Masters Dissertation

Integrated management of *Solanum mauritianum* in the Highveld of Gauteng, South Africa

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DECLARATION

I, Sanele Mtetwa, 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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Signature of candidate

28th day of May 2019

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DEDICATION

To my late two grandmothers Agnes Mtetwa and Matuzana Gladys Mteto, and my late grandfather Ephraim Mnyamana Mtetwa, who played major roles in my life.

My mother (Gloria Mtetwa), thank you for your love and support.

Thank you!

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I also thank my friends for scooping laughs all the time and for reminding me that to finish was the goal.

Most importantly I am very grateful to my grandparents for loving me always, and my mother for her big sense of humour in sunny days and the dark ones too. Thank you Ma for continuing to allow me to fall and not picking me up when I'm down.

TABLE OF CONTENTS

LIST OF FIGURES

CHAPTER ONE:

CHAPTER TWO:

CHAPTER THREE:

LIST OF TABLES

CHAPTER TWO:

CHAPTER THREE:

APPENDIX:

ABSTRACT

Solanum mauritianum (Solanaceae) Scop., bugweed, is one of the most problematic invasive alien species in South Africa. Two biocontrol agents, a sap sucker *Gargaphia decoris* Drake (Tingidae) and a flower-feeder *Anthonomus santacruzi* Hustache (Anthonomini) have been released against the weed in KwaZulu-Natal. The damage of the agents to *S. mauritianum* is considered insufficient and the agents have not provided successful control of the weed since their release. Predicted limitations of establishment are mainly from predation for *G. decoris* and climate unsuitability for *A. santacruzi*. The efficacy of the two biocontrol agents on the Highveld is unknown. An alternative to biocontrol, the conventional methods of cutting and treating trees with herbicides are very expensive and labour-intensive, and if not properly implemented, they may allow resprouting to occur. *Solanum mauritianum* is reportedly easily killed by cutting followed by a herbicide treatment but the influence of stem diameter, cutting height, multiple stems and the timing of cutting had not been thoroughly evaluated, and had not yet been assessed in Gauteng. The aim of this study was to investigate the feasibility of biocontrol, and integrated control of *S. mauritianum* in the Highveld. Biocontrol experiments were conducted at the Wits nursery and Egoli Gas (shady site) in Johannesburg, and at Gerotek (sunny site) in Pretoria. *Solanum mauritianum* trees were exposed to *G. decoris* only, *A. santacruzi* only or *G. decoris* and *A. santacruzi* together. The biocontrol agents were either released on bagged/unbagged and ant-tack protected/unprotected trees. Ant-tack is a sticky compound applied on tree stems to deter insect predators. The releases were done in summer to increase the chances of agent survival. The numbers of surviving insects were counted on a monthly basis in order to assess their establishment. Integrated control experiments were conducted at Egoli Gas, Gerotek and two additional sites in Johannesburg, namely Parktown and Houghton. *Solanum mauritianum* trees that were already growing at these sites were either cut or cut and treated with the herbicide Hatchet, in spring and autumn, and also at different cutting heights. In addition, multistemmed trees in Parktown were cut and treated with herbicide to test the effect of the number of stems on the plant's response to cutting. Monthly assessments of dead or live stumps were taken in each site. The results showed that none of the biocontrol agents in any treatment and at any site (including sun versus shade) were able to survive and establish viable populations. The predator exclusion techniques were successful at keeping ants and other insect predators off the protected trees but did not have an effect on the survival and establishment of the biocontrol agents. *Anthonomus santacruzi*'s failure to establish was attributed mainly to the lack of flowers on all experimental trees during the entire study. The low numbers of *G. decoris* released may have also influenced their survival. Nonetheless, the results suggest that the two biocontrol agents are not suitable for biocontrol of *S. mauritianum* in the Highveld. For the manual control trial, the highest mortality of trees was observed on those that were cut close to ground-level and treated with Hatchet. Cutting low and not applying Hatchet was not effective at killing *S. mauritianum* and resulted in a high percentage of resprouted stumps. The cut and herbicide treatment was much more effective in killing the trees than cut without herbicide treatment. The results also showed that stem diameter size, the number of cut stems per tree and the cutting season did not have a significant effect on stump survival. However, an increase in cutting height increased the percentage of resprouting stumps, especially on trees with thicker stems. Overall, future studies should focus on releasing *A. santacruzi* in warmer and more humid conditions, and only when the trees are flowering. Secondly future research should investigate releasing high numbers of *G. decoris* in the field to increase its chances of establishment. It is clear that cutting low and immediately treating stumps with Hatchet is the most effective method for killing *S. mauritianum* trees on the Highveld. However, challenges can be expected in cases where obstacles such as rocks and logs make it difficult to cut low and ultimately higher cuts may result in resprouting.

Keywords: Agent establishment, ant-tack, biological control, cutting height, cutting season, herbicides, resprouting, stem diameter.

CHAPTER ONE

GENERAL INTRODUCTION

Problem statement

Solanum mauritianum is one of the most invasive species of the 13 exotic *Solanum* species in South Africa (Gibbs Russel *et al*. 1987). It has been in South Africa for about 135 years and is widespread in the higher rainfall region of South Africa (Olckers 1998; Olckers 1999; Henderson 2001; Henderson and Wilson 2017). It is a declared category 1b invader species, which requires mandatory control (Department of Environmental Affairs 2014).

Solanum mauritianum causes irritation of the respiratory tract and skin (Henderson 2001; ISSG 2006; ARC-PPRI 2014 b). It also forms dense stands in various environments including pastoral land, agricultural land and urban areas, creating a high canopy cover that shades and inhibits growth of indigenous plant species (Henderson 2001; ARC-PPRI 2014 b). Commercial forestry is affected when young trees cannot grow due to *S. mauritianum* shading (Olckers and Zimmermann 1991; ISSG 2006).

Two biocontrol agents for *S. mauritianum,* namely the lace bug *Gargaphia decoris* Drake (Tingidae) and the flower-feeding weevil *Anthonomus santacruzi* Hustache (Anthonomini), are available and have been released in the higher rainfall regions of KZN Province with the aim of long-term control of *S. mauritianum* (Lotter 2004; Olckers 2008). However, their efficacy and suitability in Gauteng Province is unknown, and has been studied in this research. The alternative to biocontrol is slashing and/or herbicide spraying which is expensive and damaging to non-target plants (ARC-PPRI 2014 b; Witkowski and Garner 2008). Herbicide application can be effective at reducing infestations (Hodgson 1978; Stam 1978), however this control is not always successfully implemented allowing the plant to re-sprout (Witkowski and Garner 2008). This study will investigate the feasibility of integrating chemical and mechanical control techniques to effectively control *S. mauritianum* growing in Gauteng.

Biological invasions

Biological invasions are natural phenomena that occur when species expand their natural range into new areas (Van der Velde *et al*. 2006). This occurs when the dispersal stage of species are moved from place to place, or when geological barriers are lifted over geological time thereby allowing for mixture of species that were originally from separate places (Vermeij 1991; D'Antonio and Vitousek 1992; Shigesada and Kawasaki 1997). Introduction of exotic species into a new range facilitates their escape from natural enemies in their native range, which increases their competitiveness through redirecting resources from defence to fitness, and this ultimately allows them to dominate over indigenous species in new areas (Gordon 1998; Keane and Crawley 2002; Blossey 2011). However, not all species become invasive once introduced to new areas. Invasion success is mainly determined by; (i) species traits that are suitable for survival in the new area, (ii) climatic suitability, (iii) competition and (iv) predation (Lodge 1993; Gordon 1998; Heger and Trepl 2003). Therefore once dispersed species will either colonize or die off (Shigesada and Kawasaki 1997; Mavruk and Avsar 2007). The colonizers with suitable traits and under favourable conditions will establish, reproduce and spread into new areas thus becoming invasive (Lodge 1993). Invasive species can reach self-sustaining populations especially when geographic barriers breakdown, which can enhance seed dispersal (D'Antonio and Vitousek 1992).

Although the process of invasion occurs naturally, human influence has dramatically increased the rate of invasions on a global scale (Lodge 1993; Pysek *et al*. 2002; Seebens *et al*. 2015; Hoffmann and Courchamp 2016). There is continuously increasing trade of materials over time between various regions of the world due to globalization, which has seen countless movements of species either deliberately or accidentally (D'Antonio and Vitousek 1992; Galil 2000; Seebens *et al*. 2015). It is believed that the mobile society of humans, is responsible for redistributing species to places that they could not have otherwise reached and at a pace that raises challenges and threats to ecosystems, human health and economies (Vitousek *et al*. 1996; Grice 2006; Van der Velde *et al*. 2006; Gewin 2013). This aspect of human-related invasions deserves global attention. Invasive species can have positive and negative impacts, but the negative impacts are often overwhelming and of greater concern as they can have serious environmental and economic implications. The negative impacts may include altering the frequency and/or intensity of disturbances in new areas which ultimately replaces native flora (Vitousek 1990; Gordon 1998; Pysek *et al*. 2003; Vilà *et al*. 2006; Powell *et al*. 2011). Relatively large scale impacts can include altering habitat structure, ecosystems processes and functioning, and causing greater extinctions compared to global climate change (Vitousek 1990; Witkowski 1991; D'Antonio and Vitousek 1992; Lodge 1993; Gordon 1998).

There is no shortage of examples of invasive plants that have had detrimental effects on ecosystems and biodiversity globally; examples include water hyacinth *Eichhornia crassipes* (Mart.) Solms (Brendonck *et al*. 2003; Villamagna and Murphy 2010), tickberry *Lantana camara* L. (Verbenaceae) (Mersie and Singh 1987a; Gentle and Duggin 1997; Aravind *et al*. 2010), famine weed *Parthenium hysterophorus* L. (Mersie and Singh 1987b; Tefera 2002), *Tamarix* species (D'Antonio and Vitousek 1992) and *Solanum mauritianum* Scopoli (Solanaceae) (Henderson 2001; van den Bosch *et al*. 2004; ARC-PPRI 2014 b). Overall, this calls for global and local conservation efforts to control such invasive species in the interest of mitigating their impacts.

Biological control

There are three objectives that any programme in invasive species management should strive for; prevention, eradication and control (Christoffoleti *et al*. 2007). Preventive management is the most cost-effective approach if achieved; however it is complex, often involving the understanding of processes and practices that contribute to species introduction and proliferation (Christoffoleti *et al*. 2007). In cases where invasive species are already present eradication and control methods are employed (Christoffoleti *et al*. 2007). The knowledge of economic costs of invasive species and the costs of management are key to deciding whether or not to impose management, thereafter a suitable management strategy is evaluated and selected (Maxwell and O'Donovan 2007).

There are various management strategies/techniques that are used against invasive plants in introduced areas such as biological control (hereafter referred to as biocontrol), mechanical control and integrated control, which are discussed below. Biocontrol is the use of one or more species (natural enemies) to reduce the population of another to a threshold that is considered harmless to the environment or economy (Briese 2000). Natural enemies or biocontrol agents usually have long-evolved associations with the target species from the native regions (Hokkanen and Pimentel 1984). This is a very old technique that has been actively practised for more than 100 years (Stiling and Cornelissen 2005). Sheppard *et al*. (2006) argues that it is the only tool available for long-lasting management of invasive species that become dominant in new regions in the absence of their natural enemies. In biocontrol programmes the biocontrol agents are collected from the native area of the invasive species and released against it in the introduced areas with the hope of supressing its populations and mitigating the impacts caused (Sheppard *et al*. 2006). Although specialist natural enemies have been used successfully in most biocontrol programmes (Huffaker *et al*. 1971; De Bach and Rosen 1991), the use of generalist predators to control invasive plant populations is also sometimes advocated (van Driesche and Bellows 1996; Chang and Kareiva 1999).

Over 500 biocontrol agents have been released to control more than 200 invasive species around the world (Winston *et al*. 2014). However, these exotic agents can potentially threaten populations of non-target species that are taxonomically closely related plants to the target species and thus need to be tested to avoid such risks (Howarth 1991; Marohasy 1998). Once collected from the native range of the target invasive species the biocontrol agents undergo host specificity testing (HST) which aims to predict the field host range of the agents when released in the new area (Wapshere 1974). Host specificity testing can help to avoid dramatic non-target impacts especially to economically valuable species (van Klinken 2000; Barton Browne and Withers 2002). The fundamental assessments in HST involve choice and no-choice tests including target and non-target plant species, that are conducted in cages in laboratory (quarantine) and field environments (van Klinken and Edwards 2002). Host specificity testing is very important in biocontrol because the benefits from successful agents or the impacts from wrong decisions to non-target species are very large (Briese 2005). Old predator-prey associations between the weed and potential biocontrol agents from the native range can prevent agents from being effective based on the evolution of some degree of balance, but it is argued that new associations with predators in the introduced range yields higher biocontrol success due to lack of such evolved associations (Pimentel 1963; van den Bosch and Telford 1964; Huffaker *et al*. 1971; Hokkanen and Pimentel 1989).

Countries actively involved in biocontrol have internationally recognized standards and procedures for biocontrol programmes (Zwolfer and Harris 1971; Sheppard *et al*. 2003; OECD 2004; Briese 2005; IPPC 2005), and these strict standards can cause some potential agents to be incorrectly rejected. We have also seen a paradigm shift from host specificity testing that focuses on the safety of certain economically valuable plant species, to testing for safety of all potentially threatened species, making it more difficult to release agents in some regions (Briese 2005). Nonetheless, biocontrol agent releases after HST are usually successful and safe (Pemberton 2000; Willis *et al*. 2003) but failures have been observed in cases where the non-target species had not been included in HST (McFadyen *et al*. 2003), incorrect conclusions that non-target species are not at risk (Haines *et al*. 2004), and also misinterpretation of HST results (Gassmann and Louda 2000).

Examples of successful biocontrol programmes may include; the successful biocontrol of mist flower *Ageratina riparia* (Asteraceae) in New Zealand using the white smut fungus *Entyloma ageratinae* and the gall fly *Procecidochares alani* (Barton *et al*. 2007), more than 50 years of outstanding biocontrol of *Opuntia ficus-indica* (Cactaceae) populations in South Africa by the cactus moth *Cactoblastis cactorum* (Bergroth) (Zimmermann and Moran 1991), the significant reduction of the reproductive potential and vegetative growth of *Acacia longifolia* (Andrews) Willd resulting from damage caused by the pteromalid gall wasp *Trichilogaster acaciaelongiliae* (Pteromalidae) in South Africa (Dennill 1987a) and local extinctions of the red waterfern *Azolla filiculoides* Lamark (Azollaceae) observed in South African aquatic ecosystems due to feeding damage by the agent *Stenopelmus rufinasus* Gyllenhal (Coleoptera: Curculionidae) (McConnachie *et al*. 2004).

The initial establishment of biocontrol agents is important for a successful biocontrol project (Grevstad *et al*. 2011). The establishment of biocontrol agents in the field is not always guaranteed, the overall chance of establishment is influenced by factors that put agent populations at risk of extinction such as; the size and number of releases, developmental stage of plants and their agents, timing of releases, environmental variability, competition with other insects and predation of agents (Holt 1977; Leigh 1981; Ehler and Hall 1982; Grevstad 1999; Shea and Possingham 2000; Fisher *et al*. 2007). For example, climatic conditions may hinder biocontrol agent establishment especially when a weed occupies climatic zones that are not optimal for the agent's survival, reproduction and development (Wapshere 1985; Byrne *et al*. 2002; Fowler *et al*. 2008). Byrne *et al*. (2002) showed how lower humidity in the area of introduction causes stress to egg development of a biocontrol agent, the tortoise beetle *Gratiana spadicea* (Klug) (Coleoptera: Chrysomelidae). Another important factor is finding the optimal release strategy for successful establishment of biocontrol agents (Grevstad *et al*. 2011). Historically, less than 65% of biocontrol agents released against invasive plants were successfully established in the field (Julien *et al*. 1984); this called for an improvement in release strategies (Memmott *et al*. 1996).

There is evidence that the probability of agent establishment is higher with (i) a larger number of individuals per release and (ii) a greater total number of releases (Beirne 1975; Hall and Ehler 1979; Beirne 1985; Pimm and Redfearn 1988; Hopper and Roush 1993; Memmot *et al*. 1998). However, smaller releases are more vulnerable to variable environmental conditions and may perform poorly and ultimately become extinct (Fowler *et al*. 2008). Finding an optimal release strategy can be very difficult especially given that biocontrol practitioners often have limited numbers of agents that they hope to get established in as many sites as possible and as quickly as possible (Memmott *et al*. 1996; Grevstad 1999). Therefore decisions must be made on how to divide the releases based on available agents. Grevstad (1999) suggests that when environmental variability is high the probability of establishment is more likely with frequent small releases instead of a few large releases. Larger releases should be considered when there are a few established sites or when small releases have a lower probability of establishing, for example, when the conditions are not favourable (Shea and Possingham 2000; Memmott *et al*. 1996; Grevstad 1999). The establishment of biocontrol agents can also be improved by protecting them from predators during which period they build up their numbers (Holt 1977). Protected environments, such as cages, have been used successfully to improve agent establishment by protecting the initial low insect densities from factors that may be harmful (Fowler *et al*. 2008).

Furthermore, a frequently asked question in biocontrol programmes is the use of multiple species as agents against a weed (Stiling and Cornelissen 2005). The combination of two or more agents can generate (i) (positive) synergistic or additive effects, or (ii) (negative) inhibitory effects on agent performance compared to the individual effect of each agent separately (Hatcher and Paul 2001; Stiling and Cornelissen 2005). It is argued that multiple-species are more effective than single species releases and can improve biocontrol success (Colfer and Rosenheim 2001; Denoth *et al*. 2002). Multiple agent releases increase the chances of releasing the most effective agent (lottery model), cumulative effect on the target plant (cumulative model) (Denoth *et al*. 2002) or optimized weed control by assisting with counter pressure exerted by only one agent. Moreover, it is important to identify and select fewer but more effective agents for success in biocontrol of invasive species.

Altogether, biocontrol has generated great success in the control of invasive species and this is highlighted in a review of biocontrol literature in two major journals, *Biological Control* and *Environmental Entomology* showing that for the past decade biocontrol agents of alien invasive weeds have significantly reduced weed biomass (−82.0%), as well as flower (−98.9%), and seed production (−89.4%) (Stiling and Cornelissen 2005). Biocontrol stands as a tried and tested technique to control invasive species as it is less costly than conventional methods such as cutting and herbicide treatment (Andres 1977; Marsden *et al*. 1980); it is also environmentally friendly and proves to be sustainable over long periods provided that thorough research is done. Nevertheless, conventional methods such as mechanical control are also applicable in the control of invasive species and continue to be recommended and used.

Mechanical control

Mechanical control focuses on physical damage to plants in order to kill them or control their growth. This approach has been around for a very long time and was initially used mostly in farming (Cloutier *et al*. 2007). It involves several techniques such as removing whole plants from the soil, cutting their stems, leaves and roots, chaining or bull-dozing (Paynter and Flanagan 2004). Cutting is one of the most common techniques and can result in slower growth rates and reduced seed production in invasive plants (Cloutier *et al*. 2007). However, the emergence of herbicides in mid-20th century led to decreased reliance on mechanical control (Cloutier *et al*. 2007). Chemical control, on the other hand, involves the application of herbicides on plants by several methods such as aerial spraying, hand spraying or brush painting on cut stems (Paynter and Flanagan 2004; Goodall and Witkowski 2014).

It is reported that chemical and mechanical control techniques may be adequate for controlling small satellite weed infestations, however for larger infestations that may cover thousands of hectares, less labour-intensive techniques such as biocontrol, may be necessary (Moody and Mack 1988; Paynter and Flanagan 2004). Mechanical control has limitations that have been highlighted in clearing efforts where cutting and pulling of weeds have resulted in more vigorous regrowth (Heisel *et al*. 2001). This is particularly undesirable as it adds to the problem rather than solving it. In some instances cutting has been effective when carefully done, for example, cutting as close as possible to the ground has resulted in effective control of *Chenopodium album* L. and *Sinapis arvensis* L. (Heisel *et al*. 2001). In addition to cutting height, the effectiveness of cutting may also be affected by the biology of the plant, stem diameter, development stage of the plants, timing of cutting (seasonality) or environmental conditions. All in all, individual control techniques such as cutting may rarely be sufficient; rather a combination of techniques may be better for effective control (Cloutier *et al*. 2007). Further research has focused on the combination of various control techniques with the aim of successfully controlling weed populations across different environmental situations.

Integrated control

Given that challenges may arise in all control options, some research has focused on modelling the consequences such as integrating the control techniques (Paynter and Flanagan

2004; Chikowo *et al*. 2009; Lamichhane *et al*. 2017). Integrated weed management is a sustainable control strategy that combines different techniques such as biological, cultural, chemical or mechanical control in a way that maximizes their effectiveness while minimizing economic, health and environmental harm (Buhler 2002; Paynter and Flanagan 2004). So the question arises, could integrated control methods be more effective compared to each method separately? Integrated control should be thoroughly investigated and implemented in clearing efforts for successful outcomes.

The target weed: *Solanum mauritianum*

Solanum mauritianum biology and invasiveness

Solanum mauritianum is a perennial shrub that originates in South America, and is common particularly in northern Argentina, southern Brazil, Uruguay and Paraguay (Roe 1972). It is also known as *S. mauritianum*, a shrub that grows to heights of 2 – 4m (ISSG 2006). *Solanum mauritianum* is evergreen and known to produce flowers as early as the first year of growth (ARC-PPRI 2014 b). The stems, leaves and flower stalks of the plant are densely covered with white hairs (Kissmann and Groth 1997; Henderson 2001; PIER 2005; ISSG 2006) (Figure 1). The leaves are soft and fairly long, reaching lengths of 250mm at maturity, but can grow longer under shade (ARC-PPRI 2014). The upper surface of the leaf is dull green and velvety while the bottom surface is white with a felt-like texture (Klein 2011). The flowers are purple to lilac in colour and give rise to a cluster of green berries that are 10mm in diameter and turn yellow during ripening (ARC-PPRI 2014).

Introduced *Solanum* species in South Africa and Australia, including *S. mauritianum*, are all found to have large infestations in disturbed areas (Symon 1981). *Solanum mauritianum* invades various environments including pastoral land, forest margins, riverine areas, forestry plantations, conservation areas, agricultural land, urban areas and along roadsides (Olckers and Zimmerman 1991; Henderson 2001; ARC-PPRI 2014 b). *Solanum maurtianum* was introduced as an ornamental plant, and has since spread and become invasive (Oatley 1984; Pooley 1993; ARC-PPRI 2014 b). In addition, frugivorous birds seem to have a higher preference for *S. mauritianum* over the indigenous plant species (Olckers 1999). *Solanum mauritianum* is unpalatable to some animals and the unripe berries are also toxic, as such it is generally avoided by grazing animals, both domestic and wild (ISSG 2006).

The widespread distribution of *S. mauritianum* outside of its native range has been due to human influence and natural mechanisms (Roe 1972; ISSG 2006). *Solanum mauritianum* has become naturalized in Australia, India, and the Pacific, Indian, and Atlantic islands probably through the Portuguese trade routes during the 16th Century (Roe 1972; PIER 2005; ISSG 2006). There are 51 *Solanum* species in South Africa, 13 of which are exotic. *Solanum mauritianum* is one of the most invasive species in the genus (Gibbs Russel *et al*. 1987). It is a declared weed in South Africa and other neighbouring countries including Swaziland, Mozambique and Zimbabwe and also further north in western, central and eastern tropical Africa (Hepper and Jaeger 1986). In South Africa it occurs in all provinces except Northern Cape (ARC-PPRI 2014 b) (Figure 1). It has spread extensively in the higher rainfall region of South Africa (Olckers 1998; Olckers 1999; Henderson 2001).

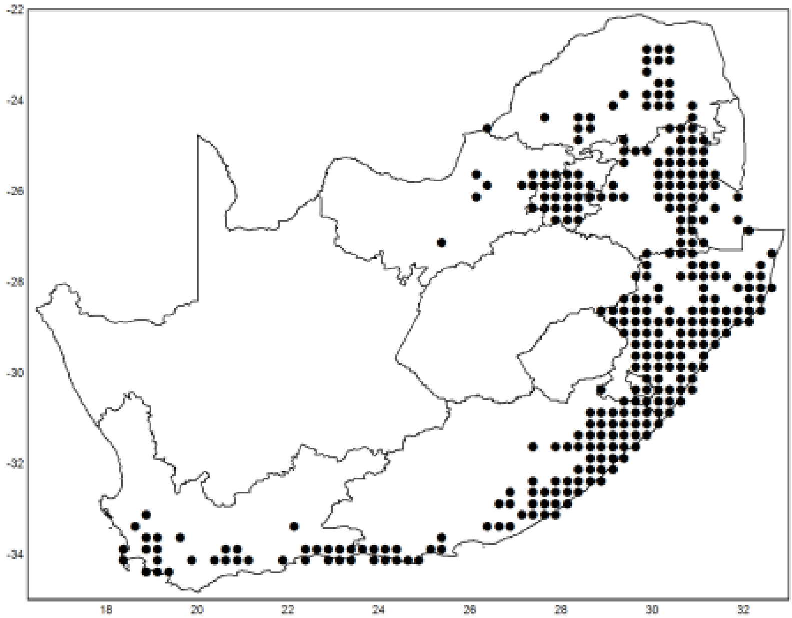

Figure 1: The recorded distribution of *Solanum mauritianum* in South Africa. (www.arc.agric.za).

Solanum mauritianum is highly invasive. According to the National Environmental Management: Biodiversity Act of 2014 (NEMBA), it is regarded as a category 1b invader (Department of Environmental Affairs 2014). All property owners are therefore legally obliged to list any of the designated invasive alien species (IAS) present and remove them before sale of the property (Shackleton and Shackleton 2016). This means that *S. mauritianum* requires mandatory control. Category 1b listed invasive species be must be controlled in compliance with sections 75(1), (2) and (3) of the Act (Department of

Environmental Affairs 2014). Failure to remove declared IAS will result in the cost of removal being subtracted from the property selling price (Shackleton and Shackleton 2016).

Solanum mauritianum reproduction

Solanum mauritianum primarily reproduces by seeds (Witkowski and Garner 2008; Olckers 2009; Goodall *et al*. 2017). The fruit contains many seeds (±150) with an average viability of 98% (Campbell and Staden 1994; Witkowski and Garner 2008; ARC-PPRI 2014 b). Frugivorous birds are responsible for dispersing *S. mauritianum* seeds over relatively long distances (Oatley 1984; Olckers 1999; Pistorius 2005; ARC-PPRI 2014 b). The fruits and flowers are produced all year round (Olckers 2009). The seeds have a dormancy of ≥ 2 years (Oatley 1984; Witkowski and Garner 2008) and seed germination is triggered by fire, floods, and clearing operations, and plant growth is rapid (Olckers 2009). *Solanum mauritianum* is also capable of self-pollination and thus isolated plants can form new populations fairly rapidly (Rambuda and Johnson 2004). Self-pollination is also facilitated by one of its biocontrol agents, a flower-bud feeding weevil *Anthonomus santacruzi* Hustache (Coleoptera: Curculionidae) (Cowie *et al.* 2017). The plants can recover from disturbances by vegetative means following being cut back, especially in the absence of follow-up treatment or correct treatment, i.e. applying herbicides to all stumps, within a few minutes of being cut and mixing Hatchet with water as recommended (Olckers 2009; BPG). In summary, the life history and ecology of *S. mauritianum* is responsible for the high invasive potential observed (Olckers and Zimmermann 1991; ISSG 2006; Witkowski and Garner 2008).

Solanum mauritianum impacts

Solanum mauritianum also forms monospecific stands that replace the native flora and ultimately cause changes in the ecosystem structure and functioning, and is thus regarded as a transformer species (Henderson 2001). In forestry plantations it out-competes young trees, particularly pine and black wattle, and ultimately inhibits their growth (Olckers and Zimmermann 1991; ISSG 2006). During uncontrolled fires in commercial forestry *S. mauritianum* produces undesirable fuel loads below the canopy and this in turn increases the costs for fire protection (Atkinson *et al.* 2014). The white hairs on the leaves and stem have negative impacts to humans, causing irritation of the respiratory tract and skin (Henderson 2001; ISSG 2006; ARC-PPRI 2014 b).

Biocontrol of *Solanum mauritianum*

Biocontrol of *S. mauritianum* in South Africa was first implemented in 1984 (Olckers and Zimmermann 1991) and numerous insect agents have been tested but rejected for release because of the potential for attack on non-target *Solanum* species in the field (Olckers 1999; Klein 2011). Only two biocontrol agents have been cleared for release in South Africa following rigorous host specificity tests (Olckers 2000; Olckers 2003). The two agents are the lace bug *Gargaphia decoris* Drake (Hemiptera: Tingidae) and the flower-feeding weevil *Anthonomus santacruzi* (Olckers 2000; Olckers 2003).

Gargaphia decoris

Gargaphia decoris is a pale brown bug that grows to an adult of about 5mm long (Figure 2) (Olckers 2000). The adults can fly and are easily identified by the black bars on their wings (Olckers 2000). The adults can live for 2 – 3 months and the females lay eggs in batches of variable size, ranging from 34 – 876 eggs (Olckers 2000). The eggs are laid on the under-surface of the leaf and females will guard them until they hatch and thereafter will guard the nymphs, which will also feed and develop to adults, and the life cycle proceeds (Olckers 2000). The eggs hatch after 9 – 14 days and undergo five nymphal instars lasting 17 – 19 days in total before moulting into adults, therefore egg to adult development takes 26 – 33 days (Olckers 2000). There are several generations during the year (Olckers 2000). Both adults and nymphs feed on the plant sap on the under-surface of the leaf and extensive feeding causes the leaves to turn dry and yellow and ultimately fall before maturity (Olckers 2000; ARC-PPRI 2015; Cowie *et al*. 2016a). *Gargaphia decoris* was imported from Argentina in 1995 (Olckers 2000). Host specificity tests showed that it has strong feeding and oviposition preference for *S. mauritianum* over other *Solanum* species in South Africa (Olckers 2000). Moreover, it showed no potential to attack non-target *Solanum* species in the field (Olckers 2000). Reduced use of conventional methods and their integration with biocontrol was enough to convince the authorities, and as a result *G. decoris* was cleared for release in South Africa in February 1999 (Olckers 1999; Olckers 2000).

Figure 2: Adults of the sap-sucking lace bug**,** *Gargaphia decoris*. (www.arc.agric.za).

Despite the high expectations of *G. decoris* to cause extensive damage to *S. mauritianum*, this has not often been observed in the field (Lotter 2004). The insect has failed to establish in most release sites in KwaZulu-Natal Province, and it is suggested that this could be due to general predators and adverse climatic conditions, such as low humidity (Lotter 2004; Cowie 2014). The insect populations were found to be highest at the end of summer and autumn, during which time the insects can cause damage to *S. mauritianum* foliage (Lotter 2004). Nevertheless, the populations drop radically during winter and this leads to less stress on the plants during the next growing season (Lotter 2004). So far, the moderate level of damage by *G. decoris* recorded on *S. mauritianum* in the KwaZulu-Natal region is not sufficient to effectively slow the growth rate and reduce seed production (Lotter 2004). Further research has showed that feeding by *G. decoris* causes a reduction in tree height, changes biomass allocation in the plant and also causes a reduction in photosynthetic rates due to removal of chlorophyll, and this might allow clearing to be done easily and cheaply (Cowie 2014; ARC-PPRI 2015; Cowie *et al.* 2016a**)**. The feeding damage inflicted by *G. decoris* on *S. mauritianum* in the Highveld region, of the Gauteng Province, is still largely unknown.

Anthonomus santacruzi

Anthonomus santacruzi occurs across the native range of *S. mauritianum* in South America (Olckers 1999). The adults are black weevils that grow to 2 – 3mm long, easily identifiable by their elongated snout (Hakizimana 2011) (Figure 3). The full life cycle of the insect can take 15 to 25 days (ARC-PPRI 2014 a). The females can live for over three months, during which time they lay tiny eggs (0.3mm) that attach to the sides of anthers of both immature and mature buds (Olckers 1999; ARC-PPRI 2014 a). A flower bud can house 1 – 3 larvae which feed and develop inside the bud for 10 – 18 days (Olckers 1999; Olckers 2003; Hakizimana 2011). This stage is followed by pupation which takes an additional 4 – 10 days (Olckers 1999). The larvae cause the most damage to the plant as they ultimately feed on the entire contents of the bud including the anthers and petals (Olckers 1999; Olckers 2003; Hakizimana 2011). The emerging adults will chew on the bud on their way out (Olckers 1999; Olckers 2003; Barboza *et al*. 2009). Adults also feed on the stamens of flowers, however in the absence of flowers they can eat apical leaflets and shoot tips thus causing further damage to the plant (Olckers 2003). Therefore, one of the reasons for the high expectations of success with *A. santacruzi* is because of its ability to reduce overall reproductive output and therefore reduce further spread of *S. mauritianum* by seed dispersal (Olckers 2003; Cowie 2014; Cowie *et al*. 2017).

Figure 3: *Anthonomus santacruzi* adult weevil. (www.arc.agric.za).

Anthonomus santacruzi was released in South Africa in 2008 following host specificity testing in quarantine that showed consistent high feeding and oviposition preference on *S. mauritianum* compared to other *Solanum* species (Olckers 2003). Ambiguous host specificity results for *A. santacruzi* suggested an expanded host range of the biocontrol agent, when in fact only two non-target species namely *Solanum cf. linnaeanum* (Hepper and Jaeger) and *Solanum tomentosum* L. were likely to be utilized (Olckers 1998; Olckers 1999; Hakizimana and Olckers 2013a). Thousands of weevils were released in 2008 in KZN and the agent continues to achieve establishment and an extended distribution (Olckers 2003; Olckers 2008; Hakizimana and Olckers 2013a). Nevertheless, despite the progress seen in KwaZulu-Natal, there has been no study done on the efficacy and suitability of *A. santacruzi* in the Highveld region of Gauteng Province. So, ultimately this study will investigate the damage caused to *S. mauritianum* trees by (a) *G. decoris* only, (b) *A. santacruzi* only, and (c) both agents combined, in Gauteng.

Integrated control of *Solanum mauritianum*

The Working for Water (WFW) Programme of the Department of Environmental Affairs has made efforts to control invasive alien species (AIPs) in South Africa mainly by clearing the plants in areas within 30m of rivers (Van Wilgen *et al*. 1998). The programme has been running since 1995 (Marais *et al*. 2004). Plant clearing by WfW involves both cutting stems and applying chemicals to kill plants and ultimately reduce infestations (Beater *et al*. 2008; Morris *et al*. 2008; Witkowski and Garner 2008). The combination of the two methods, namely cutting plant stems and treating their stumps with herbicide is an integrated approach, which combines mechanical and chemical control techniques. Nevertheless, these methods have proven to be costly and labour-intensive (ARC-PPRI 2014 b; Witkowski and Garner 2008).

Previous research has shown that *S. mauritianum* is easily killed by cutting their stems and applying herbicide (Beard 1951; Pickworth 1971; Hodgson 1978; Vermeulen and Grobler 1987). Challenges have been observed however, including rapid seedling and coppice growth, and the labour-intensive duty of cutting trees to an ideal height for spraying with herbicides (Paynter and Flanagan 2004). The highest tree mortality has been observed when herbicides are applied by paint brush on cut stumps compared to other methods such as treating frills, or angle cuts around the stem into the cambium, with herbicides (Hogson 1978; Stam 1978). *Solanum mauritianum* is also capable of coppice growth from the stump following cutting (Witkowski and Garner 2008). However, it is suggested that *S. mauritianum* is relatively easily killed by cutting and immediately applying the appropriate herbicide to the cut stump and thus high proportions of re-sprouting found in the field may be due to herbicides not being applied to all cut stumps or being incorrectly applied (Witkowski and Garner 2008). Moreover, cutting low on the stem has resulted in high mortality compared to cutting higher (Witkowski and Garner 2008). Lastly, the integrated control of *S. mauritianum* is less studied than its biocontrol and needs to be explored to generate new insights and better ways of controlling this weed in numerous other South African environments, including the Highveld.

Aim and Objectives

This study aims to investigate the feasibility of biocontrol, and integrated weed management, using chemical and mechanical methods, to control *Solanum mauritianum* growing in Gauteng.

Objectives

1. To investigate the survival of *Gargaphia decoris* and *Anthonomus santacruzi* in the Highveld, by comparing release methods.
2. To assess and compare the impact of mechanical control (cutting) and integrated control, i.e mechanical and chemical control combined, on *Solanum mauritianum* survival and re-growth.

CHAPTER TWO

Establishment of *Gargaphia decoris* and *Anthonomus santacruzi* in the Highveld

Abstract

Solanum mauritianum, bugweed, is one of the most invasive alien plant species in South Africa. Since 1984, two biocontrol agents, a sap sucker *Gargaphia decoris* and a flower-feeder *Anthonomus santacruzi* have been released against the weed in KwaZulu-Natal. *Gargaphia decoris* was first released in 1999 and *A. santacruzi* was released in 2009. These agents have so far not provided successful control of the weed since their release. The efficacy of the two biocontrol agents in the Highveld is unknown. Predicted limitations on their establishment in the Highveld of Gauteng are mainly predation for *G. decoris* and climate suitability for *A. santacruzi*. Predator exclusion for *G. decoris* and the use of more suitable microhabitats for *A. santacruzi* can increase their chances of establishment. This study aimed to investigate the establishment of the two biocontrol agents in the Highveld. The experiments were conducted at the Wits nursery and Egoli Gas (shady site) in Johannesburg, and at Gerotek (sunny site) in Pretoria. *Solanum mauritianum* trees were exposed to either *G. decoris* only, *A. santacruzi* only or *G. decoris* and *A. santacruzi* together. The biocontrol agents were either released on bagged/unbagged and ant-tack protected/unprotected trees. Ant-tack is a sticky compound applied on tree stems to deter insect predators. The releases were done in summer to increase chances of survival. The numbers of surviving insects were counted on a monthly basis in order to assess establishment. None of the biocontrol agents in all treatments and at all sites (sun versus shade) were able to persist, produce progeny or establish viable populations. The predator exclusion techniques were successful at keeping ants and other insect predators off the protected trees but did not have a significant effect on the survival and establishment of the biocontrol agents. The *A. santacruzi* failure to establish was attributed mainly on the lack of flowers on all experimental trees during the entire study. The low numbers of *G. decoris* released may have also reduced their chances of survival. The results suggest that the two biocontrol agents are not suitable for biocontrol of *S. mauritianum* in the Highveld. Future studies should focus on releasing *A. santacruzi* in warmer and higher rainfall periods when

trees are flowering, and releasing high numbers of *G. decoris* under similar climatic conditions to increase its chances of establishment.

Keywords: Agent establishment, ant-tack, bagging, biological control, predation, sun versus shade.

Introduction

The establishment of biocontrol agents is a very important phase of a biocontrol programme but is often the most difficult aspect to predict during pre-release studies (Crawley 1986; Ehrlich 1986; Simberloff 1989; Carvalheiro *et al*. 2008; Marchante *et al*. 2017). There are various factors that influence biocontrol agents' establishment and if not considered they could lead to failure of the biocontrol programme at this stage (Hall and Ehler 1979; Lawton 1990). Such factors may include climate suitability which refers to the match between the biocontrol agent and the new area where the target weed occurs, and a mismatch increases the chances of failure to establish (Stiling 1993; Grevstad 1999; Dhileepan *et al*. 2005; Hoelmer and Kirk 2005; Hughes *et al*. 2009).

Failed agent establishment could be as a result of climate compromising the agent's reproduction and development (Stiling 1993; Grevstad 1999; Dhileepan *et al*. 2005). On the contrary, a climatic match enhances the chances of establishment (Grevstad 1999; Byrne *et al*. 2004). For example, the arthropod biocontrol agents of water hyacinth *Eichhornia crassipes* (Mart.) Solms have established on water hyacinth throughout a wide range of climatic conditions in South Africa because of niche overlap between the weed and the agents (Hill and Olckers 2000; Smit *et al*. 2014). On the cool Highveld the development of the agents is slowed down, and they become dormant for five months and have low reproductive output, which results in limited impact to the target weed (Hill and Olckers 2000). In Australia the ragwort flea-beetle *Longitarsus flavicornis* (Stephens) (Coleoptera: Chrysomelidae) has established and caused significant damage to ragwort *Senecio jacobaea* L. in Tasmania which has variable rainfall (Ireson *et al*. 2000) but showed slow spread and limited impact to ragwort in high rainfall areas (McLaren *et al*. 2000). The two leaf-mining beetles against *Lantana camara* L. (Verbenaceae), namely *Octoma scabripennis* Guérin-Méneville (Coleoptera: Chrysomelidae) and *Uroplata girardi* Pic (Coleoptera: Chrysomelidae) are thought to be limited by climate (Cilliers 1987; Baars and Neser 1999). In South Africa the biocontrol of *Solanum mauritianum* has also seen variable degrees of establishment and damage to the target weed (Lotter 2004; Klein 2011; Olckers 2011; Patrick and Olckers 2014).

Since 1984 two biocontrol agents namely *Anthonomus santacruzi* and *Gargaphia decoris*, have been released against *S. mauritianum* in South Africa (Olckers 1999; Olckers 2009). *Anthonomus santacruzi* feeds mainly on the floral parts and has the potential to reduce the reproductive capacity and dispersal of *S. mauritianum* (Olckers 2003, Olckers 2008, Cowie *et al*. 2017), while *G. decoris* feeds on the plant sap from the leaves and extensive feeding causes chlorosis and reduction of photosynthesis (Cowie *et al*. 2016a). Several years after release, post-release evaluations showed that *A. santacruzi* was widely established in the higher rainfall regions in KwaZulu-Natal but its impact on Solanum mauritianum in the province remains 'unknown' (Klein 2011). Predation was initially predicted to limit the establishment of *A. santacruzi*; however further research showed that this was not true (Hakizimana and Olckers 2013a; Hakizimana and Olckers 2013b). Instead climatic constrains are likely to drive the establishment of the weevil in South Africa (Cowie *et al*. 2016b). Cowie *et al*. (2016b) suggested that *A. santacruzi* reproduction and development are constrained by low temperatures (<˚4C) and low relative humidity (<50%), which are typical climatic conditions in the Highveld. Singh and Olckers (2017) added that inland altitudes of above 1000m would not be suitable for the establishment of *A. santacruzi*. Although the predictions that *A. santacruzi* will not establish in the Highveld are based on laboratory data; this prediction was virtually never tested in field trials. Hence the current study was aimed at testing this laboratory prediction. Perhaps suitable microclimatic conditions may allow for establishment, for example under more sheltered, and shaded conditions.

The predation of biocontrol agents can also affect their establishment. Ants and spiders in particular are often blamed for predation of biocontrol agents which affects establishment (Day *et al*. 2004). For example, the larvae of the geometrid *Macaria pallidata* Warren (Lepidoptera: Geometridae), biocontrol agent for *Mimosa pigra* L. (Fabaceae), were found to be significantly reduced by ants in the laboratory and field trials that excluded ants resulted in increased larval survival (Grace 2005). Also, the exclusion of bird predators by using netting caused increased larval survival in this species (Grace 2005). The use of exclusion cages is another release method that has been successful at reducing predation in biocontrol agents such as *Crioceris* sp. (Reilly *et al*. 2004). There is no one method that excludes predation for all biocontrol agents, therefore trials to determine the most effective method for different biocontrol agents should be conducted (Day *et al*. 2004).

Gargaphia decoris was first released in 1999 in KwaZulu-Natal and has since been released in two more provinces, namely Mpumalanga and Limpopo (Olckers 2011). Future releases are encouraged at such low altitude, warmer climatic regions (Cowie *et al*. 2018); however the releases in inland areas such as the Highveld are equally as important to investigate the efficacy of *G. decoris*. *Gargaphia decoris* failed establishments in the Highveld were initially attributed to climate unsuitability, but Barker and Byrne (2005) disproved this. However, the indirect effects of low winter temperatures, i.e. reduction in plant quality via reduction in photosynthesis, poses a threat to the immature stages of *G. decoris* because their development is slowed down which makes them more susceptible to predation (Lotter 2004). Failed *G. decoris* establishment has been attributed to predation of eggs, nymphs and adults by generalist predators such as mirids, coccinellids and formicids (Lotter 2004; Olckers 2009; Olckers 2011; Patrick and Olckers 2014). Predation of released *G. decoris* needs thorough investigation, particularly the effects of predation in different habitats (Patrick and Olckers 2014). Given that *G. decoris* has the potential to severely damage *S. mauritianum* under sun and shade conditions, especially during outbreaks (Witt 2007; Cowie *et al*. 2016a; Patrick and Olckers 2014), further research on release techniques that will enhance establishment in the field is very important.

Finding an optimal release strategy is crucial to the establishment of biocontrol agents. This refers to carefully planning releases from available biocontrol agents in order to maximise the number of agents that survive and establish viable populations in the field (Grevstad 1999; Shea and Possingham 2000). Some biocontrol agents may require a single large release to establish successfully while with others, smaller and frequent releases may lead to establishment (Day *et al*. 2004). Memmott *et al*. (1998) found that a large number of small releases of <100 gorse thrips *Sericothrips staphylinus* Haliday (Thysanoptera: Thripidae) allowed successful establishment (*ca*. 83%) in the field compared to 1000 thrips which were initially suggested. However, release size has been found to be positively correlated with establishment of the agent *Laricobius nigrinus* Fender (Coleoptera: Derodontidae) released against the hemlock woolly adelgid *Adelges tsugae* Annand (Hemiptera: Adelgidae) (Mausel *et al*. 2010) and the parasitoid *Aphytis melinus* Debach (Hymenoptera: Aphilinidae) against the scale insect *Aonidiella aurantii* (Maskell) (Hemiptera: Diaspididae) (Campbell 1976).

More than a million *G. decoris* individuals have been released in South Africa with establishment confirmed in many sites in the low altitude areas (Olckers 2011); however *G. decoris* remains an ineffective agent because of its reported 'trivial' damage to *S. mauritianum* (Klein 2011). It is also suggested that fewer releases with higher numbers of *G. decoris* (*ca*. 1000) are more likely to establish compared to more frequent smaller releases (Lotter 2004; Patrick and Olckers 2014), especially when biocontrol agents are protected from predation by applying sticky barriers on *S. mauritianum* stems (Lotter 2004). In this study different release methods were tested to see which one is more effective for the establishment of the two biocontrol agents *G. decoris* and *A. santacruzi* in the Highveld.

In addition to enhancing the establishment of biocontrol agents, the method of releasing multiple agents is not new in biocontrol programmes and has been considered to control invasive plants (Klein 2011; Weyl 2011). Releasing multiple agents can produce different outcomes compared to single agent releases, which either could be detrimental or beneficial to a programme (Hatcher 1995; Stephens *et al*. 2013). Multiple agent release can result in competitive interactions that affect the reproduction and survival of one or more agents, as observed with the two leaf-feeding biocontrol agents for Lantana, *Uroplata girardi* Pic (Coleoptera: Chrysomelidae) and *Ophiomyia camarae* Spencer (Diptera: Agromyzidae) (April *et al*. 2011). April *et al*. (2011) showed that *Ophiomyia camarae* females laid more eggs on clean leaves compared to *U. girardi*-infested leaves, while *U. girardi* showed indistinguishable oviposition preference between clean and *O. camarae*-infested leaves. This oviposition preference means that *O. camarae* can avoid unfavourable interactions with *U. girardi* larvae and this ultimately enhances its development and survival (April *et al*. 2011). On the other hand, synergistic interactions between biocontrol agents are sought because they can improve the overall efficacy of control, in particularly when agents have different feeding strategies (Stiling and Cornelissen 2005; Turner *et al*. 2010; April *et al*. 2011; Weyl 2011; Buccellato *et al*. 2012; Stephens *et al*. 2013). There are various examples of successful biocontrol of weeds from the combined effect of two or more biocontrol agents (Hoffmann and Moran 1998; Hoffmann and Moran 1999; Moran 2005), but single agents can also be very effective (Denoth *et al*. 2002; Crowe and Bourchier 2006). Following this logic, the combination of *A. santacruzi* and *G. decoris*, which feed on flower structures and leaves respectively, has the potential to accelerate damage on *S. mauritianum* if both agents can establish successfully (Cowie *et al*. 2018). Hence, the current study investigated the establishment of the two agents in both single and combined treatments under field conditions.

It was hypothesized that *G. decoris* would establish better than *A. santacruzi*, not only because of higher numbers released, but also because they can tolerate lower humidity unlike *A. santacruzi* which is known to be constrained by low relative humidity (Cowie *et al*. 2017). It was also hypothesized that biocontrol agent numbers will be higher on trees that are protected by 'ant-tack' compared to unprotected trees, especially when bagged in their release; hence predators would be prevented from killing the biocontrol agents. It was also hypothesized that *G. decoris* populations would be the same on *G. decoris* only and *G. decoris* together with *A. santacruzi* trees, because the two agents do not compete for the same resources and therefore would not hinder each other's growth.

Methods and Materials

Study sites

Two biocontrol agents, *G. decoris* and *A. santacruzi* were, released at (1) the Wits University nursery and (2) Egoli Gas in Johannesburg, and (3) Gerotek Test Facilities in Pretoria. Wits University and Egoli Gas are in the Johannesburg Municipality and Gerotek is in Tshwane Municipality (Appendix). The Wits University nursery is located in Braamfontein (S26.1905; E28.0303) while Egoli Gas (S26.1882; E28.0183) is situated in Anne Rd, Cottesloe, Johannesburg. Both Johannesburg sites are characterized by relatively warm summers with mean temperatures of 21°C, during which rainfall is restricted, and cooler winters with mean temperatures of 10°C. Egoli Gas is located in Egoli Granite Grassland vegetation type typically defined by low hills dominated by heavily utilized and degraded grassland with some woody species occurring in scattered groups or solitary trees (Mucina *et al*. 2006) (Appendix). Egoli Gas site is an old industrial space that is highly disturbed by alien plants. It was originally used to produce coal gas but currently used as a gas storage site. It is a shady site dominated by Eucalyptus trees that shade *S. mauritianum* trees mostly growing below the canopy. Gerotek is located in Church Street Extension (S25.7588; E28.0070), Pretoria West, Gauteng Province. Gerotek is located in Moot Plains Bushveld vegetation type characterized by Acacia dominated thorny savanna and woodlands with varying heights and density (Rutherford *et al*. 2006) (Appendix). Pretoria experiences summer rainfall with dry winters and frequent frost occurrence (Rutherford *et al*. 2006). However Joburg has more frost than Pretoria but the Eucalyptus trees at Egoli Gas might be a better frost protector than out in the open. The mean temperature in Pretoria is 24°C in summer and 12°C in winter. Virtually all *S. mauritianum* trees used at Gerotek were located on the edges of a woodland, in a sunny site. The Gerotek site was predicted as being more likely to be suitable for the agents to establish compared to Egoli Gas because it is slightly warmer. The Highveld has relatively harsh winters with frost, therefore the summer season is the best time to release these biocontrol agents which are more likely to establish, grow in numbers and find suitable microsites in which to overwinter. However, low relative humidity on the Highveld, particularly during the very dry winter, might still hinder establishment (Cowie *et al*. 2018).

<u>Insect rearing</u>

1. *Solanum mauritianum* saplings less than 1m tall were transplanted in July 2016 from around the Wits greenhouse and grown in pots at the Wits nursery until they were about 1m tall and used for rearing *G. decoris* at the Wits Insectary. Saplings were regularly sprayed with water to remove predators such as spiders and ants. The saplings were kept in (n=5) cages of size (50(L)x50(W)x100(H)cm) with Thrip-mesh to protect them from predatory animals like birds. A sticky substance called Debello, referred to here as 'ant-tack', was applied to the stem of each seedling to prevent ants and other predatory insects from walking up the stems to feed on the biocontrol agents. Saplings were watered every two days, paying careful attention not to create pools of water which may drown *G. decoris*. Regular checks for predatory insects were conducted and if found were removed by hand. New generations of *G. decoris* nymphs were transferred to fresh saplings (five per time) in separate cages (n=5) in order to keep the culture growing. Furthermore, additional *G. decoris* insects were collected outside the Pistols Saloon and Wild Museum near Ramsgate South in KwaZulu-Natal (S30° 54' 09.0", E30° 19' 17.92") in December 2016 and 2017.
2. *Anthonomus santacruzi* insects were also collected in KwaZulu-Natal in various areas, (S27° 27' 20.4", E030° 38' 15.8"; S30° 24' 58.4", E030° 38' 05.7"; S30° 24' 04.7", E030° 38' 40.8"; S30° 23' 40.4", E030° 38' 53.0"; S30° 23' 17.2", E030° 39' 29.3"; S30° 13' 40.7", E030° 47' 19.8"; S29° 44' 47.7", E030° 41' 05.4") in November 2016 and December 2017. These particular insects are difficult to rear in the laboratory and therefore were released immediately after collection.

<u>Data collection</u>

In this study, treatment replicates are *S. mauritianum* trees that were exposed to the agents and controls are *S. mauritianum* trees not exposed to agents. All *S. mauritianum* trees taller than 1m were randomly selected in each site (detailed below). Due to differing availability of biocontrol agents and *S. mauritianum* trees in the field, equal treatment and control sample sizes were not maintained between the sites as it depended on the availability of suitable trees within each site (Table 1). The experiments were run in summer when it was warmer and most likely for the insects to survive. Furthermore, each experiment was intended to run for at least one year to investigate insect survival in different seasons, but unfortunately they ran for shorter periods because the insects died (see results).

For the first releases at the Wits nursery, 28 trees were randomly selected from 31 trees and used for this experiment. This experiment ran for 2-3 months (start date 10 November 2016, end date 20 January 2017) (Table 1). Three treatments and a control of 7 trees, each included trees exposed to *G. decoris* only, *A. santacruzi* only, *A. santacruzi* and *G. decoris* together and trees without any agents (control). The two biocontrol agents were combined to investigate whether the interaction would enhance or inhibit individual agent's establishment. A total of 20 insects were released per tree, therefore in *A. santacruzi* only (140 *A. santacruzi*), *G. decoris* only (140 *G. decoris*) and in the *A. santacruzi* and *G. decoris* treatment each tree was exposed to a combination of 10 *A. santacruzi* and 10 *G. decoris* (70 *A. santacruzi*, 70 *G. decoris*) (Table 1). Unfortunately, all the *S. mauritianum* trees were not flowering at the time of release, therefore both agents were released on a randomly selected leaf on the third apical branch, and wherever possible *A. santacruzi* were released on young foliage where they could feed on the buds. The biocontrol agents were bagged onto the release branch using fine mesh nets, and four of the trees in each treatment were protected by applying 'ant-tack' to the stem to deter predatory insects. On a monthly basis ant-tack was re-applied to tree stems, in all sites, to maintain long-lasting stickiness. Branches from adjacent trees were broken down by hand to avoid ants using them as 'bridges' to move between the trees.

At Egoli Gas in Johannesburg, releases were done in February 2017 and December 2017. In February 2017 a total of 21 trees were randomly selected and two treatments and a control were established, of 7 trees each, including trees exposed to *A. santacruzi* only, *A. santacruzi* and *G. decoris* together and without the agents (control). Twenty insects were released per tree, therefore on the *A. santacruzi* and *G. decoris* together treatment a combination of 10 *A. santacruzi* and 10 *G. decoris* were released per tree (Table 1). Overall, a total of 210 *A. santacruzi* and 70 *G. decoris* were released (Table 1). The agents were bagged onto branches. Four of the seven trees in each treatment were protected by ant-tack. This experiment was run for 3 months (start date 10 February 2017, end date 8 May 2017). In December 2017 a total of 20 trees were used at Egoli Gas for releases, of which 13 trees were exposed to *G. decoris* only (30 *G. decoris* per tree) and 7 trees exposed to *A. santacruzi* and *G. decoris* together (combination of, 15 *A. santacruzi* and 30 *G. decoris* per tree), therefore a total of 600 *G. decoris* and 105 *A. santacruzi* were released (Table 1). During the releases in December 2017, none of the trees had flowers, therefore the agents were released on non-flowering trees. The agents were unbagged onto tree branches in the releases and all the trees were protected by ant-tack. This experiment ran for 2-3 months (start date 22 December 2017, end date 2 March 2018). Adjacent trees, including trees used as control, were checked at every visit if they harboured any biocontrol agents that might have dispersed onto them.

At Gerotek in Pretoria the releases were done in December 2016 and again in December 2017. In December 2016, a total of 41 trees were used and two treatments established including trees exposed to *A. santacruzi* only (22 trees) and trees exposed to *A. santacruzi* and *G. decoris* together (19 trees) (Table 1). On the *A. santacruzi* only treatment, 20 *A. santacruzi* were released per tree (440 *A. santacruzi*) and on the treatment of *A. santacruzi* and *G. decoris* released together a combination of 20 *A. santacruzi* and 20 *G. decoris* were released together on each tree (380 *A. santacruzi* and 380 *G. decoris*) (Table 1). All available trees were used for releases and therefore there were no control trees. None of the *S. mauritianum* trees were flowering and therefore all agents were released on a randomly selected leaf from a third apical branch in each tree. All agents were bagged onto tree branches and on the *G. decoris* only treatment half of the trees (eleven) were protected by ant-tack and the other half were not protected (without ant-tack). On the *A. santacruzi* and *G. decoris* together treatment, ten trees were protected by ant-tack and nine trees were not protected (without ant-tack). This experiment ran for 1 month (start date 25 November 2016, end date 10 December 2016). In December 2017 a total of 26 trees were used for releases, however this time without bagging the agents onto trees branches upon release. The setup was 22 trees exposed to *G. decoris* only and 4 control trees (without agents). Thirty *G. decoris* were released on each tree and therefore a total of 660 *G. decoris* released (Table 1). This experiment ran for 2-3 months (start date 22 December 2017, end date 2 March 2018). Adjacent trees were checked at every visit to see if they harboured any biocontrol agents.

Figure 1 shows the different release strategies that were used including *Anthonomus santacruzi* and *G. decoris* that were released on *S. mauritianum* trees in all sites either bagged or unbagged, with or without ant-tack protection.

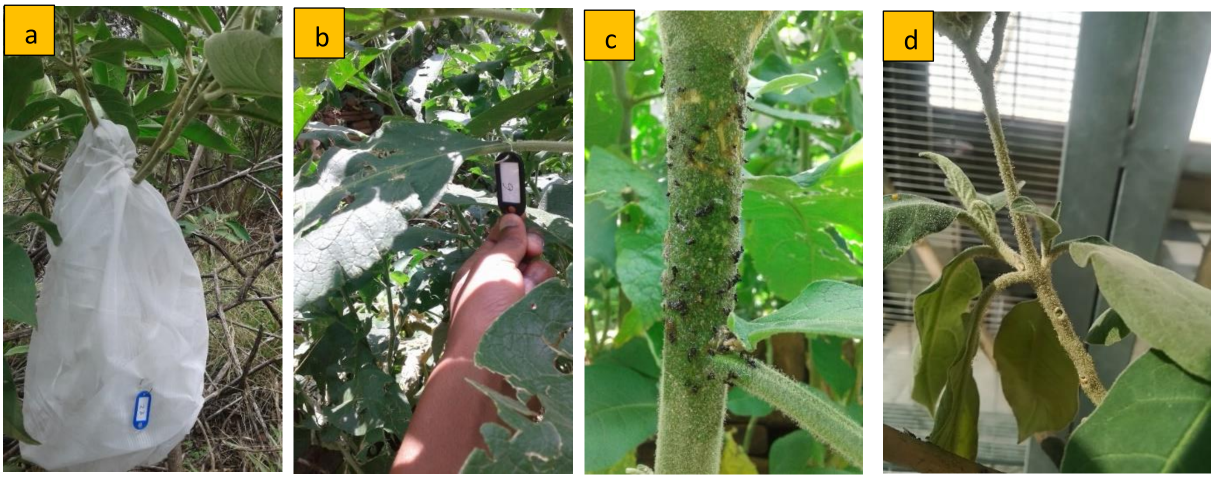

Figure 1: Different release strategies of *Anthonomus santacruzi* and *Gargaphia decoris* on *Solanum mauritianum* trees: (a) Bagged insects, (b) unbagged insects, (c) protecting the insects with ant-tack, and (d) not protecting the insects with ant-tack.

In summary, the release methods include 1) bagged agents on ant-tack protected trees, 2) bagged agents on unprotected trees (no ant-tack), 3) unbagged agents on ant-tack protected trees and 4) unbagged agents on unprotected trees. The survival of the biocontrol agent populations was measured by counting the number of insects found on the trees on a monthly basis. The nymphs were also counted if found, to see if the agents were reproducing. Table 1 provides a summary of the details of the release strategies used.

Table 1: Summary table providing size (numbers) of releases of *Anthonomus santacruzi* and *Gargaphia decoris*, number of *Solanum mauritianum* trees for each release site, duration of trial, and status of experiment at each site.

		Site			
	Wits nursery	**Egoli Gas**		**Gerotek**	
Bagging (Yes/No)	Yes	Yes	No	Yes	No
Start date	10-Nov-16	10-Feb-17	22-Dec-17	25-Nov-16	22-Dec-17
End date	20-Jan-17	8-May-17	2-Mar-18	10-Dec-16	2-Mar-18
Trees (N)	28	21	20	41	26
G. decoris/tree (n)	20	________	30	________	30
(total trees)	7	________	13	________	22
(insects released)	140	________	390	________	660
A. santacruzi/tree (n)	20	20	________	20	________
(total trees)	7	7	________	22	________
(insects released)	140	140	________	440	________
A. santacruzi + *G. decoris* (n)	10 + 10	10 + 10	15 + 30	20 + 20	________
(total trees)	7	7	7	19	________
(insects released)	70 + 70	70 + 70	105 + 210	380 + 380	________
Control trees (n)	7	7	________	________	4
Ant-tack (Yes/No)	Yes	Yes	Yes	Yes	Yes
Flowers (Yes/No)	No	No	No	No	No

Data Analysis

For the nursery trial and each of the field experimental sites, the survival of biocontrol agents was compared between treatments, and between ant-tack protected and not protected trees, using repeated measures ANOVAs.

Results

The protection using ant-tack had no significant main or interaction effects in the Wits nursery trial or in any of the experimental field trial sites (Table 2), and hence these data were reanalysed using One-way repeated measures ANOVAs without 'protection' as a main effect (Table 3). During the site visits dead ants and other predatory insects were found stuck on the ant-tacked plant stems which indicated that they were a potential threat to the biocontrol agents.

The survival of bagged insects at the Wits nursery was very poor across all treatments; this is shown by a general decrease in their numbers over time after their release (Figure 2). Biocontrol agent numbers on all *S. mauritianum* trees decreased significantly over time (Repeated Measures ANOVA, $F_{(6,48)}=15.276$, $P<0.001$) (Figure 2; Table 3). Therefore none of the biocontrol agents were able to establish viable populations.

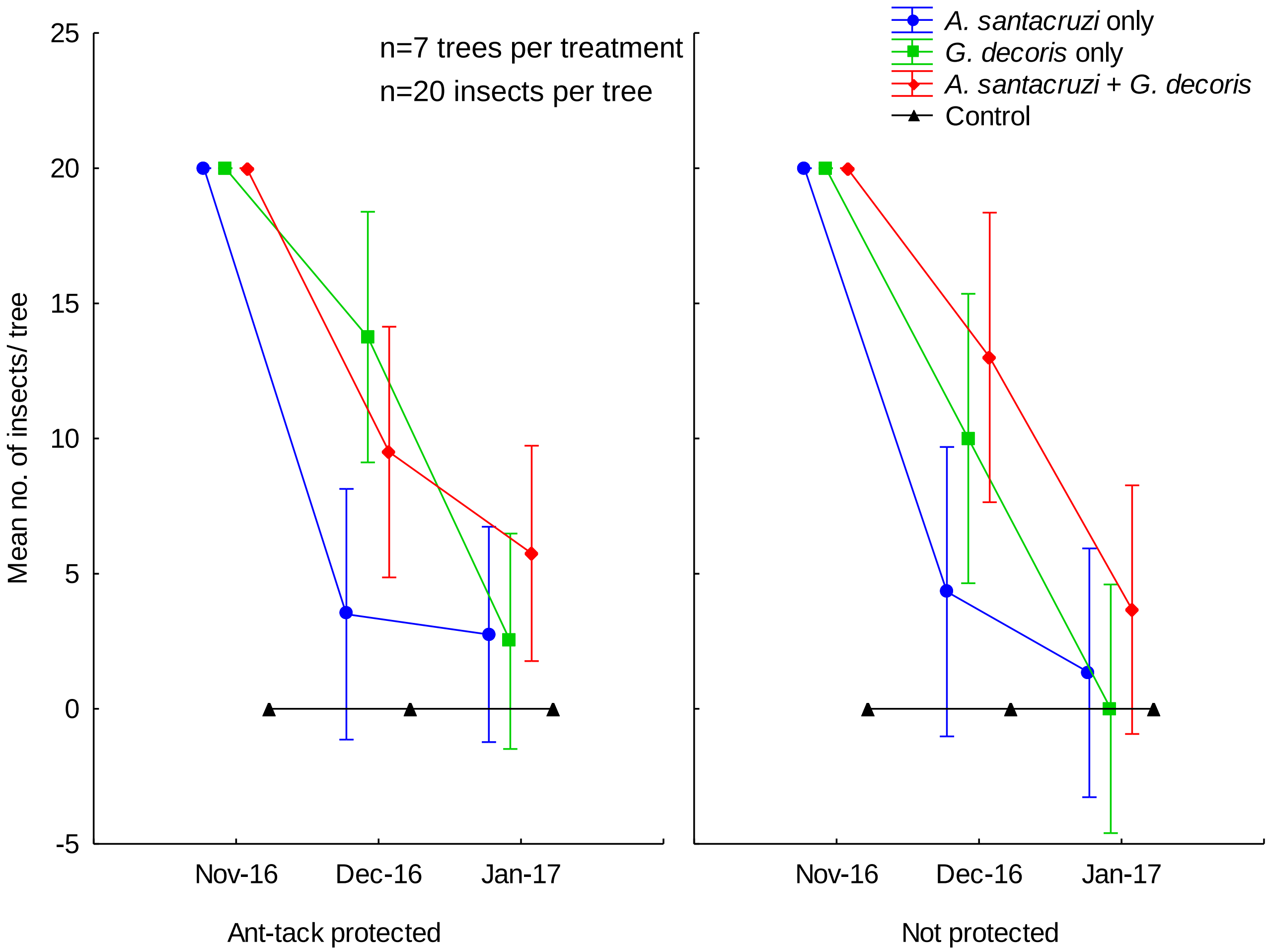

Figure 2: The survival of bagged *Gargaphia decoris* and *Anthonomus santacruzi* on *Solanum mauritianum* trees at the Wits nursery. The treatments (n=7 trees each) included trees exposed to *G. decoris* only, *A. santacruzi* only, *A. santacruzi* and *G. decoris* together, and a control (no insects). A total of n=20 insects were released per tree. *Anthonomus santacruzi* and *G. decoris* together, had n=10 *A. santacruzi* and n=10 *G. decoris* insects per tree (mean ±SE). In each treatment four trees were protected by ant-tack and three were unprotected (without ant-tack). (N=total count, n=sample count).

The assessment of bagged insects in a shady site at Egoli Gas showed that biocontrol agents in all *S. mauritianum* trees decreased significantly over time (Repeated Measures ANOVA, $F_{(6,54)}=185.796$, $P<0.001$) (Figure 3; Table 3). Insect survival on bagged and ant-tack protected and bagged and not protected trees was very poor and none of the biocontrol agents were able to establish viable populations.

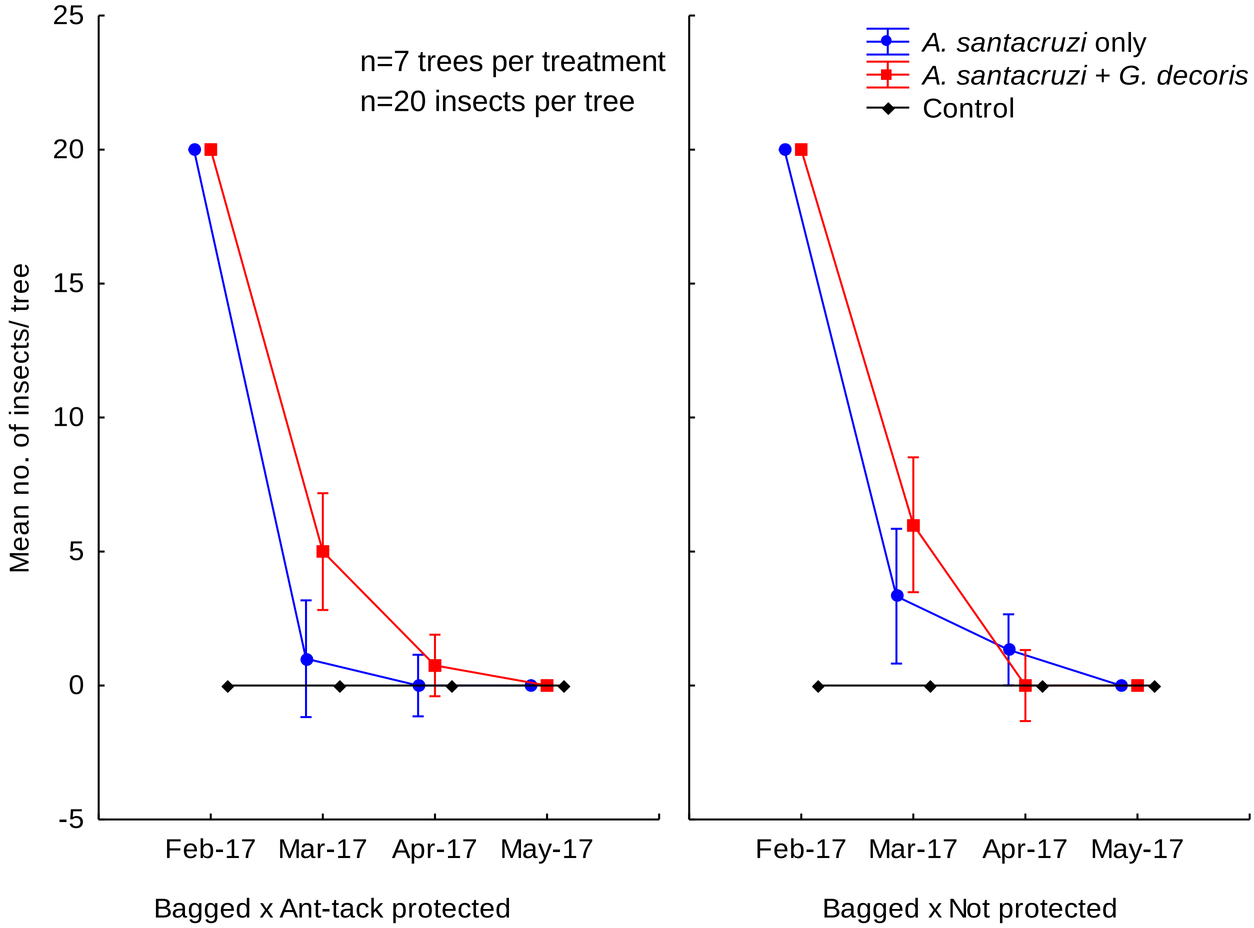

Figure 3: The survival of bagged *Gargaphia decoris* and *Anthonomus santacruzi* insects on *Solanum mauritianum* trees in a shady site at Egoli Gas. The treatments were trees exposed to *A. santacruzi* only, *A. santacruzi* and *G. decoris* together and a control (without insects) n=7 trees each, and n=20 insects released per tree. The *A. santacruzi* and *G. decoris* together n=20 was separated into n=10 *A. santacruzi* and n=10 *G. decoris* per tree (mean ±SE). In each treatment four trees were ant-tack protected and three were not protected (without ant-tack).

Insects unbagged at Egoli Gas decreased significantly in numbers over time (Repeated Measures ANOVA, $F_{(3,54)}=1860.830$, $P<0.001$) (Figure 4; Table 3). Adjacent trees were checked at every site visit if they harboured any of the biocontrol agents, but none were found. The *A. santacruzi* and *G. decoris* together treatment had a higher mean number of released insects compared to the *G. decoris* only treatment, nevertheless neither biocontrol agent established in the field (Figure 4).

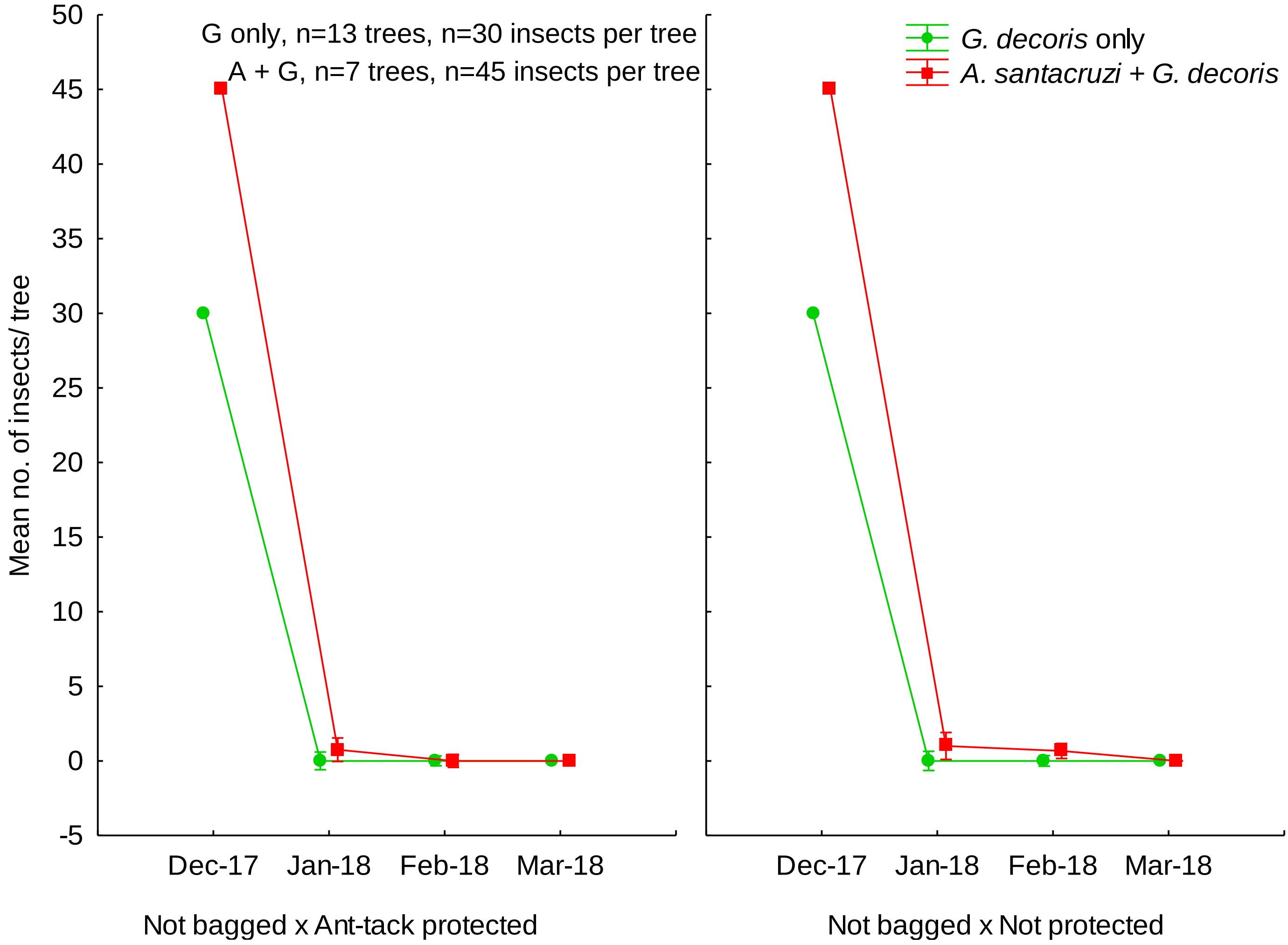

Figure 4: The survival of unbagged *Gargaphia decoris* and *Anthonomus santacruzi* on *Solanum mauritianum* trees in a shady site at Egoli Gas. Treatments of trees exposed to *G. decoris* only (n=13 trees) and *A. santacruzi* and *G. decoris* together (n=7 trees) were exposed to 30 insects, and 15 *A. santacruzi* and 30 *G. decoris* together per tree, respectively (mean ±SE). On the *G. decoris* only treatment seven trees were ant-tack protected and six were not protected (without ant-tack). On the *A. santacruzi* and *G. decoris* together treatment four trees were ant-tack protected and three trees were not protected (without ant-tack).

Bagged insects on ant-tack protected and not protected trees at a relatively warmer and sunny site in Gerotek were not found within a month after release (Figure 5). Similar poor survival was found in a shady site at Egoli Gas (Figure 3 & 4). None of the biocontrol agents established in the field.

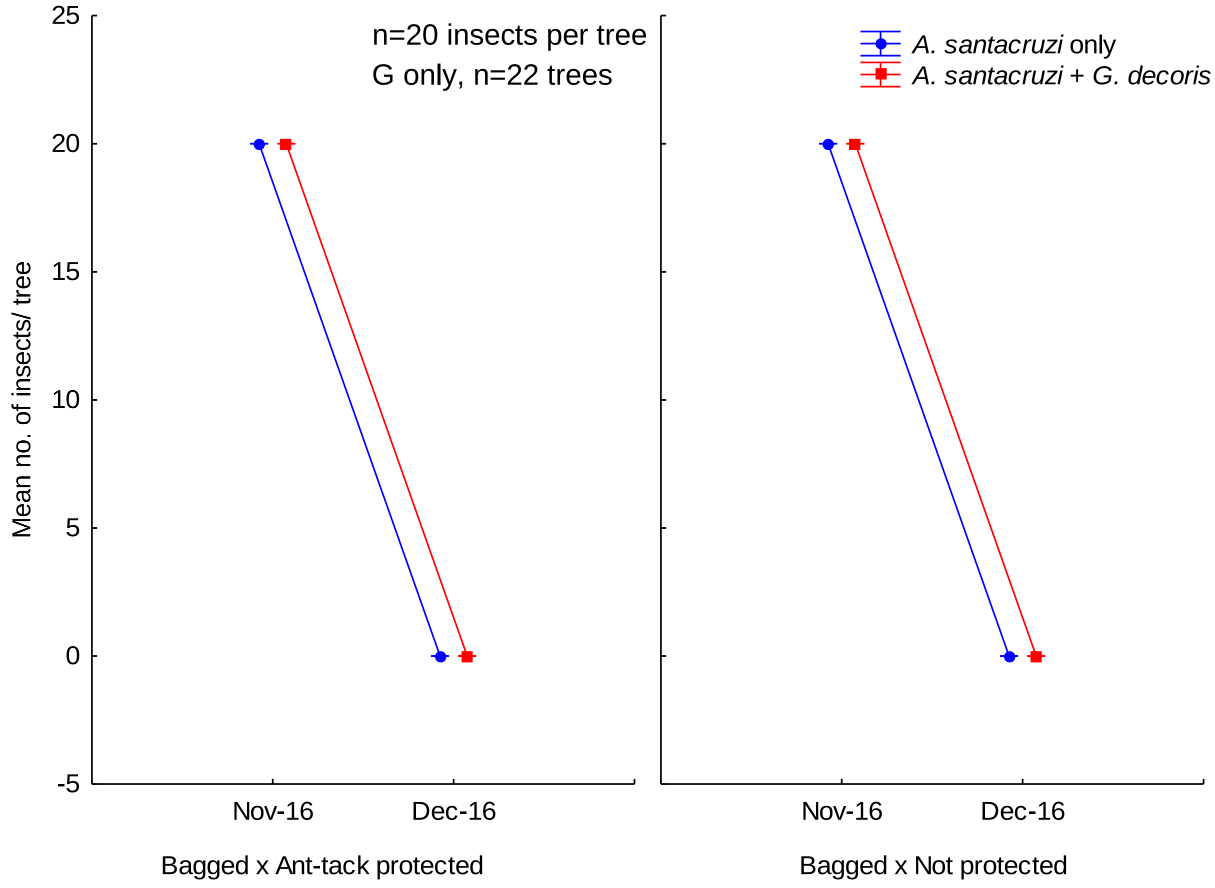

Figure 5: The survival of bagged *Gargaphia decoris* and *Anthonomus santacruzi* on *Solanum mauritianum* trees in a sunny site at Gerotek. Treatments of trees exposed to *G. decoris* only (n=22 trees) and *A. santacruzi* and *G. decoris* together (n=19 trees), were exposed to n=20 insects per tree, and for the *A. santacruzi* and *G. decoris* together treatment, n=20 *G. decoris* and n=20 *A. santacruzi* were released together per tree, respectively (mean ±SE). On the *G. decoris* only treatment half of the trees (eleven) were protected by ant-tack and the other half were not protected (without ant-tack). On the *A. santacruzi* and *G. decoris* together treatment ten trees were protected by ant-tack and nine trees were not protected (without ant-tack).

Unbagged insects at Gerotek significantly decreased in numbers over time (Repeated Measures ANOVA, $F_{(3,72)}=1232.693$, $P<0.001$) (Figure 6; Table 3). Adjacent trees were checked and did not harbour any of the biocontrol agents. None of the insects were able to establish in the field. Clearly the biocontrol agents, *A. santacruzi* and *G. decoris*, are not suitable for establishment on *S. mauritianum* in the Highveld.

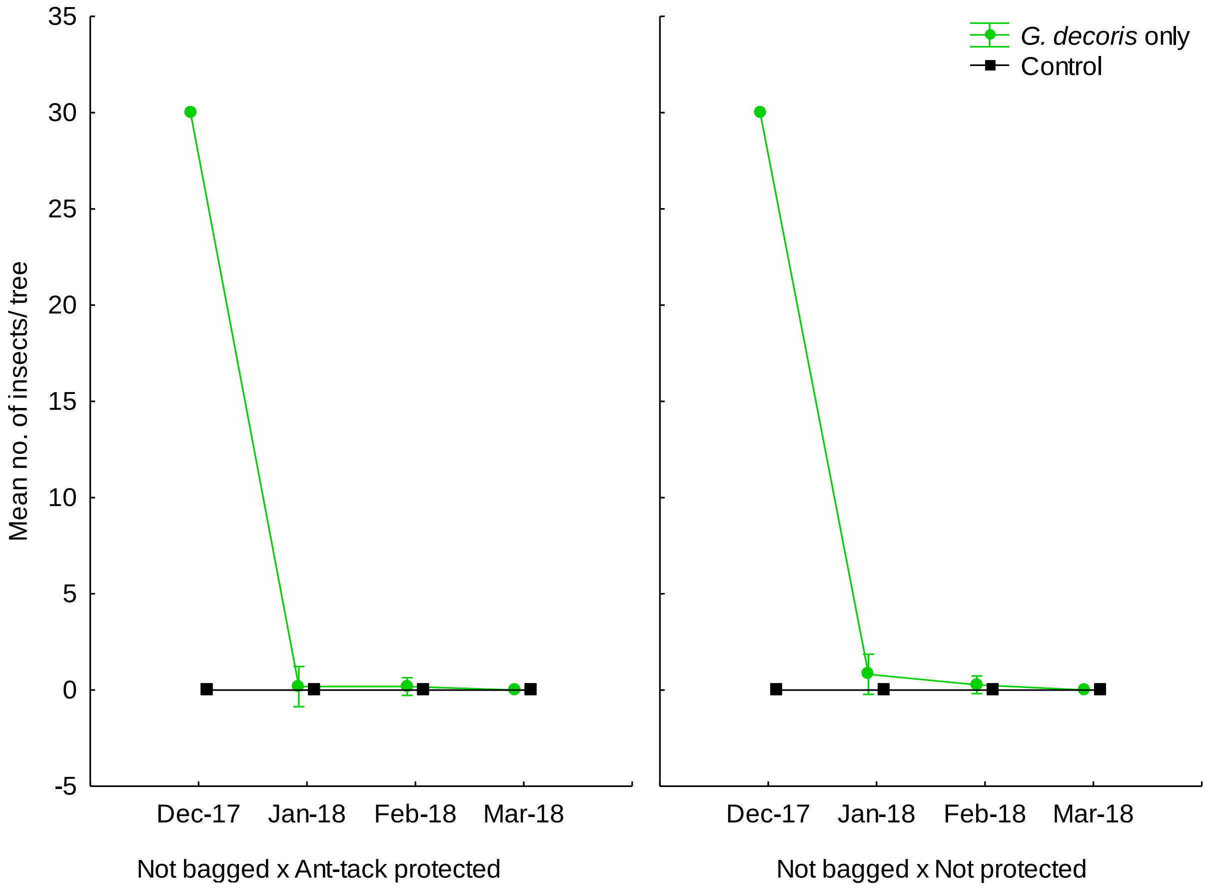

Figure 6: The survival of unbagged *Anthonomus santacruzi* and *Gargaphia decoris* on *Solanum mauritianum* after being released at Gerotek. Only one treatment of trees exposed to *G. decoris* only (n=22 trees) was established due to lack of biocontrol agents. A total of n=30 insects per tree were released and a control (n=4 trees) without insects (mean ±SE). Half of the trees (eleven) in each treatment were protected by ant-tack.

Table 2: The results of Two-way Repeated Measures ANOVAs to assess the survival of *Anthonomus santacruzi* and *Gargaphia decoris* on *Solanum mauritianum* trees. The treatments of trees exposed to *G. decoris* only, *A. santacruzi* only, and *A. santacruzi* and *G. decoris* together were established at different sites. Trees in each treatment were either protected by ant-tack, a sticky compound that deters predatory insects from walking up the stem to feed on biocontrol agents, or not protected (without ant-tack).

Wits nursery					
Effect	SS	Degr. of Freedom	MS	F	P
Intercept	5161.668	1	5161.668	496.6458	<0.000001
Treatments	1841.995	3	613.998	59.0778	<0.000001
Protection	4.191	1	4.191	0.4033	0.532591
Treatments*Protection	19.471	3	6.490	0.6245	0.607481
Error	207.861	20	10.393		
TIME	2373.050	2	1186.525	99.0031	<0.000001
TIME*Treatments	983.181	6	163.863	13.6727	<0.000001
TIME*Protection	11.383	2	5.691	0.4749	0.625413
TIME*Treatments*Protection	32.847	6	5.475	0.4568	0.835831
Error	479.389	40	11.985		
Egoli Gas bagged					
Effect	SS	Degr. of Freedom	MS	F	P
Intercept	1355.716	1	1355.716	743.4222	<0.000001
Treatments	685.788	2	342.894	188.0298	<0.000001
Protection	2.192	1	2.192	1.2017	0.290263
Treatments*Protection	3.597	2	1.799	0.9863	0.395885
Error	27.354	15	1.824		
TIME	2438.256	3	812.752	693.6178	<0.000001
TIME*Treatments	1249.585	6	208.264	177.7365	<0.000001
TIME*Protection	4.352	3	1.451	1.2381	0.307036

TIME*Treatments*Protection	4.919	6	0.820	0.6996	0.651289
Error	52.729	45	1.172		
Egoli Gas unbagged					
Effect	SS	Degr. of Freedom	MS	F	P
Intercept	6504.64	1	6504.636	21440.18	<0.000001
Treatments	294.24	1	294.235	969.84	<0.000001
Protection	0.24	1	0.235	0.78	0.391562
Treatments*Protection	0.24	1	0.235	0.78	0.391548
Error	4.85	16	0.303		
TIME	18699.29	3	6233.097	45590.65	<0.000001
TIME*Treatments	717.69	3	239.231	1749.80	<0.000001
TIME*Protection	0.33	3	0.111	0.81	0.494222
TIME*Treatments*Protection	0.33	3	0.111	0.81	0.494242
Error	6.56	48	0.137		
Gerotek unbagged					
Effect	SS	Degr. of Freedom	MS	F	P
Intercept	798.909	1	798.9093	569.473	<0.000001
Treatments	798.909	1	798.9093	569.473	<0.000001
Protection	0.112	1	0.1119	0.080	0.780272
Treatments*Protection	0.112	1	0.1119	0.080	0.780272
Error	30.864	22	1.4029		
TIME	2248.265	3	749.4218	1171.324	<0.000001
TIME*Treatments	2248.265	3	749.4218	1171.324	<0.000001
TIME*Protection	0.238	3	0.0793	0.124	0.945688
TIME*Treatments*Protection	0.238	3	0.0793	0.124	0.945688

Error	42.227	66	0.6398		

Table 3: The results of One-way Repeated Measures ANOVAs to assess the survival of *Anthonomus santacruzi* and *Gargaphia decoris* on *Solanum mauritianum* trees. The treatments of trees exposed to *G. decoris* only, *A. santacruzi* only, and *A. santacruzi* and *G. decoris* together were established at different sites. Trees in each treatment were either protected by ant-tack, a sticky compound that deters predatory insects from walking up the stem to feed on biocontrol agents, or not protected (without ant-tack).

Wits nursery					
Effect	SS	Degr. of Freedom	MS	F	P
Intercept	5312.190	1	5312.190	550.6672	<0.000001
Treatments	1894.952	3	631.651	65.4776	<0.000001
Error	231.524	24	9.647		
TIME	2387.881	2	1193.940	109.4482	<0.000001
TIME*Treatment	999.833	6	166.639	15.2757	<0.000001
Error	523.619	48	10.909		
Egoli Gas bagged					
Effect	SS	Degr. of Freedom	MS	F	P
Intercept	1368.108	1	1368.108	743.0270	<0.000001
Treatments	693.500	2	346.750	188.3219	<0.000001
Error	33.143	18	1.841		
TIME	2496.321	3	832.107	724.7367	<0.000001
TIME*Treatment	1279.928	6	213.321	185.7957	<0.000001
Error	62.000	54	1.148		
Egoli Gas unbagged					
Effect	SS	Degr. of Freedom	MS	F	P
Intercept	6594.92	1	6594.925	22766.04	<0.000001

Treatments	296.42	1	296.423	1023.27	<0.000001
Error	5.21	18	0.290		
TIME	19002.54	3	6334.180	48370.10	<0.000001
TIME*Treatment	731.04	3	243.680	1860.83	<0.000001
Error	7.07	54	0.131		
Gerotek unbagged					
Effect	SS	Degr. of Freedom	MS	F	P
Intercept	798.909	1	798.9093	606.942	<0.000001
Treatments	798.909	1	798.9093	606.942	<0.000001
Error	31.591	24	1.3163		
TIME	2248.265	3	749.4218	1232.693	<0.000001
TIME*Treatment	2248.265	3	749.4218	1232.693	<0.000001
Error	43.773	72	0.6080		

Discussion

Bagged insects at the Wits nursery showed very low survival and as a result they did not establish viable populations. Experiments at the Wits nursery were done under controlled conditions, unlike in the field, and as such high survival and establishment of the biocontrol agents were anticipated but were ultimately not found. No trees had flowers during releases and that may have affected the survival and reproduction of *A. santacruzi* which feeds, lays eggs and develops on the floral parts of *S. mauritianum* (Olckers 1999). Also, *A. santacruzi* was observed feeding on leaves on one of the trees which had very green leaves compared to the other trees, indicating quality of leaves might have affected establishment. This suggests that *A. santacruzi* may occasionally feed on *S. mauritianum* leaves when there are no flowers in the field, but this may provide limited nutrition and cannot be sustained over long periods.

The lack of *G. decoris* establishment is currently attributed mainly to predation (Lotter 2004; Olckers 2009; Olckers 2011; Patrick and Olckers 2014), however the results from the Wits nursery trials did not support this. The exclusion of predators on bagged and ant-tack protected trees was successful as no predators were found on protected trees, but this did not prevent the rapid decline of the biocontrol agents in all the treatments. The exclusion of bird predators using netting and ant predators using a sticky gel on the stem has been used successfully to enhance the survival of a biocontrol agent. For example, the geometrid *M. pallidata* was successfully protected against *M. pigra* (Grace 2005). The disadvantage of the sticky gel is that it can also block the movement of biocontrol agents (Grace 2005), but the biocontrol agents in this study were not affected by ant-tack because they were not found trapped and killed at any time. Nonetheless, the failed establishment of *A. santacruzi* is blamed on the absence of flowers on *S. mauritianum* trees during the experiments and failed *G. decoris* establishment may be due to the quality of the trees used.

However, field releases also did not show any success in biocontrol agent establishment. Bagged and unbagged insects in a shady field site at Egoli Gas showed very low survival and ultimately did not establish viable populations. Similar to the nursery trials, the ant-tack and bagging techniques kept the ants off the protected trees but did not have an effect on the establishment because of the rapid decline of the biocontrol agents. It was taken into account that the temperature and humidity fluctuations in winter in the Highveld could account for changes in plant quality and therefore impede the chances of *A. santacruzi* to survive and reproduce (Cowie *et al*. 2016b). Therefore, strategically releasing *A. santacruzi* in summer, which is warmer and rainy, was more likely to provide suitable microhabitats and increase the chances of survival. However the failed establishment of *A. santacruzi* was still attributed to the absence of flowers on *S. mauritianum* trees during the releases and for the entire duration of the experiments. *Anthonomus santacruzi* feeds primarily on the floral parts of *S. mauritianum* and therefore low survival in the absence of flowers is certain. The phenology of invasive plants, for example the timing of senescence, is important to know in order to understand plant fitness and ultimately allows for assessing the efficacy of biocontrol agents (Dennill 1987b). For example, the knowledge of flowering times of an invasive species could help with releasing flower-feeding biocontrol agents when the flowers are available. In this study it was assumed that *S. mauritianum* flowers throughout the year as suggested by Olckers (2009), however this was not the case on the Highveld plants.

The failed establishment of *G. decoris* at Egoli Gas was unexpected given that it has been suggested that its establishment is achievable in semi-shady areas when predation is excluded using sticky barriers on stems (Lotter 2004; Patrick and Olckers 2014). However, single releases are more likely to become extinct by chance compared to multiple releases (Memmott *et al*. 1998). Fewer but large releases of *G. decoris* (*ca*. 1000) have been suggested for successful establishment (Lotter 2004; Patrick and Olckers 2014); however in this study such high numbers could not be released, and hence this may have influenced the chances of survival of the agents.

The survival of bagged and unbagged insects in a relatively warmer and sunny site at Gerotek was also very low and the agents failed to establish. Again, the ant-tack and bagging deterred ants and other insect predators but did not have a big effect on the establishment because the biocontrol agents decreased rapidly in numbers. Although *G. decoris* was found to cause significant physiological damage to *S. mauritianum* under sun conditions in the lab (Cowie *et al*. 2016a), it failed to establish in a sunny site at Gerotek. Altogether, multiple releases (perhaps monthly) of *G. decoris* should still be investigated because predation alone is unlikely to be the main cause of failed establishment. *Anthonomus santacruzi* releases should be focused on flowering trees in future, and also when the conditions are warm and rainy.

The release of multiple agents can yield mixed results, however interactions that enhance the damage to the target weed are normally sought after in biocontrol programmes (Stiling and Cornelissen 2005; Stephens *et al.* 2013). The idea is that the biocontrol agents occupy different plant parts and do not compete for resources, i.e. *G. decoris* feeds on leaf sap and *A. santacruzi* feeds on flowers, and therefore will not inhibit each other's reproduction, development and establishment. However, it was found that the combination of *A. santacruzi* and *G. decoris* did not enhance the survival and establishment of the biocontrol agents in any of the sites. The fact that the agents could not establish even in single treatments suggests that the same factors that inhibited establishment in single treatments also prevailed when the agents were combined. Therefore, the interaction per se is unlikely to have resulted in non establishment of the agents. Nevertheless, in biocontrol programmes or natural systems, such interactions are known to reduce plant performance (Stephens *et al*. 2013). Overall, the biocontrol agents did not establish in the Highveld of Gauteng, therefore it is suggested that this interaction should be tested in areas where both the agents are able to establish, such as KwaZulu-Natal.

Conclusion

It is clear that the biocontrol of *S. mauritianum* using *A. santacruzi* and *G. decoris* is not currently the solution to control the *S. mauritianum* infestation in the Highveld. Both agents failed to establish under a wide range of environmental conditions, i.e. sun and shade conditions, and with various strategies employed to protect them from predation. The protection of biocontrol agents from predators seems to be common, but it does not guarantee the survival and establishment of the agents in the field. This is true for other highly invasive alien plant species such as *L. camara*. Similar to this study, the failed establishment of a flower-feeding moth *Ectaga garcia* Becker (Lepidoptera: Depressariidae) against *L. camara* revealed that the agent had low survival, even when predators were excluded using cages (Day *et al*. 2003). Indeed, there are other underlying factors and conditions under which the agents can thrive.

Gargaphia decoris is predicted to prefer shady conditions, however that was not clearly shown in this study. The quality of the trees used in this study may have influenced performance of the biocontrol agents. Lower than recommended biocontrol agent numbers were released in this study, nevertheless larger numbers could be beneficial, particularly for the establishment of *G. decoris*. Releasing low numbers of agents may not be the best decision but sometimes is the only option. The long history of biocontrol of *L. camara* highlights some failed and successful agent establishment events due to the size of releases. The failed establishment of two agents for *L. camara* namely the leaf-feeding beetle *Uroplata* *fulvopustulata* Baly (Coleoptera: Chrysomelidae) and the hispine *Uroplata lantanae* Buzzi and Winder (Coleoptera: Chrysomelidae) were due to low numbers released (Taylor 1989). For the same weed, larger releases of a different agent, a gall-midge *Zeuxidiplosis giardia* Kieffer, successfully established in the field, and of course under favourable conditions (Annecke and Neser 1977). In this study it is proposed that future research should focus on fewer releases with larger numbers of *G. decoris* (*ca*. 1000) as suggested by (Lotter 2004; Patrick and Olckers 2014). *Anthonomus santacruzi* should also be released on prolifically flowering trees, in summer, in order to increase the chances of survival. Releasing *A. santacruzi* and *G. decoris* together did not improve the efficacy of biocontrol, however the interactions between the two agents should be further investigated in areas where they can both establish successfully, such as in KwaZulu-Natal.

CHAPTER THREE

Impacts of mechanical control and, mechanical and chemical control combined, on *Solanum mauritianum* survival and re-growth

Abstract

Solanum mauritianum, bugweed, is a NEMBA category 1b invader species, requiring mandatory control. Biocontrol efforts are not sufficient at controlling the weed and the conventional methods of cutting and herbicide application are expensive and labour-intensive. *Solanum mauritianum* is reportedly easily killed by cutting and herbicide treatment but the influence of stem diameter, cutting height, multiple stems and the timing of cutting has not been evaluated. The rapid resprouting of trees after cutting usually results in ineffective control. The objective of this study was to investigate the efficacy of integrated control for *S. mauritianum* growing in the Highveld. The study was conducted at three sites in Johannesburg including Egoli Gas, Parktown and Houghton and another at Gerotek, Pretoria. *Solanum mauritianum* trees were either only cut or cut and treated with the herbicide Hatchet, in spring and autumn, and also at different cutting heights. Also, multistemmed trees in Parktown were cut and treated with herbicide to test the effect of number of stems on plant response to cutting. Monthly records of dead or live stumps were taken in each site. The highest mortality was observed on trees that were cut close to ground-level and treated with Hatchet. Cutting low and not applying Hatchet was not effective at killing *S. mauritianum* and resulted in a high percentage of resprouted stumps. The cut and herbicide treatment was effective and as such the stem diameter size, the number of cut stems per trees and the cutting season did not have a significant effect on stump survival. An increase in cutting height increased the percentage of resprouting stumps, especially on trees with bigger stems. These results indicate that cutting low and immediately treating stumps with Hatchet is the most effective method for killing *S. mauritianum* on the Highveld. However, challenges can be expected in cases where obstacles such as rocks and logs make it difficult to cut low and ultimately higher cuts may result in resprouting.

Keywords: Cutting height, cutting season, Hatchet, multistemmed, resprouting, stem diameter.

Introduction

The removal of alien invasive plant species is important to minimize the negative economic and environmental impact of these weeds. Efforts have seen more research focused on integrated control methods given that “single/individual” methods often don’t provide sufficient control of invasive species (Webster *et al*. 2006). The hope is that the combination of two or more control methods can be more effective than the individual methods separately. The cut-stump treatment is an integrated approach where mechanical and chemical methods are combined. The mechanical component involves cutting tree stems and herbicide application is typically done immediately after cutting (Ballard and Nowack 2006). The killing of plants by mechanical control (cutting trees) and integrated control (cut and herbicide treatment) is not novel (Sterrett 1915; Tjosvold and McHenry 1986; Wahlers *et al*. 1997; Butler and Britting 1998; DiTomaso and Kyser 2007). Large woody plants have been controlled using the cut stump technique, in which herbicide or no herbicide is applied on the cambial regions of cut stems (Kochenderfer *et al*. 2006; DiTomaso and Kyser 2007). Control by cut and herbicide application is often more effective compared to cutting only, because the herbicide’s movement to the plant’s growth points causes mortality (Burch and Zedaker 2003; Hartman and McCarthy 2004; Siso and Burzycki 2004; Kochenderfer *et al*. 2006; DiTomaso and Kyser 2007; Fick and Geyer 2010; Miller *et al*. 2013; Harmoney 2016; Young *et al*. 2017; Enloe *et al*. 2018). For example, the removal of aggressive invasive species such as *Ailanthus altissima*, *Ardisia elliptica* and *Ligustrum sinense* by manual cutting often stimulates stump or root resprouting and ultimately this could increase an infestation (Kelly 2001; Burch and Zedaker 2003; Siso and Burzycki 2004; Enloe *et al*. 2018).

However, plant removal by cut and herbicide treatment can provide varied levels of control. The effectiveness of cut and herbicide treatment control is limited by both biotic and abiotic factors – for example cutting height, tree stem diameter and cutting season among other factors affecting the likelihood of resprouting. For plants that can resprout after clearing, it is very important that both stems and below-ground parts including roots are killed (Burch and Zedaker 2003). The cutting height of stumps can affect the amount of herbicide required to penetrate the plant’s growth points, in that the taller the post-cut stumps are, the lower the herbicide penetration and the greater number of available buds that remain which ultimately increases the chance of resprouting (Crist *et al*. 1983; Harrington 1984; McCreary *et al*. 1991; Jobidon 1997; Witkowski and Garner 2008). Similar to cutting height, trees with greater stem diameter, which represents a larger plant size (biomass), can result in a greater probability of resprouting of stumps after clearing in numerous types of habitats (e.g. Kays and Canham 1991; Luoga *et al*. 2004; Neke *et al*. 2006; Mwavu and Witkowski 2008).

The season in which the cutting is done may also have a strong influence on subsequent resprouting following cut and herbicide treatment of stumps (Kays and Canham 1991). Hardwood trees mainly rely on root reserves to resprout after clearing, therefore the changes in plant physiology (which are predictable) over the course of the year in different seasons can influence how plants respond to clearing (Tew 1970; Schier and Zasada 1973; Kramer and Kozlowski 1979; McLaughlin *et al*. 1980). Clearing can be effective in autumn probably due to the translocation of photosynthates to roots for winter storage, which allows herbicide movement to the roots (Evans *et al*. 2006; Delanoy and Archibold 2007). Clearing in spring can also be effective due to a drop in root reserves during the start of shoot growth (Kays and Canham 1991). In the USA, plant clearing was initially thought to yield the best results when done in the growing season, but has also shown some success when done in other seasons (Ballard and Nowack 2006). Overall, answering the questions on biotic and abiotic factors that influence stump resprouting following clearing could assist land managers to improve the control of *Solanum mauritianum*.

Solanum mauritianum is an aggressive invasive species in South Africa. Biocontrol of *S. mauritianum* in South Africa has not resulted in satisfactory control of the weed (Olckers 1999; Olckers 2000; Olckers 2003; Cowie *et al*. 2018), and hence the reason to investigate other methods of control. Although physical methods are more costly, time-consuming and difficult to apply than biocontrol (Smith *et al*. 1997), they can provide good control for relatively short periods, but are likely to be ineffective for long-term control due to rapid stump sprouting (DiTomaso and Kyser 2007) and weed resurgence from seed banks (e.g. Witkowski and Wilson 2001). Whether active removal of *S. mauritianum* represents an alternative to biocontrol remains an open question. Similarly, in the US biocontrol of Tamarix populations using biocontrol has resulted in highly variable results (Hultine *et al*. 2015; Kennard *et al*. 2016) and therefore some research has focused on other control methods such as physical removal of Tamarix trees (Fick and Geyer 2010; González *et al*. 2017).

The Working for Water (WfW) Programme in South Africa is actively involved in the removal of aggressive weeds such as *S. mauritianum* (Van Wilgen *et al*. 1998; Holmes *et al*. 2005; Holmes *et al*. 2008). Olckers (1999) suggested that *S. mauritianum* is easily killed by cut and herbicide treatment. The herbicide Hatchet, a non-selective systemic herbicide with the active ingredient Imazapyr, is used for *S. mauritianum* removal. However, previous studies have shown increased densities of *S. mauritianum* after cutting due to resprouting from cut stems and seedling establishment following clearing by WfW (Garner and Witkowski 1997; Witkowski and Garner 2008). A study by Witkowski and Garner (2008) on the removal of *S. mauritianum* along the Sabie River concluded that *S. mauritianum* can be effectively controlled by cutting lower on the stem and applying the recommended herbicide (Hatchet). However, the high proportion of resprouting observed is thought to have been caused by the herbicide incorrectly applied on the stem or not at all applied as well as the high cutting height of many of the cut stems (Witkowski and Garner 2008). The high cutting may have been due to the many logs and rocks within the riparian woodland vegetation site (Witkowski and Garner 2008). This current study focused on the efficacy of the integrated control of *S. mauritianum* in the Highveld. There are various factors that can play an important role in the success of clearing efforts such as season, cutting height, the number of stems cut per tree, stem diameter and application of herbicide to the stump, and these factors were explored in this study.

Methods and Materials

Study sites

Field trials were conducted at four sites in Gauteng Province, at Gerotek, Egoli Gas, Houghton and Parktown. Gerotek is in the Tshwane Municipality close to Pretoria and Egoli Gas, Houghton and Parktown are in the City of Johannesburg Municipality close to Johannesburg. The description of Gerotek and Egoli Gas sites are provided in Chapter 2. Houghton and Parktown are both located in Johannesburg thus sharing similar climatic conditions with the Egoli Gas site, defined by summer rainfall and mean temperatures of 21°C in summer and 10°C in winter. Houghton is a residential area situated north-east of the Johannesburg city centre and Egoli Gas is in old industrial area. These two areas share the same Egoli Granite Grassland vegetation type, which is characterized by low hills dominated by heavily utilized and degraded grassland with some woody species occurring in scattered groups or solitary trees (Mucina *et al*. 2006). The Houghton site is located next to the traffic lights on the corner of Houghton Dr and Carse O'Gowrie Rd (26˚ 10' 39.12"S 28˚ 03' 05.40"E) on what looks like an area that was previously used for dumping waste and household goods. The site is dominated by scattered groups of *S. mauritianum* trees which can be easily seen growing all around the site, some reaching heights of over 6m tall. Other alien invasive species in the site include *Jacaranda mimosifolia* (Bignoniaceae: Lamiales) and *Melia azedarach* L. (Meliaceae) which were growing as solitary trees. The Parktown site is located on Queens Road, Parktown, behind the Charlotte Maxeke Hospital (26˚ 10' 33.59"S 28˚ 02' 52.73"E). *Solanum mauritianum* were the only trees in the site.

Field methods

The herbicide Hatchet, which is used by Working for Water for clearing *S. mauritianum*, was also used in this study. A 2% solution of Hatchet (2L/100L) was applied liberally to each cut stump with a paint brush immediately (within 1 minute) after cutting with a hand saw. Hatchet was mixed with a blue dye and was carefully applied on the entire surface of each stump. In April 2017 at the Gerotek and Egoli Gas sites a total of 20 trees per site of ≥ 1m in height were randomly selected and cut as close as practically possible to the ground with a 750mm long bow saw, ten of these stumps in each site were treated with Hatchet. Therefore there were two treatment groups in each site including 10 stumps cut only (mechanical control) and 10 stumps cut & treated with Hatchet (mechanical and chemical control combined - integrated control). For all cleared trees cutting height and stem diameter were measured. Cutting height was measured from the ground-level to the tip of the stump and stem diameter measurements were taken from the base of the stump using digital calipers. Two more clearing trials were conducted later in the year, at the Houghton and Parktown sites, in August and November 2017 respectively. Twenty *S. mauritianum* trees at the Houghton site were all cut and treated with Hatchet, however these were cut at various heights. Five cutting heights were tested and 4 trees were randomly selected for each cutting height including (i) closest to ground-level (<5cm tall stumps taken as 0cm), (ii) 10cm, (iii) 20cm, (iv) 40cm and (v) 50cm above ground-level. The different cut heights mirrored field conditions whereby felled logs and rocks, particularly in riverine areas, are obstacles to cutting low on the stem; e.g. on the Sabie River after WfW clearing and after major floods result in numerous fallen riparian trees which become obstacles to cutting at ground level (Witkowski and Garner 2008). At Parktown, 20 *S. mauritianum* trees were all cut as close to ground-level as possible, with 10 trees cut only and 10 trees cut and treated with Hatchet. *Solanum mauritianum* trees were also cleared by Working for Water at the Gerotek site in June 2017, and the data for resprouting and stump mortality was used in this study to compare their clearing success with the study's treatments. In this study resprouting is described as the regrowth produced on cut stumps as basal shoots that eventually grow into a single stem or multiple stems. All sites were visited monthly until March 2018 to assess the number of stumps that either (a) resprouted or (b) were dead. The numbers of dead and live plants (with resprouting shoots) were compared between treatments within sites, between sites and across different cutting seasons.

Data Analysis

Two-way ANOVAs were used to assess the effect of stem diameter and cutting height on resprouting trees between and within sites. Two-sample T-tests were used to compare stem diameter differences between killed and resprouting trees for each site separately. Contingency table 2x2 Chi-Square tests were also used to compare the numbers of dead vs live plants (with resprouting shoots) between herbicide treated and cut only (untreated) stumps.

Results

The method of cutting *S. mauritianum* as close as practically possible to the ground and treating their stumps with Hatchet killed all trees, with no resprouting observed in all the sites except one tree at one site (Figure 1). The exception was at Egoli Gas where a fire burned all but one, herbicide-treated stump, which subsequently sprouted in the following spring growing season. Controlling *S. mauritianum* by cutting low only and not treating the stumps with herbicide was not very effective, with an overall 70% of plants resprouting at Gerotek and 100% resprouting at Parktown by March 2018 (Figure 1). Clearly, cutting low and immediately treating the stumps with herbicide, the combination of mechanical and chemical control (integrated control) caused higher mortality of trees at all sites. However integrated control resulted in a higher percentage of resprouting stumps when stumps were cut higher up the stem and treated with herbicide, as observed at the Houghton site (Figure 1).

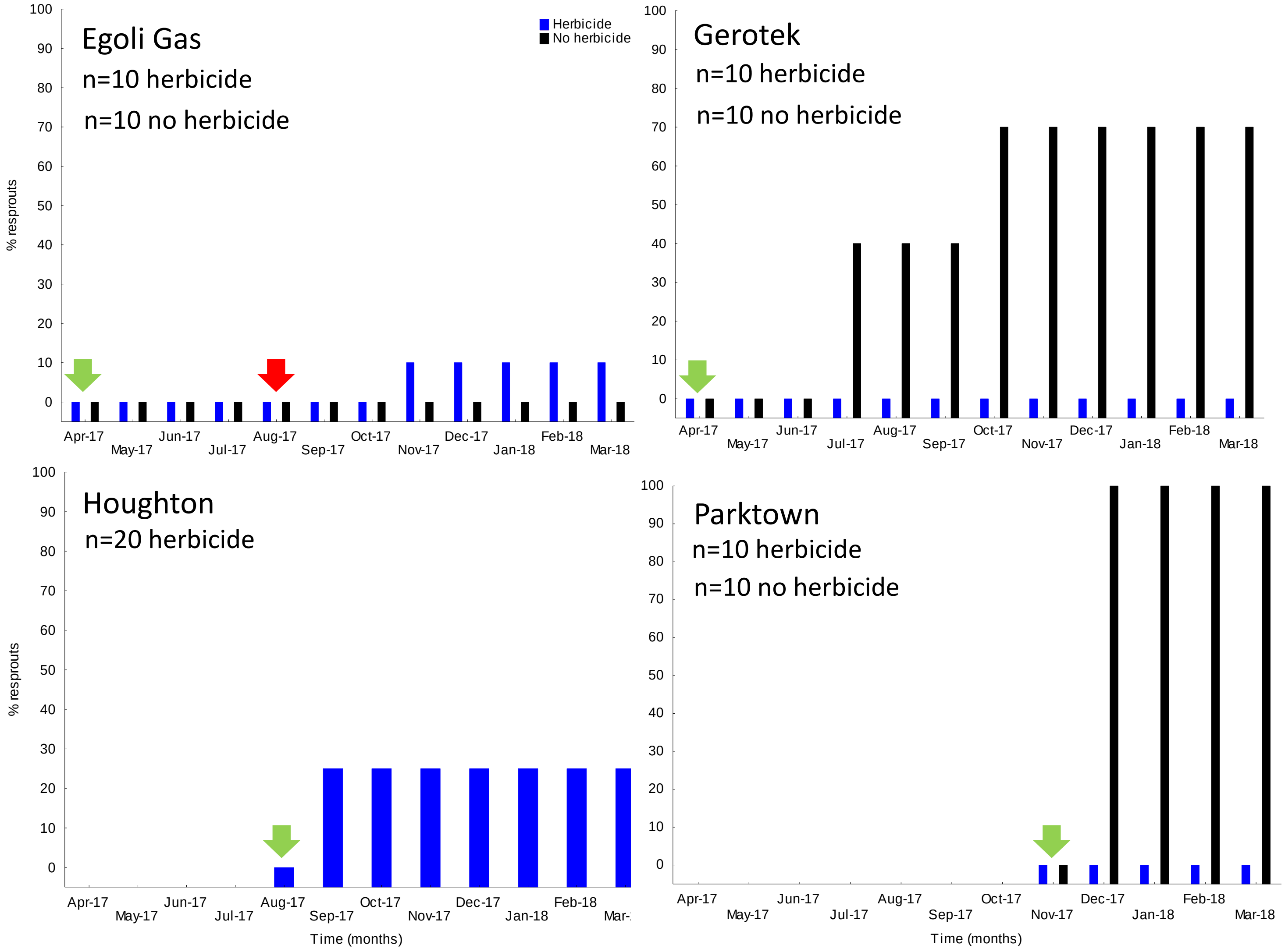

Figure 1: The percentage of *Solanum mauritianum* cut stumps that resprouted from the base at the four study sites. Cut stumps (N=20 per site) were separated into n=10 stumps that were cut only (mechanical control) and n=10 stumps that were cut and treated with 2% Hatchet solution (integrated control). All the stumps (n=20) in Houghton were treated with Hatchet (integrated control) at different cut heights to test the effect of cutting height on resprouting. Green arrows indicate the start of experiments in each site and the red arrow indicates the time when the fire occurred at Egoli Gas. (N=total count, n=sample count).

The site at Egoli Gas, located under tall Eucalyptus trees, was burned during an accidental fire that occurred for a week in August 2017. All cut stumps were burned and died, however one herbicide treated stump survived and later resprouted (Figure 2). The basal stem diameter (78.32mm) and cut height (3.7cm) above the ground of the resprouted stump is within the range of other cut and herbicide treated stumps at Egoli Gas (Table 2).

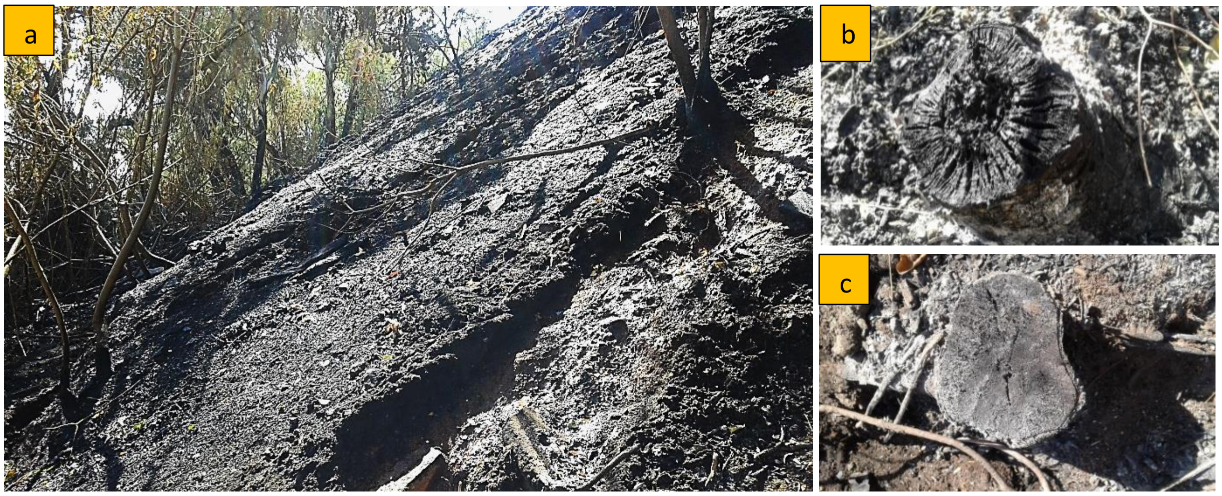

Figure 2: The remains of *Solanum mauritianum* stumps at Egoli Gas after a fire event that lasted for over a week in August 2017. (a) The burned site, (b) an example of a burned stump that died, and (c) the stump that was slightly burned but survived and subsequently resprouted. The photos were taken in August 2017.

Effect of stem diameter

The effect of stem diameter on *S. mauritianum* response to clearing was also tested. Although there were no significant differences in the mean stem diameter of dead and resprouted trees within each site (two-way ANOVA, $F_{(2,54)}$=0.473, P=0.626), the mean stem diameter of resprouted stumps at Gerotek was significantly greater than that of dead stumps at Parktown (Tukey HSD, P<0.001). Overall, stumps that resprouted in all sites had a significantly greater mean stem diameter (Least Squares means) than the dead stumps (two-way ANOVA, $F_{(1,54)}$=5.396, P=0.024). The mean stem diameter (Least Squares means) of all stumps cleared at Gerotek were significantly greater than those cleared at Parktown and Houghton (two-way ANOVA, $F_{(2,54)}$=7.795, P<0.001) (Table 1). Nevertheless, despite these differences in stem diameter, virtually all trees that resprouted were cut but not treated with herbicide (Table 2). The assessment of dead and resprouted stumps in Gerotek and Parktown showed differences, with 0% resprouting of herbicide treated stumps versus 85% in cut only (untreated) stumps (X^2_1=26.19, P<0.001). The herbicide treated stumps did not resprout except when they were cut higher, i.e. some of those at the Houghton site (Figure 3).

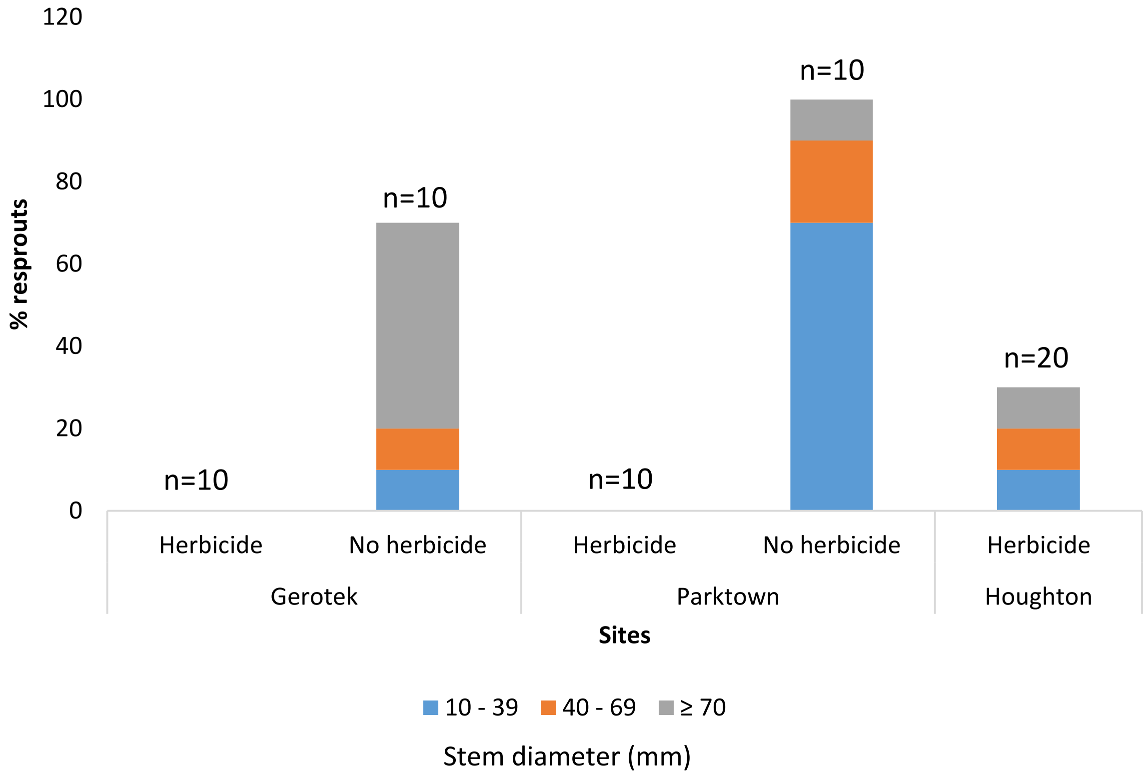

Figure 3: The effect of stem diameter on *Solanum mauritianum* response to clearing. There were N=20 stumps per site separated into n=10 cut and herbicide treated stumps (herbicide) and n=10 cut and untreated stumps (no herbicide), except at Houghton site where all n=20 stumps were treated with herbicide. The Egoli Gas results have been excluded from this analysis because of the influence of fire on the trees' response to clearing.

Table 1: Results of two-way ANOVAs to assess the influence of stem diameter and cut height on *Solanum mauritianum* response (resprouting or not) after clearing.

		Resprouted/Dead stumps			Site			Interaction		
(Figure 2)	Stem diameter (mm)	F=5.396	df=1,54	P=0.024	F=7.795	df=2,54	P<0.001	F=0.473	df=2,54	P=0.625
					Cutting season					
(Figure 5)	Stem diameter (mm)	F=8.151	df=1,36	P=0.007	F=20.287	df=1,36	P<0.001	F=0.308	df=1,36	P=0.582
					Cutting season					
(Figure 5)	Cut height (cm)	F=1.934	df=1,36	P=0.173	F=2.175	df=1,36	P=0.149	F=1.530	df=1,36	P=0.224

Integrated control, specifically cutting low and treating *S. mauritianum* stumps with 2% Hatchet was very effective, as seen at Gerotek: an example of a dead stump eleven months after clearing is shown in Figure 4. At Parktown, stumps cleared by cutting only (mechanical control) resprouted a month after clearing. Figure 4 shows an example of a stump that grew about 1m tall by four months after clearing.

Figure 4: The response of *Solanum mauritianum* to clearing by mechanical control (cut only) and integrated control (cut and treated with herbicide). The method of cutting low and treating stumps with 2% Hatchet shown (a) after cutting and (b) 11 months later at Gerotek and cutting only (c) immediately after cutting and (d) four months later.

Table 2: Assessment of post-clearing resprouting of *Solanum mauritianum* in Gauteng Province at Johannesburg and Pretoria sites. All sites had N=20 stumps separated into n=10 cut only (mechanical control) and n=10 cut and treated with herbicide (2% Hatchet) on the stumps (integrated control), except at Houghton where all n=20 stumps were cut and treated with Hatchet. An additional n=10 trees at Gerotek were cleared by Working for Water by cutting above-ground level and treating stumps with Hatchet in June 2017, were included. The stem diameter, cutting height and percentage of resprouted and dead stumps for each treatment in each site are presented.

	Johannesburg (Egoli Gas)		Pretoria (Gerotek)		Johannesburg (Parktown)		Johannesburg (Houghton)	Pretoria (Gerotek) Working for Water	**TOTAL**	
	Cut only	Cut + Herbicide	Cut only	Cut + Herbicide	Cut only	Cut + Herbicide	Cut + Herbicide	Cut + Herbicide	Cut only	Cut + Herbicide
No. of trees	10	10	10	10	10	10	20	10	30	60
Stem diameter (mm)										
Mean±SD	66.02±21.10	67.70±18.01	65.90±33.24	45.66±14.87	36.10±23.18	20.18±4.14	47.02±29.99	20.96±7.16	56.01±29.20	41.42±25.71
Range	40.37-95.46	35.65-89.69	23-119.37	21.41-70.72	11.5-78	11.5-27	19.53-134.63	13.05-36.55	11.5-119.37	11.5-134.63
Cut height (cm)										
Mean±SD	3.55±1.25	3.55±1.26	3.7±2.34	5.66±1.65	3.65±0.79	3.73±0.89	24±18.55	15.90±7.14	3.63±1.54	12.81±14.37
Range	2-5.6	1.9-5.5	1.3-9.1	2.3-7.8	2.30-4.8	2.57-5.23	0-50	9-30.5	1.3-9.1	0-50
Resprouted trees	0%	10%	70%	0%	100%	0%	30%	0%	57%	12%
Dead trees	100%	90%	30%	100%	0%	100%	70%	100%	43%	88%

Effect of the number of stems per tree

The assessment of cleared trees at Parktown showed that all herbicide-treated stumps were killed and all untreated stumps resprouted (Table 2). Furthermore, the effect of the number of cut stems per tree was assessed. The killed trees had a significantly greater mean number of cut stems compared to the resprouted trees ($t_{18}=3.918$, $P<0.001$). However, only untreated stumps resprouted and herbicide treated stumps were killed (Figure 5). This further shows that the method of cutting low and immediately treating stumps with Hatchet is the solution to killing *S. mauritianum*, regardless of the number of stems a tree may have.

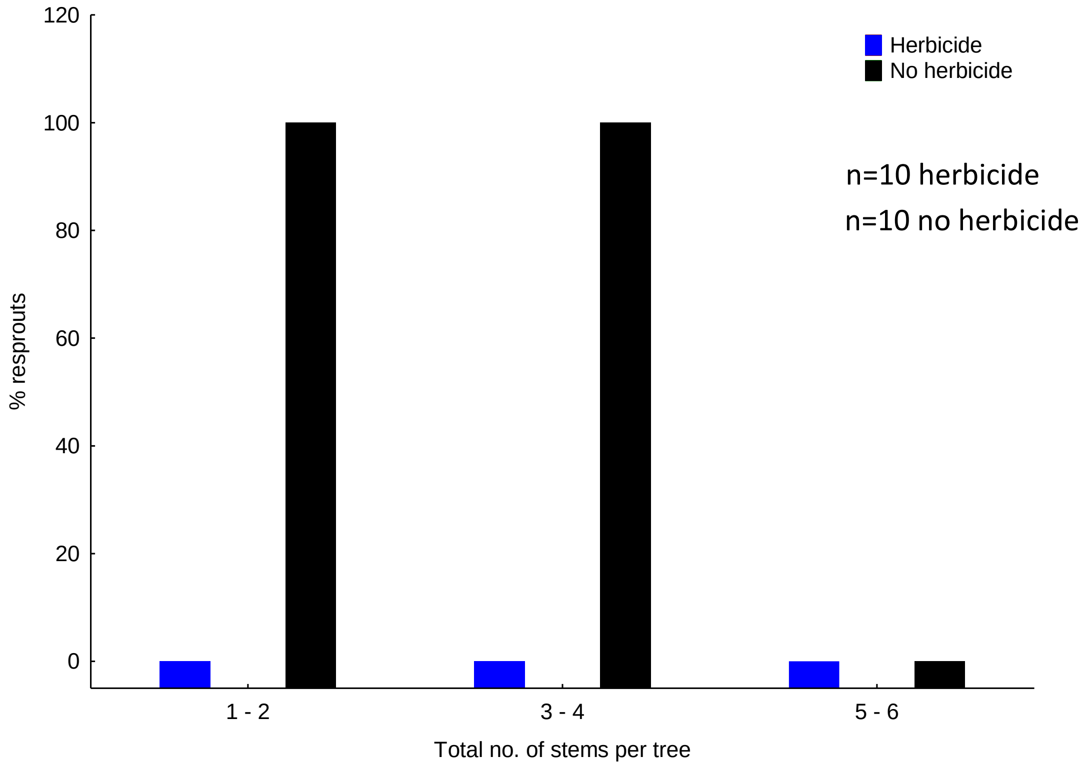

Figure 5: The effect of the number of stems per tree on how *Solanum mauritianum* responds to clearing. At the Parktown site n=10 stumps were cut only and n=10 stumps were cut and treated with Hatchet in November 2017. The percentages of resprouted stumps by March 2018 are presented.

Effect of cutting height

The assessment of the effect of cutting height was tested between Houghton and Gerotek (cleared by WfW) trees (Table 2). The trees in both sites were cut at different above-ground heights in winter (June and August 2017), however no stumps at Gerotek resprouted, whereas 30% resprouted at Houghton (Figure 6).

The mean cutting heights of dead and resprouted stumps in Houghton were not significantly different (t_{18}=0.195, P=0.847), while the mean cutting height of dead stumps was not significantly different between Houghton and Gerotek (t_{22}=1.078, P=0.293) (Figure 6). The mean stem diameter of dead stumps at Houghton was significantly greater than that of the dead stumps at Gerotek (by WfW) (t_{22}=2.395, P=0.026). Therefore for above-ground level cut and herbicide treated stumps, bigger stem diameter stumps are more likely to resprout.

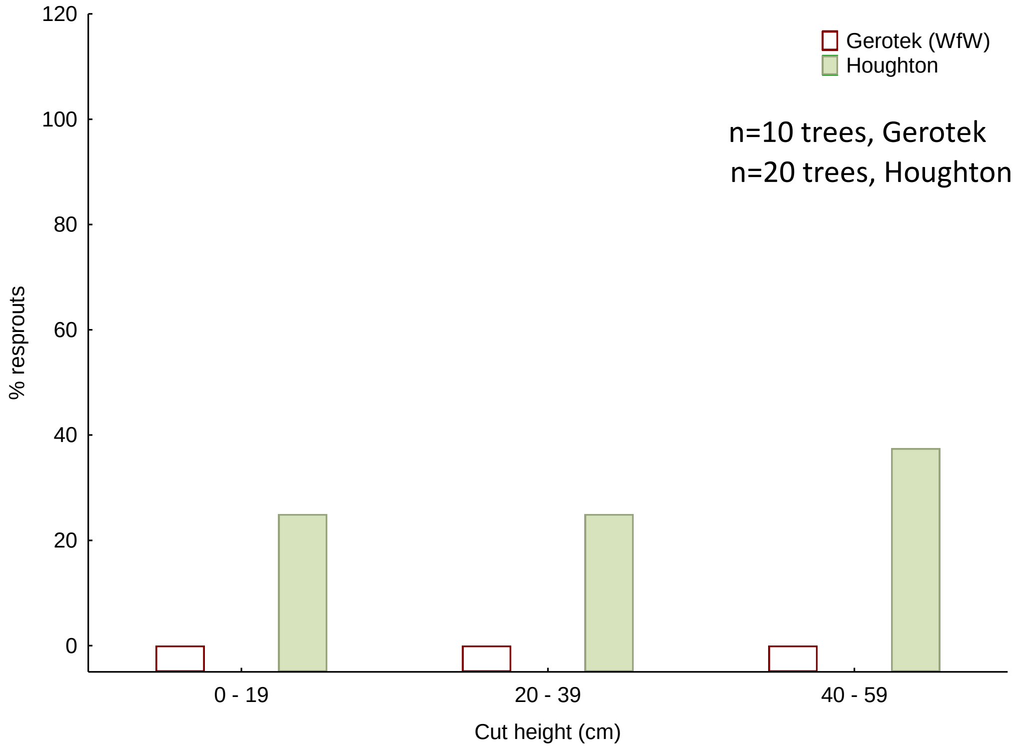

Figure 6: The effect of cutting height on resprouting of *Solanum mauritianum* after clearing. Trees at Gerotek (cleared by Working for Water) and Houghton were all cut and treated with 2% Hatchet. At Gerotek n=10 trees were cleared in June 2017 and at Houghton n=20 stumps were cleared in August 2017. All stumps were cut above-ground level, with Houghton stumps cut up to 50cm and Gerotek stumps up to 30.5cm high.

The method of cutting higher on the stem and applying herbicide on stumps resulted in resprouting of some stumps with greater stem diameter size at Houghton (Table 2) and the basal resprouts grew to about 1m tall by eight months after clearing (Figure 7). At Gerotek none of the stumps cleared by Working for Water resprouted and an example of such a stump is shown in Figure 7.

Figure 7: The effect of cutting height on the response of *Solanum mauritianum* after clearing. All cuts at Houghton and Gerotek (by Working for Water) were done at above-ground level and stumps treated with 2% Hatchet. The response of trees to clearing at (a) Houghton and (b) Gerotek is presented.

Effect of cutting season

There were no significant differences in the mean cutting height of dead and resprouted stumps between (two-way ANOVA, $F_{(1,36)}$=2.175, P=0.149) or within cutting seasons (two-way ANOVA, $F_{1,36)}$=1.530, P=0.224). The mean stem diameter of dead and resprouted stumps were not significantly different within each cutting season (two-way ANOVA, $F_{(1,36)}$=0.308, P=0.582). Nevertheless, significant differences in mean stem diameter of dead stumps between autumn and spring (Tukey HSD, P=0.022), between stumps that died in spring and stumps that resprouted in autumn (Tukey HSD, P<0.001), and between stumps that resprouted in autumn and spring (Tukey HSD, P=0.011), were observed. Overall, the mean stem diameter (Least Squares means) of resprouted stumps were significantly greater compared to dead stumps (two-way ANOVA, $F_{(1,36)}$=8.151, P=0.007) (Table 1). The mean stem diameter (Least Squares means) of stumps cleared in autumn were significantly greater than those cleared in spring (two-way ANOVA, $F_{(1,36)}$=20.287, P<0.001) (Table 1). It is clear that the clearing of *S. mauritianum*, whether done in autumn or spring, even with differences in stem diameter between the cleared trees, is more effective when the method of cutting low and treating stumps with herbicide (integrated control) is applied

(Figure 8). Furthermore, 30% of the stumps cleared in autumn by cutting at ground-level without herbicide application died compared with 0% for spring (Figure 8). Furthermore, resprouting took longer on autumn cuts (3 months) compared to spring cuts (1 month).

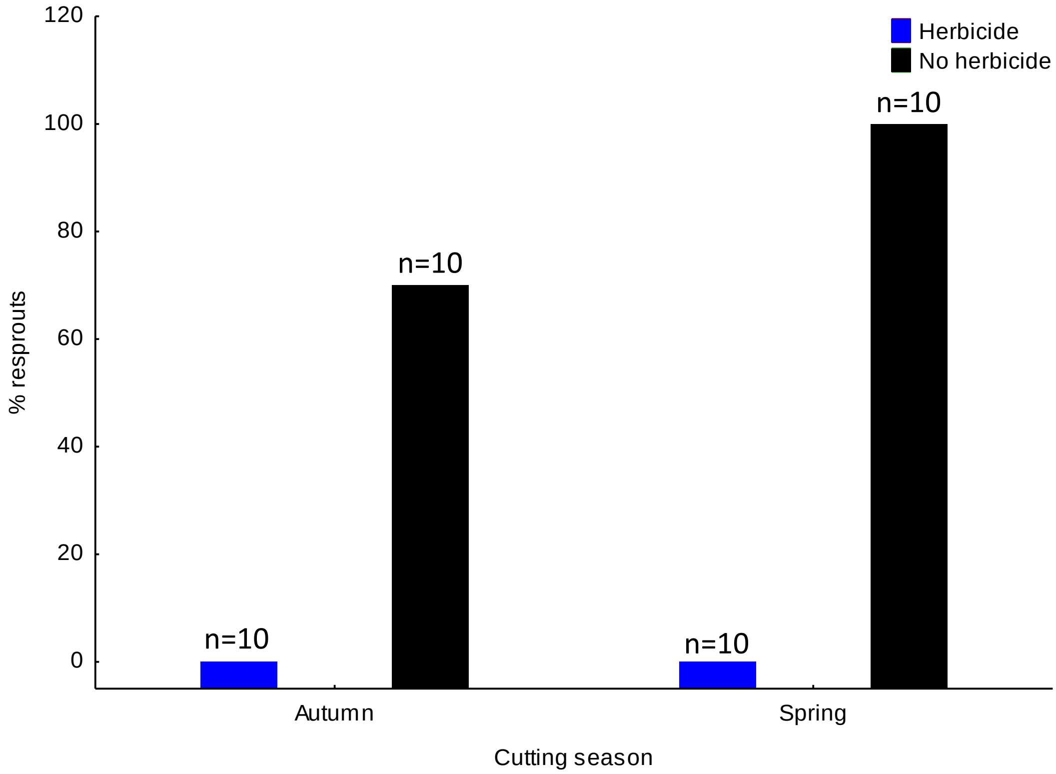

Figure 8: The effect of the cutting season on *Solanum mauritianum*'s ability to resprout after clearing. The percentage of resprouting trees is compared between two cutting seasons, autumn and spring. At Gerotek, *S. mauritianum* trees were cleared in autumn (April 2017), while at Parktown clearing was done in spring (November 2017). Each site had a total of N=20 trees divided into n=10 trees that were cut only (mechanical control) and n=10 trees that were cut and treated with 2% Hatchet (integrated control). The percentages of resprouted trees by March 2018 were compared.

Discussion

Initial analysis showed that virtually all the stumps in all sites that were cut at ground level and treated with Hatchet did not resprout. This was a clear indication that mortality was strongly driven by cutting low and applying the herbicide treatment. Significant mortality has been shown in other softwood species such as *Ligustrum sinense* Lour (Oleaceae) when stumps are cleared by cutting low (2.5cm above-ground level) and herbicide-treated as compared to untreated stumps (Enloe *et al*. 2018). Resprouting of stumps cleared by cutting only was observed at Egoli Gas and Gerotek. Other studies have also shown that the method of clearing stumps of *Alianthus altissima* (Miller) Swingle, a softwood trees, by cutting only increases the percentage of resprouts compared to cut and herbicide treatment (Butler and Britting 1998; DiTomaso and Kyser 2007). Unexpectedly, a stump cleared by cutting low and herbicide treatment at Egoli Gas resprouted after a fire occurred that killed all other stumps. Fire was an unpredicted factor in this study; it is unclear why the stump resprouted especially given that fire can be very effective at controlling some weeds such as *Chromolaena odorata* (L.) King and Robinson [Asteraceae] in grassland (Goodall and Erasmus 1996; Mbalo and Witkowski 1997; Witkowski and Wilson 2001). Fire is known to stimulate germination of *S. mauritianum* seeds (Olckers 2011). Previously, *G. decoris* outbreaks in Mpumalanga near Sabie were destroyed by extensive and severe forest fires (Olckers 2011), but the response of *S. mauritianum* to fire was not noted, and in general needs more extensive research across a range of different ecosystems.

Stem diameter did not have a significant effect on plant response when trees were cut low and treated with herbicide or cut low and untreated. Rather, cutting low and herbicide treatment consistently killed more trees compared to cutting only, and this further highlights that this is the solution to killing *S. mauritianum*.

Parktown was the only site where cleared trees, including cut only and cut and treated with herbicide, were multi-stemmed. Dead trees had significantly more stems compared to resprouted trees. Multi-stemmed trees could possibly have increased chances of resprouting because of bark inclusions, the area where two stems or branch and trunk meet and are joined by bark (Enloe *et al*. 2018). Bark inclusions may limit herbicide translocations if cuts are made where the bark inclusions are located (Enloe *et al*. 2018). However, this was not true for *S. mauritianum*, hence despite the differences in stem numbers between dead and resprouted stumps, the result of 100% mortality of cut low and herbicide treated stumps remained consistent with single-stemmed trees. Therefore having more stems does not influence the outcome of clearing for *S. mauritanum* trees. The cutting and herbicide treatment of a single stem within a multi-stemmed softwood shrub called European buckthorn *Rhamnus cathartica* L. (Rhamnaceae) can result in the death of an entire shrub, thereby also reducing material and labour costs (Pergams and Norton 2006).

Cutting height as a possible influence to resprouting was tested. More trees resprouted at Houghton although there were no differences in cutting height compared to Gerotek. Witkowski and Garner (2008) found that for *S. mauritianum* clearing by cutting and herbicide application, cutting low <18cm caused 100% mortality while cutting higher increased resprouting with 100% resprouts for stumps cut at >50cm, and that stem diameter of stumps had no effect on the outcome of stumps post-clearing. For above-ground level cuts the stem diameter size had an effect on whether trees died or resprouted, which ultimately means that when cutting higher and treating stumps with Hatchet the stumps with bigger stem diameter sizes are more likely to resprout compared to the smaller stems. Witkowski and Garner (2008) suggested that cutting higher may not allow the herbicide to penetrate down the plant sufficiently and thus increases the chance to resprout, and this study adds that this is particularly true for stumps that have bigger stem sizes as seen in Houghton. Increased resprouting of a softwood species such as *L. sinense* after clearing by cutting low and herbicide treatment or no treatment has been attributed to larger rootstocks (Enloe *et al*. 2018). Also, the clump size of resprouts is known to be positively correlated with stem diameter in the hardwood tree *Acer macrophyllum* Pursh (Aceraceae) (Tappeiner *et al*. 1996). Perhaps bigger stumps, by any measure, limit the penetration of the herbicide to growing points of the plant, such as roots, and this can cause stumps to reprout. DiTomaso and Kryser (2007) showed that a relatively high cut (60cm) and herbicide treatment of *A. altissima* stumps causes high mortality compared to cut only stumps, and perhaps the low level of resprouting observed with this method could also be attributed to bigger stem diameter sizes. Khan and Tripathi (1986) found a negative correlation between resprouting and stem diameter and a positive correlation between resprouting and stump height, and the reason for decreased resprouting of bigger stems was because bigger trees mainly reproduce sexually and not by vegetative means. The low resprouting of tall stumps was attributed to few available buds for resprouting (Hook and DeBell 1970; Khan and Tripathi 1986).

The differences in seasons (spring and autumn) and stem diameter sizes in dead and resprouted trees between the seasons did not show a significant effect on *S. mauritianum* response after clearing. This suggests that the timing of the cuts has no significant influence on the outcome of the stumps following clearing, but rather herbicide treatment has a strong influence. Similarly, Boudreau and Wilson (1992) found that *R. cathartica* stumps cleared by cutting and herbicide treatment, had 100% mortality for both autumn and summer cuts. Herbicides should move to growing points in the plant in order to successfully kill it by disrupting the balance of biochemical processes, but plant physiological processes change in different seasons and this may influence the effectiveness of clearing (Ballard and Nowack 2006). In summer rainfall areas during the autumn season plant sugars move down to the roots for winter storage, and this could allow for sufficient herbicide movement to the roots for effective control, but in spring the phloem transport is upward and may push the herbicide out and thus make it ineffective (Ballard and Nowack 2006; Tjosvold and McHenry 1986). This may explain the mixed results that have been observed in other studies, whereby effectiveness of clearing changes when cuts are done in different seasons (Enloe *et al*. 2018; Delanoy and Archibold 2007; Kochenderfer *et al*. 2006; Harrington 1984; Petrice and Haack 2011; Kays and Canham 1991; Johansson 1992; Bell *et al*. 1999).

Resprouting of stumps cut only was delayed (3 months) on stumps that were cleared in autumn compared to only 1 month in those cut in spring. The downward flow of photosynthates to the roots in autumn for winter storage during which period they are not assimilated for above-ground growth can also be attributed to the delayed resprouting of stumps (Ballard and Nowack 2006; Enloe *et al*. 2018). The reason for the 30% mortality of stumps that were cut low only is unclear but such mortality has also been observed in prolific sprouters such as *L. sinense* and *A. altissima* (Burch and Zedaker 2003; Enloe *et al*. 2018).

Conclusion

The management of *S. mauritianum* in the Highveld will be significantly improved by cutting very low and applying Hatchet. This method is less damaging to indigenous species if carefully applied, compared to foliar spraying (Krupek *et al*. 2016). Cutting higher on the stem will have implications of trees resprouting and ultimately the infestation will not be controlled. The immediate application of Hatchet, within a few minutes, is very important for effective herbicide penetration to stumps. Unfortunately in cases where cutting low is impossible due to obstacles such as logs and rocks, trees with bigger stems are more likely to resprout. Tree mortality may increase with increased herbicide dosage at higher cutting heights however the effect of herbicide dosage at different cutting heights was not tested in this study, but it is suggested as a potential future study. The management of *S. mauritianum* with this technique will be effective when done in autumn and spring in the Highveld however it is expected that it will also be successful in summer and winter but this needs to be directly tested first. However, the challenge is that *S. mauritianum* trees produce many seeds, which can be dormant for >2 years in the soil, that ultimately will contribute to the emergence of seedlings after clearing and become the new invasion foci (Witkowski and Garner 2008). Post-clearing resprouts are fast-growing and reach reproductive maturity within a year, therefore adding to the recovery and spread of the weed (Witkowski and Garner 2008). Nonetheless, in the Sabie region, where *G. decoris* populations have established, their outbreaks are known to completely defoliate *S. mauritianum* trees and significantly reduce seed production (Witt 2007). Therefore the integration of this biocontrol agent with the cut and herbicide treatment is strongly recommended in the Lowveld where it has established.

CHAPTER FOUR

Synthesis and conclusion

Solanum mauritianum is highly invasive in South Africa and has negative effects on agriculture and biodiversity (Olckers and Zimmermann 1991; Olckers 1998; Olckers 1999; Henderson 2001; ISSG 2006). It has a long history of invasiveness in the country and some efforts have been made to control the infestations (Olckers 1999; Witkowski and Garner 2008; Olckers 2011; Cowie *et al*. 2018). Biocontrol efforts in South Africa have seen the release of two biocontrol agents, namely *G. decoris* and *A. santacruzi* (Lotter 2004; Olckers 2008). Both agents have established in many sites in KwaZulu-Natal but *G. decoris*' impact is trivial (Lotter 2004; Olckers 2011; Patrick and Olckers 2014) while *A. santacruzi* impact remains unknown (Klein 2011). This is troubling especially given that the expectation in biocontrol programmes is to ultimately control infestations.

The efficacy of *G. decoris* is thought to be strongly restricted by predation (Lotter 2004), but Cowie *et al*. (2016a) showed that it can be highly damaging to *S. mauritianum*. Given its high potential it follows that *G. decoris* should be tested in different ecological and climatic situations to control *S. mauritianum*. The establishment of *G. decoris* in the Highveld failed even though predators were excluded from trees with biocontrol agents as suggested by Lotter (2004). Predation was avoided and rather poor plant quality may be linked to low survival and failed establishment. Environmental factors affect plant growth and plant physiological condition such as chlorophyll content, i.e plant quality, and in turn this affects the performance and populations dynamics of herbivorous insects in the field. Plants with high growth vigour can be more favourable to insects or physiologically stressed plants can be more susceptible to insects (De Bruyn *et al*. 2002). These factors may affect insect fecundity, diapause and insect outbreaks among others (Baylis and Pierce 1991; Hunter and McNeil 1997; Awmack and Leather 2002). For example, the larvae of the Australian weevil *Oxyopsvitiosa* (Pascoe) (Coleoptera: Curculionidae) which is the biocontrol agent of the tree *Melaleuca quinquenervia* (Carv.) ST Blake (Myrtaceae: Myrtales), had low survival when feeding on poor quality leaves with high leaf toughness and percentage dry biomass (Wheeler 2001). This knowledge helped to select suitable sites and facilitated the establishment of *O. vitiosa* (Wheeler 2001). Wheeler (2001) described leaf quality from the field by leaf colouration but later confirmed this in the lab. In the Highveld *S. mauritianum* leaves have a lighter green colour and are smaller compared to darker green and larger leaves found in the Lowveld i.e KwaZulu-Natal (personal observation), and this could mean that Highveld trees have poor quality compared to Lowveld trees.

The insect predators found killed by ant-tack and excluded by bagging was evidence that predation could be a potential threat to the survival of the biocontrol agents, therefore predator exclusion should always be considered in future releases. Although *G. decoris* failed to establish, given these insights, the research on *G. decoris* establishment in the Highveld should still be pursued. Larger and frequent releases of *G. decoris* are important to consider for successful establishment in future, because they can increase the chances of finding a mate and successful reproduction. Releases of at least 1000 individuals are also recommended for successful establishment of *G. decoris* (Winks 2014). Large numbers of biocontrol agents will also inflict more damage to the trees. In New Zealand *G. decoris* has successfully established on the North Island, but their impact on *S. mauritianum* is not well understood (Winks 2014). A single outbreak in New Zealand has occurred and was very damaging, similar to outbreaks reported in South Africa (e.g. Witt 2007).

Anthonomus santacruzi has established in KwaZulu-Natal but is predicted to not establish in the Highveld because of low temperature and humidity in winter (Cowie *et al*. 2016b). It may however utilize suitable microhabitats in the Highveld to survive. Its feeding on the floral parts can significantly reduce the reproductive capacity of *S. mauritianum* (Olckers 2003; Cowie 2014; Cowie *et al*. 2017), and with successful establishment and increase in abundance it could result in adequate control of the weed. Given that *A. santacruzi* is a flower-feeder but it was released here on trees that did not produce flowers for the duration of the experiments probably explains why it failed to survive and establish. It was assumed that releases could be done at any time of the year given that *S. mauritianum* is known to flower throughout the year (Olckers 2009). However this is not necessarily true at each location of occurence, so often *S. mauritianum* does not flower throughout the year, at least in the Highveld. The phenology of *S. mauritianum* on the Highveld should be studied and this will help with the timing of future releases to increase chances of successful agent establishment. Also, the collection of *A. santacruzi* from established populations in KwaZulu-Natal can be costly and may not always be successful given that the local municipality in KwaZulu-Natal has programmes that cut down the trees to control infestations, which in turn affects agent populations. Therefore, ideally the agents should be collected before the trees are cut down. The importation of *A. santacruzi* that is better suited to Highveld conditions from its native range could also be considered (Cowie *et al*. 2016b; Singh and Olckers 2017). Prospective agents considered for testing such as the weevil *Anthonomus morticinus* Clark (Coleoptera: Curculionidae) and the mite *Aponychus schultzi* Blanchard (Acari: Tetranychidae) should be introduced to the country for further host-specificity testing (Cowie *et al*. 2018).

In addition to being released on flowering trees, the releases should be done in the warm and rainy season (summer) and at more sites around Gauteng. Release sites should also include areas that experience frost, in order to understand the limitations of the agent in the field. Altogether, once the sites with a higher chance of establishment are known, the release of both *A. santacruzi* and *G. decoris* should be done to assess their combined effect on *S. mauritianum*. More importantly, the efficacy of the combined agents should be assessed at sites where both *A. santacruzi* and *G. decoris* have already established such as the KwaZulu-Natal sites.

Integrated control, the combination of two methods, namely the cutting and herbicide treatment methods can easily kill *S. mauritianum*. But trees can resprout if the method is not done properly (Beard 1951; Pickworth 1971; Hodgson 1978; Vermeulen and Grobler 1987; Witkowski and Garner 2008). Many factors must be taken into account to achieve high mortality of the trees and these include; cutting height, stem diameter, cutting season and the number of stems on cut trees (Kramer and Kozlowski 1979; McLaughlin *et al*. 1980; Crist *et al*. 1983; Harrington 1984; Kays and Canham 1991; McCreary *et al*. 1991; Jobidon 1997; Witkowski and Garner 2008). Cutting low and immediately applying Hatchet was found to be the solution to killing *S. mauritianum*, regardless of stem size, the number of stems per tree and cutting season. This means that programmes directed at controlling *S. mauritianum* can focus on cutting low and applying Hatchet in order to kill the weed. This method might be useful to control *S. mauritianum* in many areas in South Africa outside of the Highveld as well. Programmmes such as Working for Water can now implement, as part of their strategy, that cut and herbicide treatments can be done successfully in spring and autumn seasons. Although this seems to be the solution to killing *S. mauritianum*, the method remains costly compared to biocontrol. It will probably be ideal on a smaller scale i.e. in farms, private homes and other small infestations. Given that the control of *S. mauritianum* using a cut and herbicide treatment is expensive and may not be feasible particularly for large areas, the next step would be to aim for integrated control including the cut and herbicide treatment together with biocontrol in order to achieve sustainable control of the weed (Olckers 2011). However, *Solanum mauritianum* produces enormous seedbanks with extensive seedling recruitment when the conditions are favourable, making it difficult to supress the weed (Campbell *et al*. 1992; Goodall *et al*. 2017). Therefore integrated approaches should include appropriate follow-ups to increase their effectiveness (Goodall *et al*. 2017).

To date great efforts have been made on biocontrol of *S. mauritianum*, from searching for biocontrol agents, to host specificity testing and finally to releasing some biocontrol agents in the field, but more work should be done. The integration of biocontrol together with the cut and herbicide application treatment is something to look forward to in future research (Olckers 2011). Perhaps biocontrol agents can be released on trees that resprout after being cut and treated with Hatchet. If *G. decoris* can establish in the Highveld, which it did not, then having a biocontrol reserve would be ideal, but this would be very difficult and costly for *A. santacruzi*. It is also imperative that more damaging natural enemies be sought from the native range to complement the two biocontrol agents. Therefore, research aimed at developing new *S. mauritianum* agents should be prioritized by relevant research institutions.

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Appendix

Table 1: Description of the study sites, using Mucina *et al*. (2006) and Rutherford *et al*. (2006).

Site	GPS coordinates	Municipality	Altitude	Mucina and Rutherford Vegetation Types
Egoli Gas	26˚ 11' 17.52"S 28˚ 01' 05.88"E	City of Johannesburg	1280 - 1660m	Egoli Granite Grassland
Gerotek	25˚ 45' 34.68"S 28˚ 00' 25.20"E	City of Tshwane	1050 - 1450m	Open to closed, low, often thorny savanna
Houghton	26˚ 10' 39.12"S 28˚ 03' 05.40"E	City of Johannesburg	1280 - 1660m	Egoli Granite Grassland
Parktown	26˚ 10' 33.59"S 28˚ 02' 52.73"E	City of Johannesburg	1280 - 1660m	Egoli Granite Grassland