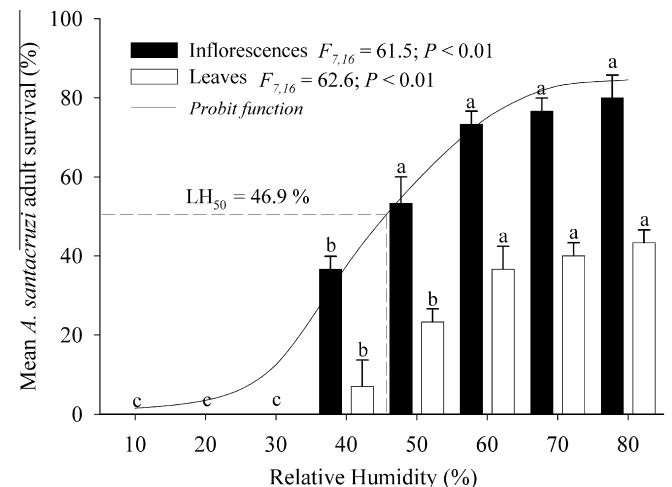


Fig. 4. *Anthonomus santacruzi* adult survival (%) on *Solanum mauritianum* inflorescences ($n = 240$) and leaves ($n = 240$), after a 14 day period at relative humidity (RH) treatments of 10, 20, 30, 40, 50, 60, 70 and 80%. Inflorescence survival data were fitted with a probit function to determine lethal humidity (LH_{50}). Lowercase letters indicate significant differences in *A. santacruzi* survival between RH treatments on inflorescences ($p < 0.05$) and leaves ($p < 0.05$). F_{stat} and P -value indicate overall difference in *A. santacruzi* survival between different RH treatments using repeated measures ANOVA for inflorescences and leaves.

fat reserves to improve their ability to endure unfavourable conditions (Chown and Nicolson, 2004). The greater nutrient content of floral material in comparison to leaves (Wackers et al., 2005), such as *S. mauritianum* inflorescences upon which *A. santacruzi* feed, may provision for these larger energy stores. The incorporation of leaf-feeding by florivorous insect species to supplement diets is not uncommon in the absence or limited availability of flowers (Smallgange et al., 2007) and may be displayed by *A. santacruzi*. Secondly, unfavourable conditions may be avoided by insects through actively seeking and exploiting microclimates found on plants (Willmer, 1982; Danks, 2002; Dzerefos et al., 2015). The small size of *A. santacruzi* is advantageous in gaining access to inflorescences, flowers, buds and rolled leaves on *S. mauritianum* plants. These microclimates may offer *A. santacruzi* adults a temporary refuge from both cold and moisture stresses. However, the utilisation and availability of these inflorescences may be strongly influenced by the climatic conditions experienced by the plants.

Indirectly the Highveld's seasonal climate will impact on the quality and availability of plant material upon which *A. santacruzi* feed. *Solanum mauritianum* is predicted to experience severe reductions in photosynthesis at temperatures below 20 °C (Cowie et al., 2016), with rates dropping as low as 3.8 $\mu\text{mol m}^{-2} \text{s}^{-1}$ during winter (Cowie unpublished data). In the case of the Highveld, the coldest temperatures and lowest humidities co-occur, which will exacerbate cold and moisture stresses to *A. santacruzi* as well as slowing plant growth and diminishing quality. The growth and quality reduction suffered by *S. mauritianum* during winter on the Highveld, would limit the availability and quality of new plant material for *A. santacruzi* to feed on, reproduce and take refuge within. The prolonged exposure of *A. santacruzi* to low temperature and humidities during the Highveld winter may result in the inhibition of reproduction or more likely the inability to persist as adults.

In conclusion, the effect of climate, namely low winter temperature and low relative humidity, should be considered major factors impeding the adult longevity, survival and establishment of *A. santacruzi* in South Africa. *Anthonomus santacruzi* displays susceptibility to cold and moisture stress, particularly as adults on the South African Highveld. Overall, the Highveld regions of the country match poorly with the agent's sub-tropical collection sites. The Highveld's seasonal fluctuations in temperature, humidity and plant quality during winter are predicted to severely impede the ability of *A. santacruzi* adults to persist, reproduce and establish. Thus, the existence of a climatic threshold may account for the failure of *A. santacruzi* to establish and spread into the drier and cooler inland regions of South Africa. This study also highlights the importance of climatic considerations and analyses in the identification and collection of biological control agents prior to their release (see Robertson et al., 2008; Manrique et al., 2014). Although the establishment of a species is often gauged by the presence of permanent adult populations, which actively reproduce, the susceptibility of the egg and larval stages should not be overlooked and future studies should assess the climatic restrictions posed to these life stages of *A. santacruzi*. Furthermore, the research presented here may aid in feasibility studies assessing the potential use of *A. santacruzi* as a biological control agent in New Zealand (Hakizimana and Olckers, 2013c).

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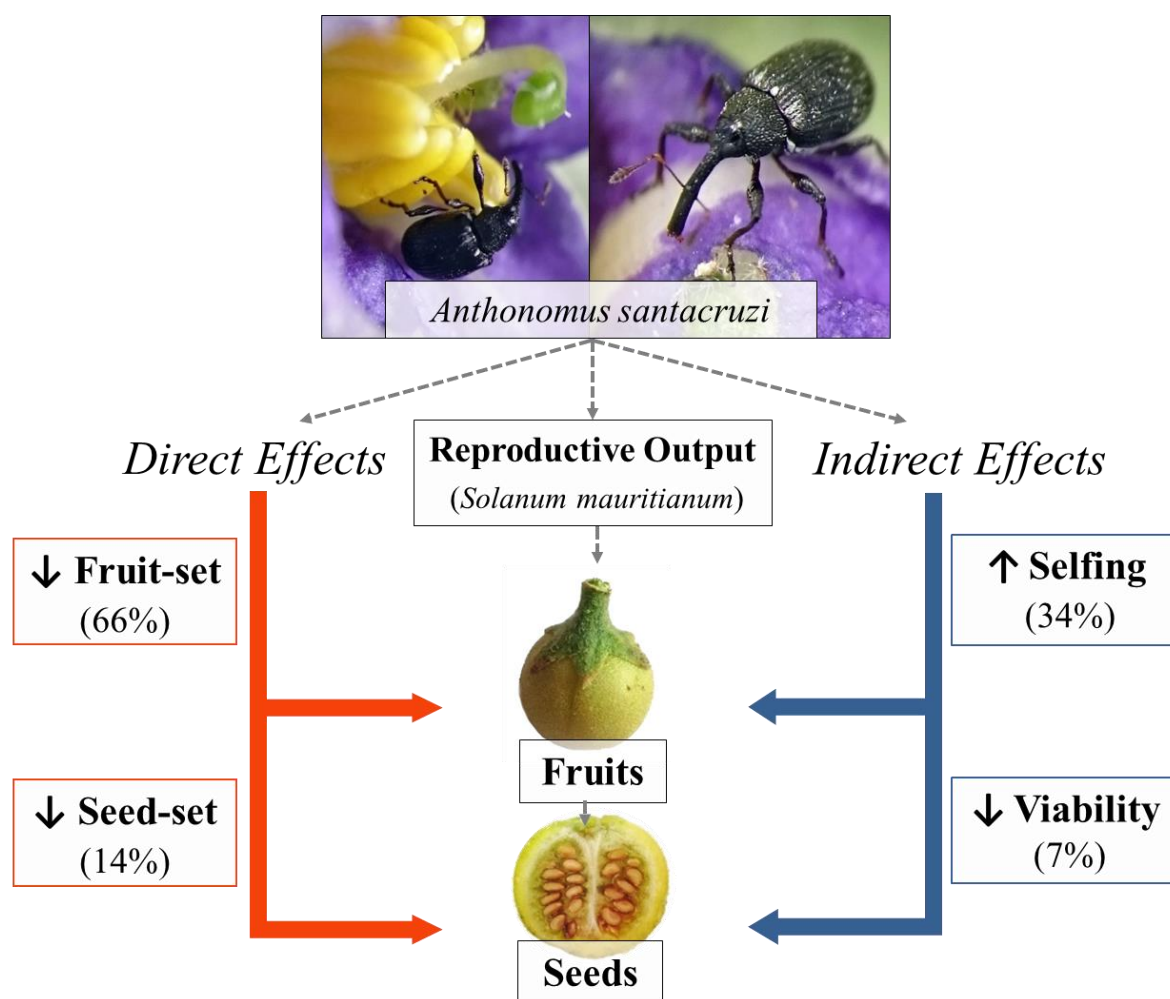
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CHAPTER FOUR:

A villainous hero: Does the biological control agent, *Anthonomus santacruzi*, pollinate its target weed, *Solanum mauritianum*? *

(* to be submitted: *Journal of Biological Control*)



Highlights

- *Anthonomus santacruzi* reduced the overall reproductive output of *Solanum mauritianum*.
- Florivory by *A. santacruzi* enhances self-pollination of *S. mauritianum*.
- Flower-feeding by *A. santacruzi* may deter visitation by other pollinators.
- This study advocates for the increased consideration of indirect agent-weed interactions in biocontrol programmes.

Abstract

Weed biological control programmes frequently make use of flower feeding agents to reduce the fruit/seed output of invasive plants. Florivorous species have the potential for direct and indirect effects on the reproductive success of their target plants. The flowerbud-feeding weevil, *Anthonomus santacruzi*, was first released in South Africa during 2008 for the biological control of the invasive tree, *Solanum mauritianum*. This study assessed the impact of *A. santacruzi* florivory as well as the potential of the agent to act as an indirect pollinator. Overall floral damage caused by *A. santacruzi* was trivial, with only ~5% of the anther and ~2% of petal areas being removed. However, the subsequent effects of *A. santacruzi* were considerably more damaging, with 25% and 66% reductions in the flowering and fruiting of *S. mauritianum* inflorescences respectively. Additionally, fruit produced from inflorescences exposed to *A. santacruzi* were smaller in size, with fewer, less viable seeds. Floral damage by *A. santacruzi* is likely to deter other potential outcrossing pollinators and subsequently enhance self-pollination of *S. mauritianum*. This suggests that in areas with well-established *A. santacruzi* populations, the weevils may simultaneously facilitate the self-pollination and potential inbreeding of *S. mauritianum*, which may be beneficial to the ongoing biological control efforts against the weed.

Keywords: Bugweed; Florivory; Indirect effects; Insect-plant interactions; Self-pollination

1. Introduction

Flower feeding agents are frequently utilised in weed biological control programmes, with the aim of reducing the fruit or seed set of invasive plants and their subsequent spread (Van Klinken et al., 2004). Florivory, the feeding on any floral plant material, maintains the potential to impact on the reproductive output and success of plants, both directly and indirectly (McCall and Irwin, 2006; Eliyahu et al., 2015). Directly, floral feeding may affect the number of flowers and seeds produced, whereas the indirect effects of florivory, tend to influence a plant's fitness by affecting plant-pollinator interactions, namely floral attractiveness to pollinators (McCall and Irwin, 2006; Willmer, 2011). Although the exact effects experienced by plants and flowers in response to florivory are debated, several studies suggest that florivores severely limit the production and viability of fruits/seeds (Rohdes and Ashman, 2010). In certain cases, florivorous insects have the potential to enhance the self-pollination of attacked plants (Penet et al., 2009). However, plants possess a wide variety of responses to florivory, which include alterations in flower phenology, excessive flowering, changes in fruit development, emissions of repellent scents and sexual segregation of flowers (Irwin et al., 2004; Willmer, 2011; Eliyahu et al., 2015). South Africa in particular, has experienced a relatively high success rate (67%) with the use or inclusion of florivorous agents in many of its weed biological control programmes (Klein, 2011).

The South American tree, *Solanum mauritianum* Scopoli (Solanaceae), is a prominent invader in South Africa (Henderson, 2001; Olckers, 2011). Since its introduction during the 1860s, the tree has spread throughout most of the country, being found in eight of the country's nine provinces (Henderson, 2001). Declared a major weed, *S. mauritianum* is problematic in a variety of habitats, ranging from natural ecosystems to commercial forestry plantations (Schor et al., 2015). The widespread distribution of this species is largely due to

the tree's prolific fruit and seed production as well as its dispersal by native frugivores, predominately birds (Olckers, 1999; Jordaan et al., 2011; Schor et al., 2015). This fast-growing tree may reach maturity as early as six months, flowering and fruiting continually thereafter, with mature trees capable of producing in excess of 200 000 seeds per annum (Witkowski and Garner, 2008). The flowers are hermaphroditic (1 stigma: 5 anthers) and are typically short lived, occurring in compact peduncles (Henderson, 2001). The seeds are key to *S. mauritianum*'s invasion success, with viabilities as high as 98%, and seedbank dormancies of up to two years (Campbell and van Staden, 1990, 1994; Witkowski and Garner, 2008). Given the environmental and economic damage caused by this species, biological control efforts were initiated during 1984 when conventional controls were deemed ineffective and impractical (Olckers, 1999; Olckers and Zimmermann, 1991).

South African biological control efforts have seen two agents released against *S. mauritianum*, namely *Gargaphia decoris* and *Anthonomus santacruzi* (Klein, 2011; Olckers, 2011). Releases of the flower-bud feeding weevil, *A. santacruzi* Hustache (Coleoptera: Curculionidae), were an attempt to reduce the spread of *S. mauritianum* by diminishing the fruit and seed set of the tree (Olckers, 2003, 2008). This reduction in fruit and seed output occurs as a result of the florivorous life cycle of *A. santacruzi* (Olckers, 2009). Adult weevils feed primarily on the floral material, including anthers, petals and buds, which may cause the premature abscission and deformation of flowers (Olckers, 2003; Gillespie and Besaans, 2014). Female weevils oviposit eggs into the newly developing buds of inflorescences, in which the endophagous larvae hatch and develop (Olckers, 2003, 2009). These buds ultimately fail to develop due to extensive feeding by the larvae, consequently reducing flower, fruit and seed production (Olckers, 2003; Olckers, 2011). Overall the destructive nature of *A. santacruzi* is suggested to have serious implications for the reproductive capabilities of *S. mauritianum* (Olckers, 2003; Hakizimana, 2011).

Assessments of the breeding systems of *S. mauritianum* in South Africa by Rambuda and Johnson (2004), found that the tree is self-compatible, displaying partial autogamy (selfing) at low levels. Self-compatibility in invasive species offers a degree of ‘reproductive assurance’ (Rodger and Johnson, 2013). However, invasive trees are suggested to be more susceptible to the detrimental effects of inbreeding following self-pollination (Barrett et al., 1996; Scofield and Schultz, 2006; Rodger and Johnson, 2013). *Solanum mauritianum* is known to be buzz pollinated by both social and solitary bee species (Olckers, 2011; Thorne and Peter, 2013), meaning that access to the pollen stores is gained through the dehiscing of anthers in response to a particular sonication or vibration (De Luca and Vallejo-Marín, 2013). Although the Solanaceae offer no nectar rewards, they produce copious amounts of nutrient rich pollen, as much as 200 000 grains per flower, which serves to attract pollinators (Buchmann and Cane, 1989). Anther feeding by *A. santacruzi* allows them access to the internal pollen stores of *S. mauritianum* by puncturing the anthers with their elongate rostrum (Gillespie and Besaans, 2014). The preference of *A. santacruzi* adults for feeding and residence on the weed’s inflorescences, as well as the floral morphology of *S. mauritianum*, raises questions about the agent’s potential to act as a pollinator.

Given the limited amount of post-release research on *A. santacruzi* and the fact that biological control programmes are often criticised for their lack of studies addressing indirect effects, it is important that these aspects be better understood. Furthermore, few pollination studies exist in a biological control context, with none investigating the potential of flower-feeding agents to act as pollinators of their target weeds. This study aimed to assess the impacts of *Anthonomus santacruzi* florivory on the reproductive output of *Solanum mauritianum*, as well as the potential of the weevil to act as an indirect pollinator.

2. Methods & Materials

2.1 *Anthonomus santacruzi* plant site selection

Relative densities of *A. santacruzi* adults on *S. mauritianum* plants, particularly inflorescences, were assessed using periodic scan sampling, adapted from Cowie et al. (2016a). Thirty adult individuals of *A. santacruzi* were placed onto flowering *S. mauritianum* plants. The weevils were randomly assigned to six flowering *S. mauritianum* plants (five weevils per plant). Plants were then divided amongst three large cages, totalling 15 weevils and two plants per cage. Adults were marked, using a non-toxic paint, in accordance with the plant (inflorescence) upon which they were released. Individuals were observed at half-hourly intervals, between 06:00 and 18:00, for a period of five days. Cages were housed in a glasshouse section of the Wits Insectary and maintained at 24 ± 1 °C, with a relative humidity of between 70-80%. Weevils were scored as either ‘inflorescence’ or ‘leaf’ dwelling when found on *S. mauritianum*. When located on an inflorescence weevils were scored as persisting (located on the same inflorescence as initially released) or dispersed (located on a different inflorescence to original release). Furthermore, the locality of each inflorescence dwelling weevil was further divided into ‘bud’ (unopened or developing flowerbud), ‘flower’ (non-reproductive material) or ‘anther’ (reproductive material) dwelling. Individuals that were not found on any part of the *S. mauritianum* plant were not scored for that particular observation. In the case that a dead weevil was noted, a replacement weevil was marked and released onto the corresponding plant’s inflorescence.

2.2 Floral feeding damage by *Anthonomus santacruzi*

2.2.1 Anther feeding damage

Twenty-five flowers were randomly selected from *S. mauritianum* inflorescences exposed to feeding by *A. santacruzi* adults for a week. Flowers selected had a single anther removed at random for damage assessment. Feeding damage was assessed by calculating the surface area removed by *A. santacruzi*. The surface area of *S. mauritianum* anthers (SA_{Anther}) was calculated using a modified cylinder formula, in which the two cylindrical columns of the anther were measured individually, using photographic analysis, ImageJ software (Ver. 1.8, Wayne Rasband, National Institutes of Health, USA), and summed (Equation 1). Feeding holes ($SA_{Feeding}$) created by *A. santacruzi* adults were treated as ellipses and totalled per anther (Equation 2). Holes located on the dorsal anther tip, which were a result of the anther dehiscing, were excluded from the $SA_{Feeding}$ calculation.

Equation 1: $SA_{Anther} = [(2\pi \cdot r_1 \cdot h_1) + (2\pi \cdot r_1^2)] + [(2\pi \cdot r_2 \cdot h_2) + (2\pi \cdot r_2^2)]$

$$r = \text{radius of anther (mm)} \quad h = \text{height of anther (mm)}$$

Equation 2: $SA_{Feeding} = \sum (rl_1 \times rs_1 \times \pi) + (rl_2 \times rs_2 \times \pi) + \dots (rl_n \times rs_n \times \pi)$

$$rl = \text{largest radial distance of ellipse (mm)} \quad rs = \text{smallest radial distance of ellipse (mm)}$$

2.2.2 Flower feeding damage

Using the same 25 flowers as selected for anther feeding damage, *A. santacruzi* damage to floral (petal) material was assessed by photographic analysis (ImageJ software). Flowers had all reproductive (stigmas, styles, anthers and stamens) and sepal material carefully removed prior to photographing. Flowers were then flattened using a clear sheet of glass and

photographed under a dissecting microscope (*Leica WILD M3B*) at 6x magnification. Total petal area removed (%) by *A. santacruzi* adults was calculated using ImageJ.

2.3 *Solanum mauritianum* pollination trials and breeding systems assessment

2.3.1 Pollination trials

Twenty five mature *S. mauritianum* trees, approximately two meters in height, were selected for pollination experiments. Three developing inflorescences per tree were selected at random and bagged using fine mesh. Inflorescences, in bud, were bagged prior to flowering to prevent uncontrolled pollinator access. Each inflorescence was assigned to one of three treatments, namely (i) “No pollinators” (bagged to test for autogamous selfing), (ii) only “*A. santacruzi*” (bagged) and (iii) “All pollinators” (open pollination – bag removed). The “*A. santacruzi*” treatment had 10 adult weevils per inflorescence, added to the bag for the duration that it was in flower, after which all weevils were removed. Lastly, the ‘All pollinators’ treatment had the mesh bag removed for the duration that the entire inflorescence was in flower, permitting all naturally occurring pollinators (i.e. no *A. santacruzi*) access to the inflorescence during flowering. After pollination, these inflorescences were re-bagged to prevent frugivore interference.

For each of the treatment inflorescences the number of buds, flowers and fruits produced were recorded. All fruits were harvested once mature (when the entire fruit had turned yellow). In the case that an inflorescence had developed more than five fruits, only five randomly selected fruits were harvested and used a representative sub-sample for the inflorescence. Fruits were then weighed and measured (widest diameter). Once weighed and measured, fruits were rinsed with sterile distilled water and dissected. Seed counts were conducted on each of the dissected fruits and their seed batches were placed into sterile petri

dishes, weighed and tested for viability (Section 2.3.3). Seed batches were then oven dried at 35 °C for a period of 72 hours and re-weighed.

2.3.2 Breeding system assessments

In addition to the pollination assessments, the potential reproductive avenues of *S. mauritianum* were investigated. Using the same *S. mauritianum* trees as the pollination trials, newly developing inflorescences were selected at random and bagged prior to the flowers opening to prevent pollinator access (interference). The inflorescences were divided amongst four treatments, namely i) selfed from the same flower (SF), (ii) selfed from the same inflorescence (SI), (iii) outcrossed and (iv) emasculated (test for apomixis). The selfing treatments were hand pollinated with pollen from the same flower / inflorescence. The outcrossed treatment involved the hand pollination of flowers with pollen collected from freshly dehiscent anthers from different trees. The emasculation (apomixis) treatment involved the removal of all anthers (emasculation) of flowers, whilst preventing pollen exposure to the stigmas.

Subsequent to the above treatments, flowers were left to fruit and allowed to develop until mature. Once fully mature, fruits/seeds were harvested, weighed, measured and tetrazolium tested following the same procedures as in the ‘pollination trials’ (Section 2.3.1), [in the case that no fruit formation occurred then no fruit/seed data were recorded].

2.3.3 Seed viability

Seed viability for all *S. mauritianum* samples was assessed by means of ‘tetrazolium testing’ adapted from Witkowski and Garner (2008). All seed batches from each of the pollination and breeding system treatments were washed in a 2% sodium hypochlorite solution and rinsed with sterile distilled water. Seeds batches were then covered in a 2%,

2,3,5-triphenyltetrazolium chloride (tetrazolium) solution for a period of 48 hours and kept out of direct light. Seeds which displayed staining (pale red) were classified as viable, whilst seeds that showed no change in colouration were classed as non-viable (dead).

2.4 Data Analysis

Total anther area removed (%) by *A. santacruzi* feeding was calculated as $(SA_{Feeding} / SA_{Anther}) \times 100$. A Pearson product correlation was used to assess the association between the number of *A. santacruzi* feeding holes and their size on *S. mauritianum* anthers. *Anthonomus santacruzi* density and inflorescence selection were each assessed using a paired samples t-test. Density on inflorescences was further assessed by means of a One-way Analysis of Variance (ANOVA) with repeated measures and a Tukey HSD post-hoc. A One-way ANOVA with a Tukey HSD was used to test for significant differences between pollination treatments for the number of buds, flowers and fruits per inflorescences as well as the number of seeds per fruit. Linear regressions, with zero intercepts, were fitted to seed number (per fruit) plotted against berry diameter for the 'All pollinators', '*A. santacruzi*' and , 'No pollinators' pollination treatments. A Standardised Major Axis (β_{SMA}) test with berry size (diameter) set as a continuous predictor was used to detect heterogeneity amongst fitted slopes for the pollination assessment treatments (Warton et al., 2006). Lastly, multiple One-way ANOVAs with a Tukey HSD post-hoc were used to test for significant differences between all fruit and seed parameters of the pollination and breeding system treatments.

3. Results

3.1 *Anthonomus santacruzi* plant site selection

Overall, *A. santacruzi* adults occurred in significantly higher densities on *S. mauritianum* inflorescences than leaves ($t_{124}=12.2$; $P < 0.001$) (Fig 1A.). Densities of *A. santacruzi*

occurring on the inflorescences indicated that the weevils have a preference for the flowers rather than buds. Furthermore weevils occupying flowers were predominately located on the anthers of *S. mauritianum* ($F_{2,72} = 18.3$; $P < 0.001$) (Fig.1B.). *Anthonomus santacruzi* also displayed a preference to remaining within a single flowering inflorescence, rather than moving between multiple inflorescences on or between plants (Fig 2C.), with 87.8% of weevils remaining on the same inflorescence as first released, 7.9% of weevils moving to different inflorescences on the same plant and only 4.3% of weevils moving to different inflorescences on different plants. The movement of *A. santacruzi* individuals was only noted when a majority of the flowers on an inflorescence had abscised or begun senescing. Throughout the assessment *A. santacruzi* experienced only 3% mortality.

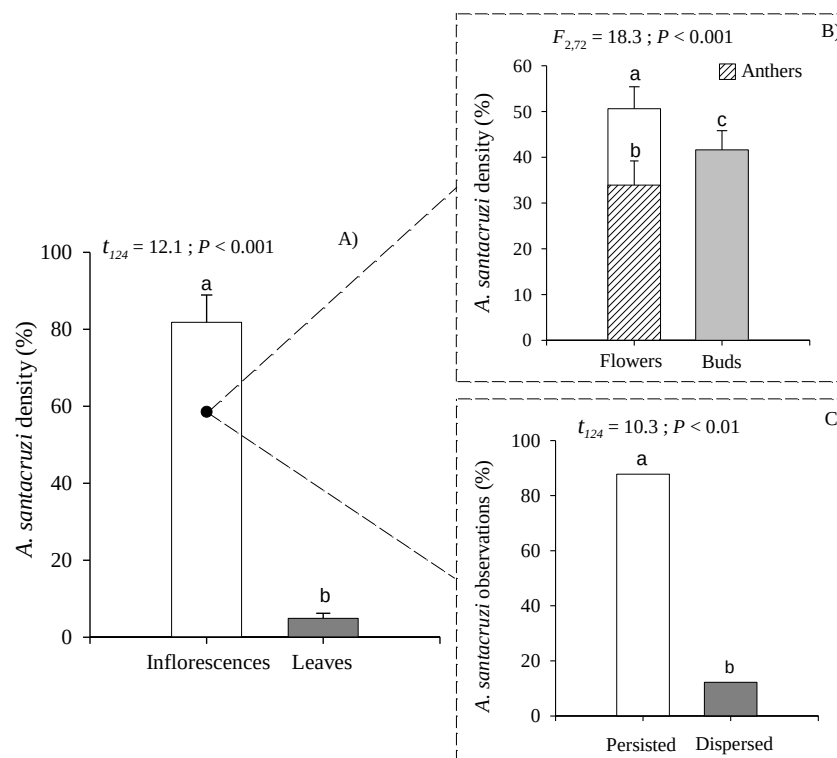


Fig. 1. A) Relative density $\pm$ SE (%) of *Anthonomus santacruzi* on the inflorescences and leaves of *Solanum mauritianum* plants. (B) Weevil preference within inflorescences was further assessed on the flowers, anthers and buds. (C) *Anthonomus santacruzi* fidelity: persistence (remaining upon the same inflorescence as released) vs dispersal (movement onto different inflorescences, on the same or different trees). Different lowercase letters indicate significant differences between weevil densities / observations.

3.2 *Anthonomus santacruzi* floral feeding damage

The mean surface area ($SA_{Anthers}$) of *S. mauritianum* anthers (Fig 2A) was calculated as $17.07 \text{ mm}^2 \pm 0.27$ ($n=25$). A strong positive correlation ($r = 0.76$; $n = 25$) was found regarding the number of feeding holes made per anther and the size of the holes, with *A. santacruzi* frequently producing fewer but larger holes per anther (Fig. 2B-D). On average, feeding by *A. santacruzi* adults ($SA_{Feeding}$), resulted in four feeding holes (3.88 ± 0.24) per anther and removal of approximately 5 % ($0.82 \pm 0.04 \text{ mm}^2$) of the anther's total area ($n=25$) (Fig. 2B-D). Floral feeding by *A. santacruzi* adults removed, on average just over 2 % ($3.41 \text{ mm}^2 \pm 0.67$) of the total area of *S. mauritianum* petals ($167.22 \text{ mm}^2 \pm 33.43$) (Fig 2E).



Fig. 2. *Solanum mauritianum*: undamaged anther (A), anthers damaged by *Anthonomus santacruzi* feeding (B-C) *A. santacruzi* feeding hole on anther (D) and feeding damage to *S. mauritianum* petal (E) by *A. santacruzi*.

3.3 *Solanum mauritianum* pollination trials and breeding systems assessment

Bud production, per inflorescence, did not differ for any of the three pollination treatments (Fig. 3A). Inflorescences exposed to only *A. santacruzi* displayed a significant decrease (25%) in the number of mature flowers per inflorescence ($F_{2, 72} = 40.4$; $P < 0.001$) when compared to the ‘No pollinators’ and ‘All pollinators’ treatments (Fig. 3B). Mean floral lifespan, from flower opening to closing, was significantly shorter in inflorescences exposed

to *A. santacruzi* ($F_{2,72} = 15.6$; $P < 0.01$) when compared to the ‘All pollinators’ and ‘No pollinators’ treatments. Fruit production differed significantly between all three of the pollination treatments ($F_{2,67} = 58.9$; $P < 0.001$), with the ‘No pollinators’ treatment displaying the lowest fruit set, followed by the inflorescences exposed to *A. santacruzi* and lastly by the all pollinators treatments, which showed the highest fruit set (Fig. 3C). Seed number, per fruit, was significantly greater ($F_{2,67} = 21.5$; $P < 0.001$) in the ‘All pollinators’ treatment when compared to the ‘*A. santacruzi*’ and ‘No pollinators’ treatments (Fig. 3D).

Fruit and seed development differed significantly between the ‘All pollinators’ and the ‘No pollinators’ and ‘*A. santacruzi*’ treatments (Fig. 4). Overall the ‘All pollinators’ treatment showed the largest fruits which produced the most seeds, maintaining a significantly greater shift in slope elevation ($\beta_{SMA} = 5.9$; $P < 0.05$) when compared to the ‘No pollinators’ and ‘All pollinators’ treatments, which shared common slopes (Fig. 4). Additionally, the strongest fruit size (diameter) and seed production relationship was noted in the ‘All pollinators’ treatment ($y=93.33x$; $R^2=0.79$). Whilst the ‘*A. santacruzi*’ ($y=88.22x$; $R^2=0.68$) and the ‘No pollinators’ ($y=86.39x$; $R^2=0.66$) treatments displayed moderately strong relationships.

Overall the pollination trials and breeding assessments carried out on *S. mauritanum* displayed similar patterns regarding their fruit and seed outputs (Table 1). Mean fruit size was found to be smallest in the ‘No pollinators’, ‘*A. santacruzi*’ and Selfed (SF/SI) treatments when compared to the ‘All pollinators’ and Outcrossed treatments ($F_{5,131} = 10.6$; $P < 0.01$). Whereas the greatest mean seed set and seed weight (total/wet/dry) was found in the ‘All pollinators’ and Outcrossed treatments. Similarly seeds in the Outcrossed and ‘All pollinators’ treatments displayed significantly greater viabilities than the ‘No pollinators’, ‘*A. santacruzi*’ and selfed (SF/SI) treatments ($F_{5,131} = 8.4$; $P < 0.01$).

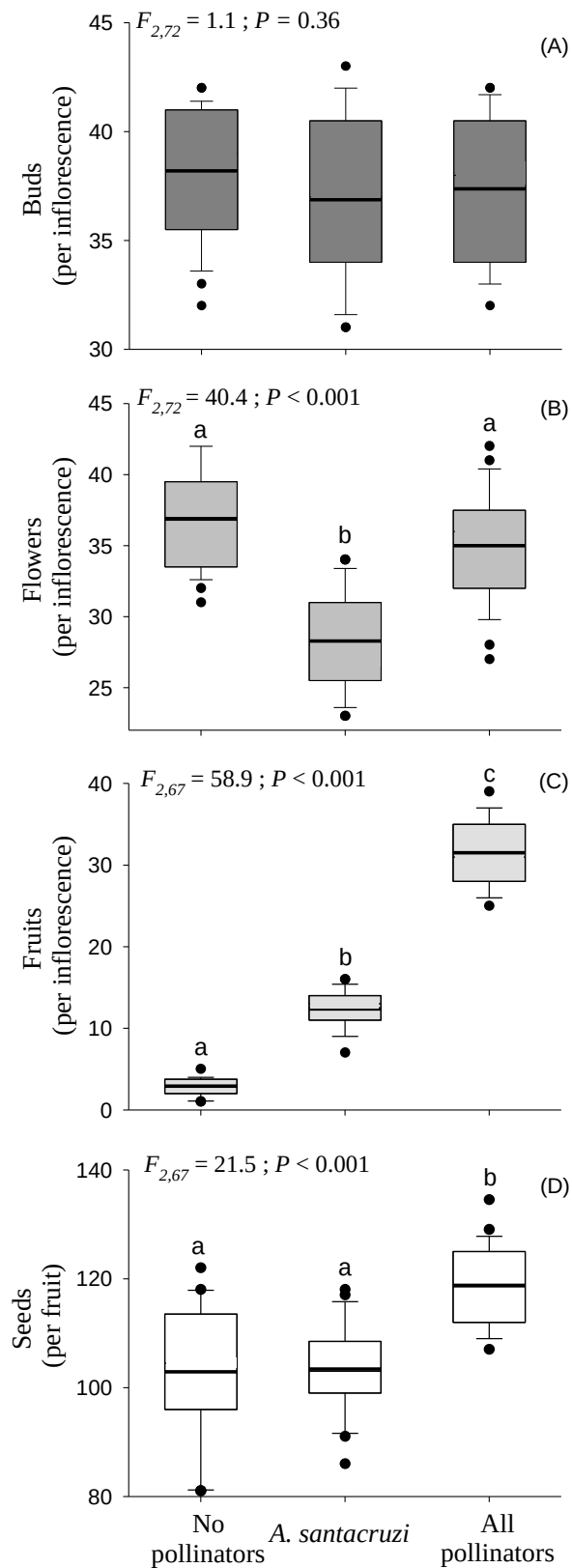


Fig. 3. Reproductive consequence for *Solanum mauritianum* inflorescences exposed to differing pollination treatments: 'No pollinators', '*A. santacruzii*' and 'All pollinators'. Number of buds (A), flowers (B), fruits (C) and seeds (D) produced per inflorescence (Boxplot of means (**bar**), 10th, 25th, 75th, 90th percentiles and predicted outliers). Different lowercase letters indicate significant differences between treatments.

Table 1. Mean reproductive outputs of *Solanum mauritianum* inflorescences exposed to the pollination treatments: ‘No pollinators’, ‘*A. santacruzi*’ and ‘All pollinators’ and the breeding system assessments: Selfed SF (same flower), Selfed SI (same inflorescence), Outcrossed and Apomixis (emasculation). Different lower case letters indicate significant differences between treatment parameters (ANOVA; Tukey HSD; $P < 0.05$).

	Pollination Assessment			Breeding Systems			
	<i>No pollinators</i>	<i>A. santacruzi</i>	<i>All pollinators</i>	<i>Selfed SF</i>	<i>Selfed SI</i>	<i>Outcrossed</i>	<i>Apomixis</i>
Fruit Parameters $\bar{x} \pm SE$	(n=20)	(n=25)	(n=25)	(n=22)	(n=22)	(n=23)	(n=25)
Fruit set (%)	10.1 $\pm$ 2.1 ^a	44.2 $\pm$ 3.3 ^b	89.9 $\pm$ 1.8 ^c	88	88	92	0
Fruit size (cm)	1.21 $\pm$ 0.04 ^a	1.24 $\pm$ 0.03 ^a	1.28 $\pm$ 0.03 ^b	1.23 $\pm$ 0.04 ^a	1.25 $\pm$ 0.02 ^a	1.30 $\pm$ 0.02 ^b	-
Fruit weight wet (g)	1.10 $\pm$ 0.05 ^a	1.19 $\pm$ 0.03 ^b	1.27 $\pm$ 0.04 ^c	1.22 $\pm$ 0.04 ^b	1.24 $\pm$ 0.04 ^b	1.33 $\pm$ 0.05 ^c	-
Seed Parameters $\bar{x} \pm SE$							
Seed set (per fruit)	108 $\pm$ 6 ^a	112 $\pm$ 5 ^a	121 $\pm$ 3 ^b	113 $\pm$ 4 ^a	115 $\pm$ 4 ^a	125 $\pm$ 5 ^c	-
Total seed weight (mg)	124.3 $\pm$ 6.9 ^a	135.5 $\pm$ 8.8 ^b	153.4 $\pm$ 8.2 ^c	135.6 $\pm$ 7.4 ^b	140.3 $\pm$ 9.4 ^b	160.7 $\pm$ 4 ^c	-
Seed weight wet (mg)	1.16 $\pm$ 0.05 ^a	1.21 $\pm$ 0.04 ^a	1.29 $\pm$ 0.05 ^b	1.21 $\pm$ 0.05 ^a	1.22 $\pm$ 0.04 ^{ab}	1.31 $\pm$ 0.03 ^c	-
Seed weight dry (mg)	1.04 $\pm$ 0.03 ^a	1.07 $\pm$ 0.03 ^{ab}	1.15 $\pm$ 0.03 ^c	1.10 $\pm$ 0.03 ^b	1.09 $\pm$ 0.03 ^b	1.18 $\pm$ 0.04 ^c	-
Seed viability (%)	67.9 $\pm$ 4.1 ^a	70.9 $\pm$ 2.8 ^{ab}	75.6 $\pm$ 2.4 ^c	71.8 $\pm$ 1.9 ^b	73.4 $\pm$ 3.2 ^b	79.7 $\pm$ 4.8 ^d	-

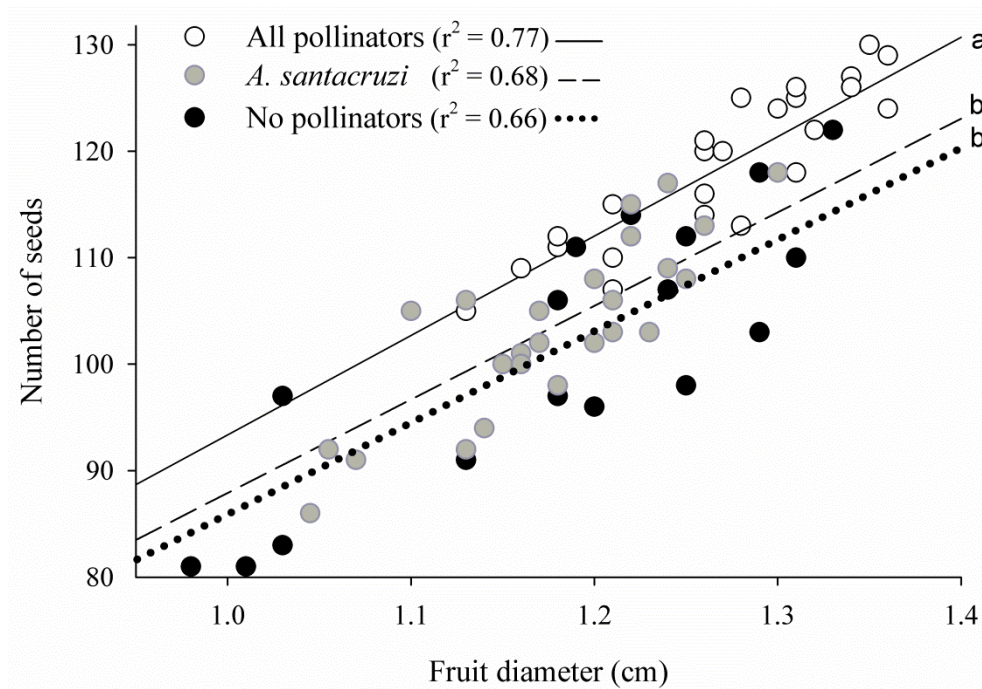


Fig. 4. Relationship of fruit size (diameter) to seed production (per fruit), compared amongst the pollination treatments: ‘All pollinators’ ($n=25$), ‘*A. santacruzi*’ ($n=25$) and ‘No pollinators’ ($n=20$), carried out on mature *Solanum mauritianum* trees. Linear regressions fitted with zero intercepts ($P < 0.05$). Different lowercase letters indicate significant differences between treatment slopes ($\beta_{SMA} = 5.9$; $P < 0.05$).

4. Discussion

Directly, damage caused by *A. santacruzi* feeding on the inflorescences of *S. mauritianum*, namely the flowers and anthers, may be classified as trivial (see Klein, 2011). However, the subsequent effects and interactions of *A. santacruzi*, with its target plant, suggest this agent has the potential to be considerably damaging, particularly to the reproductive success of *S. mauritianum*. Significant reductions in flowering and fruiting are a consequence of florivory by *A. santacruzi*. Feeding by both adults and larvae is known to terminate bud development, causing the premature abscission of flowers and in certain cases the abortion of fruiting (Olckers, 2003, Barboza et al., 2009). Consequently the production of smaller fruits and fewer seeds may be a result of abnormalities accrued from feeding damage experienced by flowers prior to fruiting (Cardel and Koptur, 2010). Plants may compensate

for florivory by accelerating fruit development, resulting in the formation of smaller less viable fruits, as opposed to the detrimental abortion or complete loss of all fruit set (Wise and Cummins, 2006; Cardel and Koptur, 2010).

The preference of *A. santacruzi* for feeding and residence on the inflorescences of *S. mauritianum* creates the potential for numerous indirect effects, particularly regarding the pollination of *S. mauritianum* (Althoff et al., 2013; Eliyahu et al., 2015). Puncturing of the anthers by *A. santacruzi* creates a risk of pollen dispersal and consequent pollination of *S. mauritianum* by the adult weevils. Given the preference of *A. santacruzi* to remain on the same inflorescence, whilst in flower, strongly suggests that should any pollination occur it is likely to be done with pollen from the same flower / inflorescence (i.e. self-pollination). Given the copious pollen production of *S. mauritianum* as well as the puncturing of anthers by *A. santacruzi*, resulting in large holes, there may be a possibility of pollen ‘leakage’ from damaged anthers onto the stigmas of the same flower. Although pollen feeding by *A. santacruzi* increases the likelihood of self-pollination, this may also serve to limit the pollen (male-gamete) availability for outcrossing (Carper et al., 2016), and result in fewer, inferior seeds from the weed.

Reductions in the floral lifespan of inflorescences exposed to *A. santacruzi* are not unexpected given the florivorous nature of the weevils (Olckers, 2003, 2008). Such reductions in flower availability and longevity are advantageous in restricting the outcrossing of *S. mauritianum* by generalist pollinators, particularly social and solitary bee species (Olckers, 2011; Thorne and Peter, 2013). Reductions in flower number and lifespan may not only limit the number of flowers available for pollination but also restrict the time available to other potentially outcrossing pollinators (Botto-Mahan et al., 2011). Indirectly, damage to flowers influences pollinator visitation, as floral damage detracts from the overall attractiveness of flowers and the visibility of their pollen guides (McCall and Irwin, 2006;

Botto-Mahan et al., 2011; Eliyahu et al., 2015), which will negatively impact their pollination/reproductive success. Numerous studies have documented that generalist pollinators, including bees, are less likely to visit damaged flowers given the likelihood of poorer energy returns (Wilmer, 2011; Eliyahu et al., 2015). Regardless of these effects *A. santacruzi* enhances the likelihood of self-pollination in *S. mauritianum*. This enhancement may be facilitated by *A. santacruzi* (pollen vector) or through the proportional increase in *S. mauritianum*'s autonomous selfing (Penet et al., 2009).

The potential self-pollination of *S. mauritianum* by *A. santacruzi* may seem counter-intuitive to the agent's intended role. However, this enhancement of self-pollination will ultimately impact on the invasive ecology of *S. mauritianum*. Rodger and Johnson (2013) found that self-pollinated invasive trees may initially gain an improved invasion success, through 'reproductive assurance', but subsequently they experience the detrimental effects of inbreeding through the production of less vigorous progeny. The effects of inbreeding, which may occur after varying successive generations, include reductions in plant fitness, such as reductions in growth, fruit size, seed production, seed viability and progeny fitness (Rohdes and Ashman, 2010; Rodger et al. 2013; Rodger and Johnson 2013). This suggests that in areas where *A. santacruzi* is established, such as the KwaZulu-Natal South Coast and Sabie region of Mpumalanga (Cowie et al., 2016b), the weevil may facilitate the self-pollination and potential inbreeding of *S. mauritianum* populations.

In conclusion, *A. santacruzi* significantly reduces the overall flowering, fruiting and seed outputs of *Solanum mauritianum*, maintaining the ability to be a considerably damaging agent. However, there is a strong likelihood that *A. santacruzi* contributes towards the self-pollination of *S. mauritianum* in South Africa. The indirect effects of self-pollination may ultimately prove beneficial in reducing *S. mauritianum* through inbreeding depression. Although this study assessed the potential for *A. santacruzi* to contribute as a potential

pollinator of *S. mauritianum*, field studies should be undertaken to determine the contributions of *A. santacruzi* in relation to generalist pollinators. Additionally, the indirect effects of *A. santacruzi* on pollinator guilds, particularly bees, may be further investigated. This study advocates for increased consideration of the indirect effects of agent-weed interactions in biological control programmes, particularly the implications of florivorous species on the reproductive ability of their target weeds.

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CHAPTER FIVE:

SYNTHESIS & CONCLUSION

General overview

Solanum mauritianum is a problematic and widespread invasive tree of global significance, prevalent in many sub-tropical and tropical countries, particularly New Zealand and South Africa (Olckers, 2009, 2011). The long-standing and detrimental invasion of *S. mauritianum* in South Africa is well documented, having seen several control measures initiated.

Mechanical and chemical controls were quickly deemed costly, unsustainable and in certain cases counter-productive (Olckers et al., 2002; Witkowski and Garner, 2008). Research efforts then shifted towards biological control, one of the few sustainable options for controlling *S. mauritianum* (Olckers and Zimmermann, 1991; Olckers, 1999).

Biological control efforts against *S. mauritianum* in South Africa have suffered a troubled history (Olckers, 2004), with numerous factors constraining the programme's efficacy and performance in the field (Klein, 2011; Olckers, 2009, 2011). At present biocontrol efforts against *S. mauritianum* have largely been discounted, as far as the agents' impact on the weed is concerned. Thus the recent management of *S. mauritianum* is largely being driven towards integrated control measures. Although biocontrol is understood to be a crucial factor in slowing and reducing the spread of *S. mauritianum* in South Africa (Olckers, 2009), it has yet to be fully integrated with the current mechanical and chemical control programmes (ISSG, 2006; Olckers, 2011).

Releases of *Gargaphia decoris* and *Anthonomus santacruzi* were anticipated following their promising pre-release assessments. Unfortunately, both *G. decoris* and *A. santacruzi*

have suffered respective constraints, which have hindered biocontrol efforts against *S. mauritianum*. This study represents a post-release assessment of the biocontrol agents of *S. mauritianum* in South Africa, particularly the factors believed to constrain their efficacy and establishment as well as their indirect effects. This synthesis aims to draw conclusions from the preceding chapters, whilst highlighting the potential implications of the findings and recommendations for the future of *S. mauritianum* biological control research in South Africa.

Biocontrol implications for *Gargaphia decoris*

Biocontrol agents may often disappoint, they underperform, fail to establish, or only establish in localised areas, typically in numbers too low to impact their target weeds. Numerous generalisations exist as to why agents establish poorly and consequently underperform, but species-specific studies are often required to identify the particular constraining factor(s). The current classification of *Gargaphia decoris* as a trivial agent is largely due to the low densities at which populations establish and persist in the field (Withers et al. 2002; Klein, 2011; Olckers, 2009, 2011). However, ecophysiology assessments presented in this dissertation (Chapter 2) suggest that *G. decoris* has the potential to be an extensively damaging agent of *S. mauritianum*. Previous research, particularly Barker and Byrne (2005), in conjunction with this ecophysiology data (Chapter 2), suggests that abiotic factors are unlikely to constrain the establishment and performance of *G. decoris*. This suggests that biotic factors, such as predation, may be responsible for the low numbers at which *G. decoris* populations establish and persist, and consequently their largely trivial damage to *S. mauritianum* in the field (Lotter 2004; Olckers, 2011; Patrick and Olckers, 2014).

Predation is known to be highly detrimental to the establishment and efficacy of agents in biocontrol programmes, second only to climatic unsuitability (Stiling, 1993; Snyder and Ives, 2001). Although introduced agents are freed from their native predators, indigenous predators (generalists) pose a continual threat to the establishment and spread of both founder and existing populations in the field (Snyder and Ives, 2001; Patrick and Olckers, 2014). It is recommended that future studies on *G. decoris* should identify and address the factors constraining populations and their subsequent performance in the field. However, a temporary mitigation against the suspected effects of predation may be attained through modifications to the release strategies of *G. decoris*. Fewer releases with higher numbers of *G. decoris*, particularly in semi-shaded areas, have an improved probability for successful establishment when compared to multiple, smaller releases (Lotter, 2004; Patrick and Olckers, 2014).

Interestingly, the erratic but damaging outbreaks of *G. decoris* are a recurring trend documented in both South Africa and New Zealand (Patrick and Olckers, 2014; Winks, 2014). Other agents within the Tingidae, such as the leaf-sucker *Teleonemia scrupulosa* released against *Lantana camara*, have displayed similar outbreaks in South Africa and Australia (DAFF, 2013). This suggests that outbreaks of *G. decoris* may have some underlying seasonal or biological trigger, which should be further investigated, with particular attention to agent biology and predator–prey systems/dynamics.

Biocontrol implications for *Anthonomus santacruzi*

Climate and *Anthonomus santacruzi*

Climate is known to be one of the most influential factors in the establishment and success of biocontrol programmes (Stiling, 1993; Byrne et al., 2002, 2004; Dhileepan et al.,

2005). Numerous studies highlight that successful agent establishment is frequently correlated with sites displaying similar climates to those experienced by the agents in their native ranges (Grevstad, 1999; Byrne et al., 2004). In the case of *A. santacruzi*, thermal studies, relative humidity assessments and climate matching highlighted that *A. santacruzi* (adults) are likely to experience both cold and moisture stresses at many of the inland regions of South Africa, particularly on the Highveld (Chapter 3). Thus failure of *A. santacruzi* to establish at the Highveld release sites is likely due to the areas' poor climatic suitability, particularly during the cool, dry winter season (May-September) (Schulze et al., 1997; Maywald et al., 2015).

The climatic requirements of *A. santacruzi* should be considered when redistributing and releasing weevils at potential sites. Sites with prolonged minimum temperatures below 4 °C and average relative humidities of less than 47% should be avoided. Releases or re-dispersals should largely focus on areas with warmer and more humid conditions throughout the year, particularly over winter, such as coastal or forested regions. Although the presence of permanent adult populations is an important factor in agent establishment, the climatic tolerances of the egg, larval and pupal stages of *A. santacruzi*, should be included in future research. Additionally, climatic modelling data (Chapter 3) may be projected back onto the native range of *A. santacruzi* (Argentina/Brazil) using CLIMEX, to identify potential areas that may present weevil populations ('biotypes') better suited to the cooler and drier regions of South Africa (e.g. Robertson et al., 2008; Kriticos et al., 2015). Climatic models predict that changing climates are likely to affect biocontrol efforts, particularly the potential range and effectiveness of released species (Hellmann et al., 2008; Gerad et al., 2013). These predicted changes to South African climate should be assessed in the context of agent distribution and efficacy, particularly regarding species which are climatically constrained at present, such as *A. santacruzi*.

Potential indirect effects of *A. santacruzi*

Growing demand for biological control will undoubtedly see an increase in the release of agents (Pimentel, 2011), inadvertently increasing the potential for indirect effects (Downey and Paterson, 2016). The use of flower-feeding biocontrol agents has become increasingly popular, given their potential to reduce the reproductive output and recruitment of invasive plants (van Klinken et al., 2004; Hakizimana, 2011). Research into the indirect effects of *A. santacruzi*, suggests that florivores have the potential for numerous beneficial indirect effects regarding their target weeds (Chapter 4). These effects may include decreases in the weed's pollination through the deterrence of outcrossing pollinators, enhancements in selfing and subsequent inbreeding depression resulting in inferior progeny. Despite contentions surrounding the effectiveness of florivorous agents, such findings may advocate for increased consideration of indirect agent-weed interactions, particularly regarding the implications of using flower-feeding agents.

Assessments of *A. santacruzi* provided valuable biocontrol insights, but also highlighted the need for further research into the weevil's other indirect effects. Future research should (i) investigate the possibility of non-target interactions of *A. santacruzi* in relation to pollinator guilds (bees) associated with *S. mauritianum*, (ii) conduct field assessments to quantify reductions in the fruiting, seeding and viability of *S. mauritianum* in regions where *A. santacruzi* is well established, such as the KwaZulu-Natal South Coast and the Sabie region of Mpumalanga, and (iii) assess the potential accrual and severity of inbreeding depression in *S. mauritianum* populations subsequent to self-pollination by *A. santacruzi*.

Prospects for the biological control of *S. mauritianum* in South Africa

Combining agents to improve effectiveness

South African biocontrol programs frequently involve the release of multiple agents against problematic weeds (Klein, 2011; Weyl, 2011). This combination of agents is not new, but has nevertheless yielded mixed results in the past (Marlin et al., 2013, Stephens et al., 2013). Agent combinations may result in a variety of effects and interactions, which are broadly classified as synergistic, additive or inhibitory (Hatcher, 1995; Stiling and Cornelissen, 2005; Weyl, 2011). Originally proposed by Hatcher (1995), these agent interactions are defined as (i) Synergistic: the agent combination is significantly more damaging than the addition of each individual agent's damage, (ii) Additive: the damage caused by the agent combination is equal to the addition of the damage caused by each individual agent and (iii) Inhibitory: the agent combination is equal to or significantly less damaging than the damage caused by the weakest of the individual agents. Synergistic interactions are frequently sought after in biocontrol given their potential to enhance overall efficacy (Stiling and Cornelissen, 2005; Stephens et al., 2013). Whereas the negative implications associated with agent competition (inhibition), are avoided due to the reduction in effectiveness (Hatcher 1995; Hatcher and Paul, 2001). Successful, namely synergistic, agent combinations frequently occur when agents occupy separate niches (feeding strategies) on the target plant (April et al., 2011; Weyl, 2011; Stephens et al., 2013). Although the potential agent interactions are predicted on the basis of theoretical models, they may not always be expressed in the field.

A prime example highlighting the success of multiple agent biocontrol programmes in South Africa was that against *Sesbania punicea* “Red Sesbania”. Releases of the first agent, *Trichapion lativentre*, a flowerbud-feeding weevil, in the 1970s, were extensively damaging,

substantially reducing seed production, but remained unable to effectively control *S. punicea* (Hoffmann and Moran, 1991, 1999). However, complete control of *S. punicea* was later attained through the addition of two more agents, a stem-boring weevil, *Neodiplogrammus quadrivittatus*, and a seed-feeding weevil, *Rhyssomatus marginatus* (Hoffman and Moran, 1999; Klein, 2011). In the case of *S. mauritianum*, the interactions of *G. decoris* (attacking leaves: reducing photosynthetic capacity) and *A. santacruzii* (attacking flowers: reducing reproductive output) have the potential to be synergistic, but are yet to be assessed in the field. This may advocate for the simultaneous release of *G. decoris* and *A. santacruzii* into suitable infested areas. This highlights the potential for agent synergy through the addition of new agents. These releases should focus on species which inhabit different niches on the plant (Olckers, 1999). The addition of agents may allow for improved biocontrol damage and the potential to reduce *S. mauritianum*'s fruiting, growth, recruitment and widespread invasion.

Potential agents to be revived

The shelving and erroneous rejection of potentially safe and effective agents (false positives) are a concern frequently expressed in biocontrol programmes (Fowler et al., 2012; Downey and Paterson, 2016). The rejection of agents typically occurs due to inconclusive or ambiguous host-specificity data, which raises concerns for the potential of non-target damage (McFadyen, 1998; Briese, 1999). However, the lack of demonstrated host-specificity by agents in quarantine is often an artificial expansion of an agents host range, and one which is unlikely to be utilised in the field (Marohasy, 1998; Briese and Walker, 2002; Olckers, 2003). Despite minor host-specificity concerns surrounding *A. santacruzii*, the weevil was released in 2008, with no reported incidences of non-target damage. This may advocate for the reconsideration of other promising *S. mauritianum* agents that were previously rejected or

shelved on similar grounds. Thus the revival of promising agents which were untested, shelved or rejected, may be of benefit (Table 5.1). Should research into new agent(s) be approved, it is recommended that it focus on either optimising biocontrol efforts, through synergistic interactions or by establishing new agent populations in regions that are seemingly unsuited to the current agents.

The synergistic biocontrol of *S. mauritianum* is likely to be gained through research into agents that attack the tree's vegetative structure, namely the stem-borers *Adesmus hemispilus* Germar (Coleoptera: Cerambycidae) and *Conotrachelus squalidus* Boheman (Coleoptera: Curculionidae), or the shoot-galler, *Collabismus notulatus* Boheman (Coleoptera: Curculionidae) (Olckers, 2009). Despite failure to establish laboratory cultures on two occasions (1995/ 1998) (Olckers, 1999), *C. squalidus* is still viewed as the most promising of these candidates (pers. comm. T. Olckers). This precedence is largely due to the widespread distribution of *C. squalidus* in its native range, as well as its documentation on only *S. mauritianum* plants in the field during agent surveys (Olckers, 1999; Pedrosa-Macedo et al., 2003). Whereas preliminary observations on *A. hemispilus*, showed the species possessed unfavourable traits, including an extensive larval developmental period of 7-12 months and an aggressive nature towards its conspecifics (Olckers, 1999, 2009). In the case of *C. notulatus* very little is known about the biology and effects of the shoot-galling weevil (Olckers et al., 2002; Olckers, 2009).

The rejection of potential agents has been a consistent hindrance to biocontrol efforts against *S. mauritianum*. In the cases of *Acrolepia xylophragma* Meyrick (Lepidoptera: Acrolepiidae), *Acallepitrax* sp. Bechyne (Coleoptera: Chrysomelidae) and *Platyphora* spp., renewed research may promote for the reconsideration of their previous rejection. *Acrolepia xylophragma* and *Acallepitrax* sp. both showed promising damage during laboratory trials but

were rejected due to ambiguous host-specificity (Olckers, 1999, 2004). More rigorous host-specificity trials, including expanded choice tests and open-field trials in the native range, may clarify ambiguities and promote for reconsiderations. The *Platyphora* spp. initially held promise, with surveys and literature studies suggesting high levels of damage and monophagy (Olckers, 1999). However, contradictory findings surrounding host-specificity as well as establishment concerns halted research into these species (Olckers, 1999). Thus the revival of *Platyphora* spp. should be considered a low priority, until the other more promising agents are reviewed and the host-specificity issues resolved (Olckers, 1999, 2000).

Additionally, the use of competitive agents against *S. mauritianum*, although seemingly counter-intuitive, should not be dismissed without prior assessment. The considerable control of a closely related weed species, *Solanum elaeagnifolium*, was attained using three agents, two of which, *Leptinotarsa texana* and *L. defecta*, occupy identical leaf-feeding niches (Olckers et al., 1995, 1999; Klein, 2011). This may support research into agents such as the mite, *Aponychus schultzi* Blanchard (Acari: Tetranychidae) and the weevil *Anthonomus morticinus* Clark (Coleoptera: Curculionidae). In the case of *A. schultzi*, host-specific biotypes are promising, however, major concerns exist surrounding the mite's risk as a potential crop pest (spider-mite), after surveys found the species on 17 host plant species in its native range (Pedrosa-Macedo et al., 2003), meaning that the species is unlikely to be reconsidered (Pedrosa-Macedo et al., 2003). Similarly, the shelving of *A. morticinus* is unlikely to be revised at present, given the likelihood of competition and potential hybridisation with *A. santacruzi* (Olckers, 2009). However, *A. morticinus* may possess better climate tolerances (temperature and humidity), which should be assessed to ascertain if this species is better adapted to areas unsuited for *A. santacruzi*.

Table 5.1 Shortlist of potential candidate agents which may be revived / reconsidered for the biological control of *Solanum mauritianum* in South Africa (data from Pedrosa-Macedo et al., 2003; Olckers, 1999, 2009; Klein, 2011) and predicted interaction(s) with current biocontrol programme (released agents).

<i>Species</i>	<i>Order: Family</i>	<i>Damage</i>	<i>Status</i>	<i>Concern(s)</i>	<i>Interaction(s)</i>
<i>Priority candidates</i>					
<i>Collabismus notulatus</i>	Coleoptera: Curculionidae	Shoot-galler	Shelved	Untested (Agent biology?)	Synergistic
<i>Conotrachelus squalidus</i>	Coleoptera: Curculionidae	Stem-borer	Shelved	Untested (Culturing issues)	Synergistic
<i>Reconsideration (promising)</i>					
<i>Acallepitrix</i> sp.	Coleoptera: Chrysomelidae	Leaf-miner	Rejected	Host-specificity	Additive (Inhibitory)
<i>Acrolepia xylophragma</i>	Lepidoptera: Acrolepiidae	Leaf-miner	Rejected	Host-specificity	Additive (Inhibitory)
<i>Adesmus hemispilus</i>	Coleoptera: Cerambycidae	Stem-borer	Shelved	Untested (Agent issues)	Synergistic (Additive)
<i>Reconsideration (low priority)</i>					
<i>Anthonomus morticinus</i>	Coleoptera: Curculionidae	Flower-bud feeder	Shelved	Hybridisation (<i>A. santacruzi</i>)	Inhibitory (Additive)
<i>Aponychus schultzi</i>	Acari: Tetranychidae	Leaf-sucker	Shelved	Untested (Potential pest)	Additive (Inhibitory)
<i>Platyphora biforis</i>	Coleoptera: Chrysomelidae	Leaf-feeder	Rejected	Host-specificity/Establishment	Inhibitory (Additive)
<i>Platyphora nigronotata</i>	Coleoptera: Chrysomelidae	Leaf-feeder	Rejected	Host-specificity/Establishment	Inhibitory (Additive)
<i>Released</i>					
<i>Anthonomus santacruzi</i>	Coleoptera: Curculionidae	Flower-bud feeder	Released	Establishment (Localised)	Synergistic
<i>Gargaphia decoris</i>	Hemiptera: Tingidae	Leaf-sucker	Released	Establishment (Trivial)	Synergistic

Integrated management and research incorporating biological control

The current research suggests that *S. mauritianum* is unlikely to be controlled solely by biological control at present, but may be better suited for integrated control measures which incorporate biocontrol (Fig. 5.1). The inclusion of biocontrol in an integrated management programme should aim to reduce the spread of *S. mauritianum* in areas too large or costly to clear mechanically or chemically. Furthermore, the inclusion of biological control in integrated management should aim to reduce the costs of future clearing operations by increasing their sustainability and minimising the amount of labour required as well as the cost and number of follow-up programmes (Olckers, 2009, 2011; Pimentel, 2011).

The widespread global invasion of *S. mauritianum* also presents the opportunity for collaborative research on *S. mauritianum* biological control, as previously seen between South Africa and New Zealand (e.g. Olckers and Borea, 2009; Hakizimana and Olckers, 2013c), and South Africa and Brazil (e.g. Pedrosa-Macedo et al., 2003; Barboza et al., 2009). Advancing South African-Brazilian relations is particularly desirable, as they offer the opportunity for open-field tests and in-field observations of potential biocontrol agents in their native ranges (Hakizimana, 2011). Such collaborations might yield conclusive support towards the reconsideration of potentially effective agents, previously shelved or rejected pending additional host-specificity data e.g. *Acallepitririx* sp., *Acrolepia xylophragma* and *Platyphora* spp. (see Olckers, 1998, 1999, 2000, 2004 and 2009). Additionally, these relations may allow the importation of climatically better suited agents given the similarity of Brazilian regions to South Africa, particularly high altitude areas such as the Paraná plateau (Pedrosa-Macedo et al., 2003).

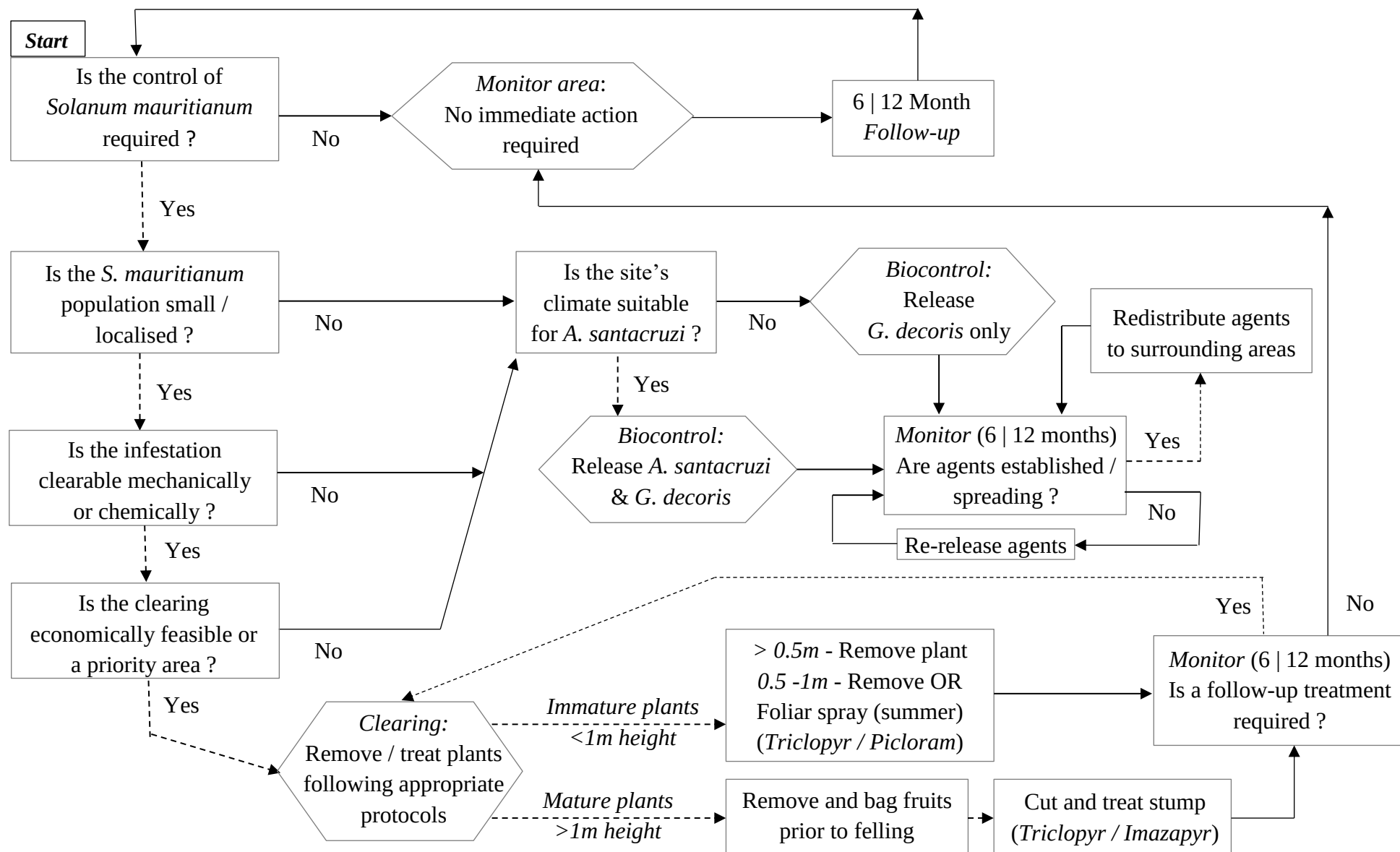


Fig 5.1. Conceptual decision chart for the integrated management of *Solanum mauritianum* in South Africa, with the incorporation of biological control (biocontrol) using the agents *Gargaphia decoris* and *Anthonomus santacruzi*.

Conclusion

Overall this research forms part of the post-release assessment of the biocontrol agents, *G. decoris* and *A. santacruzi*, released against *S. mauritianum*, providing insights towards the current biocontrol efforts and their constraints (Table 5.2). The work presented here highlights that both *G. decoris* and *A. santacruzi* have the potential to be effective agents against *S. mauritianum*, suggesting that these agents are deserving of renewed attention and investment, particularly in terms of their release strategies. The incorporation of plant ecophysiology and climate modelling offers additional techniques for the assessment of biocontrol agents' efficacy. This research may also aid in identifying constraints to biological control programmes against other Solanaceae weeds in South Africa and internationally (e.g. New Zealand). Although the incorporation of these additional techniques has enhanced the research as a whole, *S. mauritianum* presents itself as a challenging invader, and one which will require ongoing biological control efforts if we are to attain successful control.

Table 5.2 Summary of published literature pertaining to the invasion and control of *Solanum mauritianum* in South Africa. * - Knowledge areas addressed and contributed to by this dissertation.

Species	Author(s)	Overall focus
<i>Solanum mauritianum</i>	(Olckers and Hulley, 1989)	Insect herbivore diversity
	(Olckers and Hulley, 1995)	Pre-introductory surveys
	(Olckers and Zimmermann, 1991)	Biocontrol prospects
	(Olckers, 1999)	Review: Candidate agents
	(Witkowski and Garner, 2008)	Impacts of clearing efforts
	(Barboza et al., 2009)	Biocontrol prospects
	(Olckers, 2009)	Review (book chapter)
	(Klein, 2011)	Biocontrol agent review
	(Olckers, 2011)	Review: Biocontrol efforts
	(Atkinson et al., 2014)	Remote-sensing
	(Schor et al., 2015)	Land use and invasion
<i>Gargaphia decoris</i>	(Olckers, 2000)	Host-specificity
	(Olckers and Lotter, 2004)	Non-target feeding
	(Barker and Byrne, 2005)	Thermal biology (biotypes)
	(Hope and Olckers, 2011)	Non-target effects
	(Patrick and Olckers, 2014)	Abiotic conditions (sun/shade)
	(Cowie et al., 2016a)*	Ecophysiology (damage assessment)*
<i>Anthonomus santacruzi</i>	(Olckers, 2003)	Risk-assessment
	(Olckers, 2008)	Non-target feeding
	(Hakizimana and Olckers, 2013a)	Native predator impact
	(Hakizimana and Olckers, 2013b)	Crab-spider impacts (predation)
	(Cowie et al., 2016b)*	Climatic constraints*
	(Cowie et al., unpublished)*	Florivory damage (indirect effects)*

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