Native range studies on insect herbivores associated with fireweed (*Senecio madagascariensis*) in KwaZulu-Natal, South Africa, with prospects for biological control in invaded countries

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CONTENTS

DECLARATION	……	2
ACKNOWLEDGEMENTS	……	3
LIST OF PUBLICATIONS	……	4
ABSTRACT	……	5
INTRODUCTION	……	8
CHAPTER 1:	Insect herbivore taxa associated with the invasive *Senecio madagascariensis* (Asteraceae) in its native range in KwaZulu-Natal, South Africa and their potential as biological control agents for Australia and Hawaii ……	23
CHAPTER 2:	Seasonal abundance of insect herbivore taxa associated with the invasive *Senecio madagascariensis* (Asteraceae) in its native range in KwaZulu-Natal, South Africa ……	41
CHAPTER 3:	Establishment and impact of insect agents deployed for the biological control of invasive Asteraceae: Prospects for the control of *Senecio madagascariensis* ……	57
CHAPTER 4:	DNA barcoding provides insight into the field host range of endophagous insects associated with *Senecio madagascariensis* (Asteraceae) in its native range in KwaZulu-Natal, South Africa ……	76
CHAPTER 5:	Potential efficacy of biological control of fireweed in Australia: No difference in plant biomass or native insect assemblages between native and invasive populations, despite differences in pyrrolizidine alkaloids ……	102
DISCUSSION	……	133
APPENDIX:	Supplementary material for Chapter 3 ……	150

DECLARATION

The research described in this thesis was carried out in the School of Life Sciences (Pietermaritzburg campus), College of Agriculture, Engineering and Science, University of KwaZulu-Natal, from February 2011 to July 2017 under the supervision of Dr T. Olckers and Dr K. Harvey.

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

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LIST OF PUBLICATIONS

This thesis represents a conversion from an earlier dissertation for which the degree of Master of Science was awarded. Chapters from this dissertation have been synthesized and incorporated into this thesis in revised form (Chapters 1-3). An additional two chapters have been added to the thesis, based on additional work undertaken (Chapters 4-5). Data from some of the chapters from the original dissertation have since been published. These outputs are listed as follows:

Egli D. 2013. Insect herbivores associated with species of Senecio in KwaZulu-Natal, South Africa with emphasis *on Senecio madagascariensis*, a native plant that has become invasive in Australia. MSc thesis, University of KwaZulu-Natal, Pietermaritzburg, South Africa.

Egli D, Olckers T. 2015. Abundance across seasons of insect herbivore taxa associated with the invasive *Senecio madagascariensis* (Asteraceae), in its native range in KwaZulu-Natal, South Africa. African Entomology **23**: 147–156. [Chapter 2]

Egli D, Olckers T. 2017. Establishment and impact of insect agents deployed for the biological control of invasive Asteraceae: Prospects for the control of *Senecio madagascariensis*. BioControl **62**: 681–692. [Chapter 3]

ABSTRACT

Senecio madagascariensis Poir. (fireweed; Asteraceae), native to southern Africa and Madagascar, has invaded Australia, Hawaii, South America and Japan, reducing pastoral productivity and poisoning livestock. Interest in biological control by Australia and Hawaii led to initial surveys for potential insect agents in Madagascar. However, molecular evidence revealed that both the Australian and Hawaiian populations originated from KwaZulu-Natal Province, South Africa. Efforts to find suitable biocontrol agents have since been focussed within this region. Studies on potential biocontrol agents in the weed's native range, which consider its centre of origin, closely related plants and seasonal variation in the abundance of its natural enemies, can provide valuable information for selecting host specific and effective agents. This study aimed to prioritise potential insect agents for Australia and determine their response to possible changes in alkaloid concentrations in plants from within the invaded Australian range.

The insect herbivore fauna associated with *S. madagascariensis* was quantitatively surveyed across 21 sites in KwaZulu-Natal to provide a comprehensive list of herbivores and identify potential agents. A total of 64 herbivorous taxa were recorded. Many of these were recorded rarely, but at least 17 taxa were considered as potential agents having been successful in previous biological control programs. Of these, the most promising were a capitulum feeder (Lepidoptera: Pyralidae), three stem borers (Coleoptera: Curculionidae; Diptera: Tephritidae; Lepidoptera: Tortricidae) and a root-feeding flea beetle (Coleoptera: Chrysomelidae: Alticinae).

Biological control programs are more successful when agents attack the target plants throughout the year. The abundance of the insects associated with *S. madagascariensis* was sampled once per season at two sites in Pietermaritzburg to determine which are present throughout the year and are thus capable of inflicting sustained damage. There was significant seasonal variation in the abundances of the main insect taxa. Of the most promising potential agents, four were recovered all year round, two during three seasons, two during two seasons and one during summer only. Releasing a complement of natural enemies that attack the plant at different times may thus be required to ensure that *S. madagascariensis* is attacked throughout the year.

A number of invasive Asteraceae have been targeted for biological control. An evaluation of the successes and failures of different insect taxa and feeding guilds used in previous programs was carried out to prioritise agents that are most likely to be successful on fireweed. The most effective insect taxa for the biocontrol of species of Asteraceae were Coleoptera (Curculionidae and Chrysomelidae) and Lepidoptera (Pterophoridae and Tortricidae), while root-feeding and stem-feeding species were the most effective guilds. This verified that the root-feeding flea beetle (Chrysomelidae), stem-boring moth (Tortricidae) and stem-boring weevil (Curculionidae) should be prioritized as candidate agents for *S. madagascariensis*.

Agent host specificity is particularly important for Australia which has 87 native *Senecio* species (Hawaii has none). The field host range of endophagous Coleoptera, Lepidoptera and Diptera associated with fireweed was assessed by comparisons of these taxa across 18 *Senecio* species native to South Africa. Ten plants of each *Senecio* species were collected from each of three sites. The COI gene of insect larvae recorded within the tissues of the various *Senecio* species was sequenced to assess their host specificity. Stem-boring Curculionidae, capitulum-feeding Diptera and stem-boring and capitulum-feeding Lepidoptera that were recorded on *S. madagascariensis* were restricted to the *Senecio madagascariensis* species complex, and could thus be suitable for release in Australia and almost certainly in Hawaii. Laboratory host-range tests in Australia are needed to confirm the specificity of these insects.

Invasive species have a large adaptive capacity to establish successfully in new environments and may evolve in response to the new range. It is imperative to understand whether any adaptive or evolutionary response can influence a weed's interaction with natural enemies from the native range. In particular, increased alkaloid concentrations in Australian fireweed populations may affect the efficacy of insect biocontrol agents. A field experiment compared the biomass, insect assemblages recruited in the field, and alkaloid profiles between invasive Australian and native South African populations of *S. madagascariensis*. Minimal variation in plant biomass and insect community composition was detected, despite some variation in alkaloid composition and concentrations, between the regions and countries. There was no relationship between alkaloid concentrations and insect communities indicating that potential insect biocontrol agents are unlikely to be affected by increased plant defences in Australian fireweed populations.

Several insect agents were prioritised for further consideration in Australia based on the different criteria examined in this thesis. The root-feeding flea beetle (*Longitarsus basutoensis* Bechnyé), stem-boring weevil (probably *Gasteroclisus tricostalis* (Thunberg)) and stem-boring tortricid moth (unidentified species) will be the focus of additional field and laboratory studies that examine their life cycles and host range, prior to their introduction into quarantine in Australia.

Key words: agent selection, DNA barcoding, field host range, invasive plants, phytophagous insects, pyrrolizidine alkaloids

INTRODUCTION

Invasive alien plants

The recent increase in human global movements has resulted in a drastic increase in the number of species introduced to foreign countries, both intentionally and accidentally (Mack *et al*. 2000; Mack 2003; Ceddia *et al*. 2009; Hulme 2009). This has led to a worldwide increase in the number of invasive species (Mack *et al*. 2000; Mack 2003; Hulme 2009). Most of these introduced species are plants (Pimentel *et al*. 2001). Although few introduced plants establish and only around 1% become invasive, invasive plants have considerable negative impacts in their introduced range (Williamson & Fitter 1996; Pyšek *et al.* 2012). This problem is likely to get worse due to the increasing extent of global trade and human movement, but also climate change (Le Maitre *et al*. 2004).

In natural environments, invasive plants pose significant threats to biodiversity by displacing native plants, which can result in their extinction and can indirectly affect other organisms (Pimentel *et al*. 2001). Invasive plants can interfere with ecosystem services such as water and nutrient cycles (Stock *et al*. 1995; Callaway & Aschehoug 2000; Mack *et al*. 2000), and can alter fire regimes and ecosystem energy budgets (Mack *et al*. 2000). While the economic costs of losses to biodiversity are difficult to quantify, the control of alien invasive plants is expensive. In 2001, the USA spent an estimated US$ 148 million on the control of environmental weeds (Pimentel *et al*. 2001), and environmental damages and losses caused by invasive species added up to just under 120 billion per year (Pimentel *et al*. 2005).

Invasive plants also cause greater losses to agriculture than any other group of organisms (Pimentel *et al*. 2001). Annually, agricultural weeds have cost the economy of the USA an estimated US$ 26.4 billion (Pimentel *et al*. 2000) and the Australian economy an estimated A$ 4 billion (Briese *et al.* 2004). Pasture weeds cause huge economic losses by decreasing productivity, replacing desirable pasture species, and in the case of some toxic species, poisoning livestock (Mack *et al*. 2000; Pimentel *et al*. 2000, 2005). The estimated losses to pastures in the USA, Brazil, the UK, South Africa, India and Australia was US$ 7.52 billion per year for all countries combined (Pimentel *et al*. 2001).

Invasive plants are commonly controlled chemically, mechanically (including cultural techniques) or biologically (Culliney 2005). Chemical and mechanical control methods are

often effective, especially in smaller areas, but neither is without drawbacks (see Culliney 2005 and references therein). Both are short-term, labour intensive, and expensive. Chemical control can have negative impacts on the environment (e.g. killing non-target plants) and on human health, and plants can become resistant to herbicides (Culliney 2005). Mechanical control can disturb natural habitats if clearing operations are extensive and poorly implemented (Culliney 2005). Due to the expense and harmful impacts of chemical and mechanical control, there has been an increased interest in biological control (Culliney 2005).

Biological control

Biological control is based on the premise that plants are invasive in their introduced range due to the lack of natural enemies that control them in their native range (i.e. the Enemy Release Hypothesis) (Guretzky & Louda 1997; Callaway & Aschehoug 2000; Keane & Crawley 2002; DeWalt *et al*. 2004). By introducing natural enemies from the native range, plants could potentially be controlled in their invaded range (Keane & Crawley 2002). Because host-range tests on the natural enemies (agents) are carried out under the guidance of strict legislation, biocontrol is deemed to be a safe and an environmentally sustainable method of control (McFadyen 1998; Denslow & D'Antonio 2005; Clewley *et al*. 2012). Successful biological control programmes are self-sustaining, long-term solutions to invasive alien plants (McFadyen 1998; Zimmermann *et al*. 2004) and are more cost effective than other control methods because costs are independent of the extent of the weed infestation (Culliney 2005). Initial costs of research are high but after release of a successful agent there are no more significant costs involved, while the benefits continue to accrue (Zimmermann *et al*. 2004; Culliney 2005).

Biocontrol has come a long way, with stringent host-specificity testing to reduce the risk of non-target damage (Louda *et al*. 2003a, b; Sheppard *et al*. 2005). However, biological control agents may cause negative indirect effects. For example, they may serve as a supplementary food source for native predators and parasitoids, thereby increasing their numbers and impacts on their native hosts (Simberloff & Stiling 1996; Pearson & Callaway 2003, 2005). Indirect effects are more difficult to predict than the risk of direct effects of non-target feeding, but risks can be reduced by only releasing agents which are likely to be effective (Pearson & Callaway 2005). Despite these risks, very few weed biocontrol programmes have been detrimental, and most have either been successful or have had a

negligible effect on the target (Culliney 2005; Hinz *et al*. 2014). It is therefore important to weigh the potential risks of biological control against the costs and negative impacts of the target plant and of other control methods (Culliney 2005).

Senecio madagascariensis

Senecio madagascariensis Poir. (fireweed; Asteraceae) is a small (± 50 cm) herbaceous annual or short-lived perennial (Hilliard 1977) that is characterised by its yellow flowers (Figure 1). The plant's native range extends from Madagascar to the coast of Mozambique and much of South Africa, where it is found throughout large parts of KwaZulu-Natal, as well as in the Eastern Cape, Western Cape, Northern Cape, Gauteng, and Mpumalanga provinces (Hilliard 1977; Lafuma *et al*. 2003), and Lesotho and Swaziland (Lafuma *et al*. 2003; Csurhes & Navie 2010). It grows predominantly in disturbed or degraded areas and along roadsides at altitudes lower than 1500 m above sea level (Hilliard 1977; Csurhes & Navie 2010). Plants can flower throughout the year, beginning as early as six weeks after germination and on average can produce over 100 flower heads in the invaded range (Radford & Cousens 2000; Prentis *et al*. 2007; Csurhes & Navie 2010; DEEDI 2011). The seeds are dispersed by wind, animals, and vehicles, or in agricultural produce (Csurhes & Navie 2010). The plant has become invasive in a number of countries where it was introduced.

Senecio madagascariensis was introduced to the Hunter Valley, New South Wales (NSW), Australia, by 1918, and has since spread through the eastern parts of Australia, including coastal NSW, north to northern Queensland and south to isolated parts of Victoria (McFadyen & Morin 2012). Accidental introduction of *S. madagascariensis* to the Hawaiian Islands occurred in the 1980s, probably via Australia (Le Roux *et al*. 2006). It currently occurs in the north-eastern, western and southern parts of the island of Hawaii, and on the eastern part of Maui (Le Roux *et al*. 2006, 2010). In 1976, *S. madagascariensis* was introduced to Japan; it is now found from southern Kyushu in the south to southern Tohoku in the north, occurring mostly on the Pacific coast and the Seto Inland Sea coast (Tsutsumi 2011). In South America, *S. madagascariensis* is found in Brazil (Cruz *et al*. 2010), Uruguay, Columbia (Le Roux *et al*. 2010) and northern Argentina, where it was first collected in 1940 (Lopez *et al*. 2008). According to climate matching, habitat suitability and niche-modelling research, it has not yet reached its full potential distribution in any of these countries and if left uncontrolled will

spread further (Sindel & Michael 1992; Le Roux *et al*. 2010; Tsutsumi 2011; McFadyen & Morin 2012).

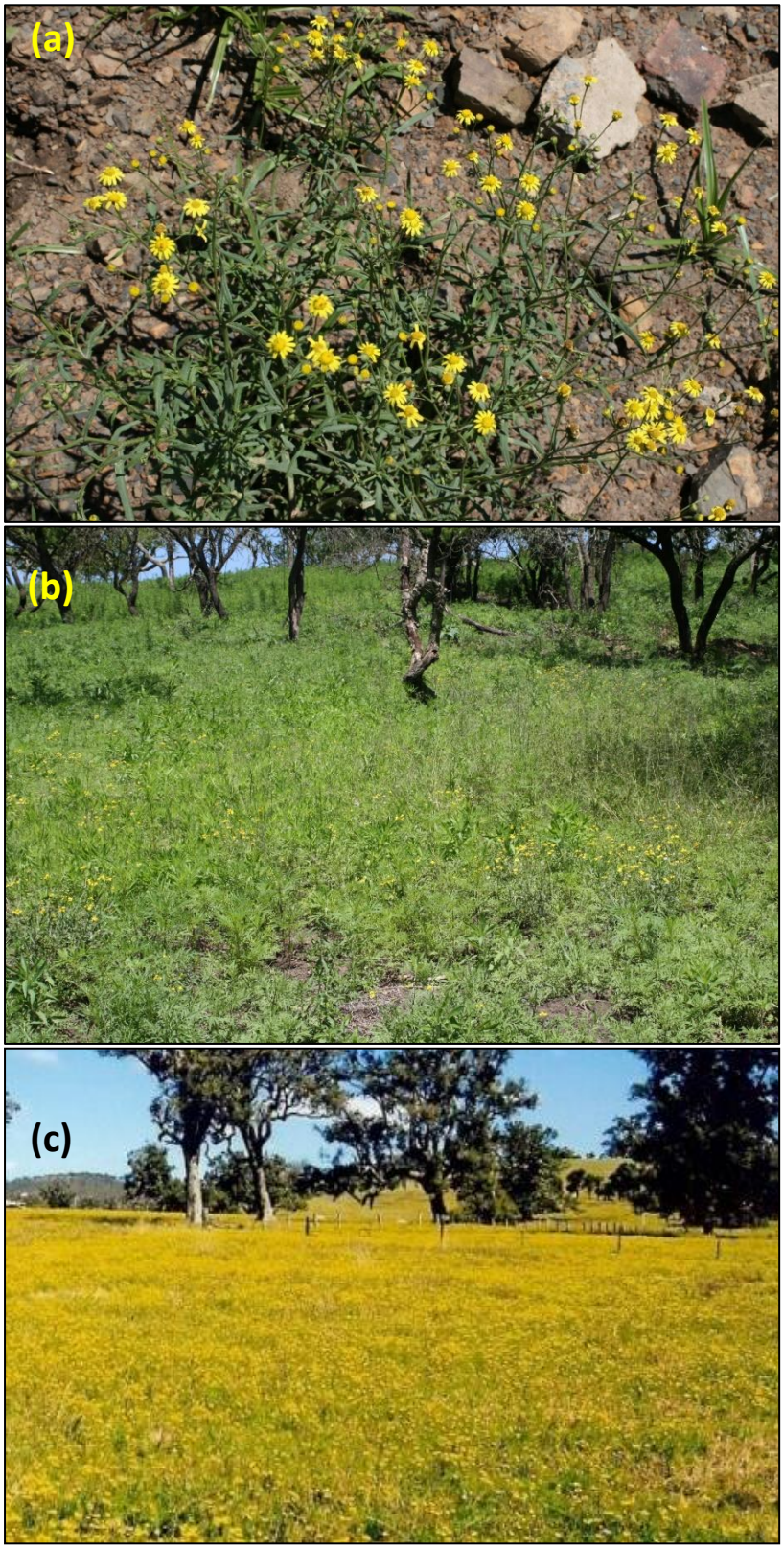

Figure 1: *Senecio madagascariensis* (a) whole plant, (b) natural abundance in South Africa, and (c) infestation in Australia. Image (c) courtesy of A.W. Sheppard.

Senecio madagascariensis is an important agricultural weed in countries where it has been introduced (Csurhes & Navie 2010). It invades pastures (Figure 1) and competes with pasture species for light, moisture and nutrients, resulting in a decline in other pasture species and thus carrying capacity (McFadyen & Sparks 1996; Thorne *et al*. 2005; Le Roux *et al*. 2006; Prentis *et al*. 2010). It can cover up to 60% of the land and reduce pasture productivity by 30-40% (Thorne *et al*. 2005). Fireweed contains at least 13 pyrrolizidine alkaloids (Gardner *et al*. 2006) that are toxic to livestock (Thorne *et al*. 2005; Csurhes & Navie 2010). Poisoning by *S. madagascariensis* can decrease animal production by slowing growth in young cattle, reducing milk production, causing sensitivity to sunlight, and causing liver damage which can result in death (Csurhes & Navie 2010; DEEDI 2011). Livestock tend to avoid *S. madagascariensis* and are more likely to ingest it when forage is limited, when the plants are small and difficult to avoid, or when it is a contaminant in hay and silage (Csurhes & Navie 2010). The avoidance of *S. madagascariensis* by cattle contributes to the plant out-competing other species, dominating pastures and reducing pasture productivity (Sindel *et al*. 2008).

Reductions in profits due to invasion by *S. madagascariensis* in Australia have been estimated to be between 15 and 50% (Sheppard *et al*. 2013). Annual economic losses have been variously estimated at A$ 5.4 million for farmers in NSW, A$ 250 000 to the NSW dairy industry (Csurhes & Navie 2010), and up to US$ 2 million overall in Australia (Le Roux *et al*. 2006). *Senecio madagascariensis* also negatively impacts the environment as it is able to hybridise with the Australian native *Senecio pinnatifolius* A.Rich. (Csurhes & Navie 2010). *Senecio pinnatifolius* produces fewer viable seeds in areas where it grows sympatrically with *S. madagascariensis*, which has a hybridization advantage and produces proportionally more progeny (Prentis *et al*. 2007). It is thus possible that *S. madagascariensis* could result in the local extinction of *S. pinnatifolius* in areas where they co-occur (Prentis *et al*. 2007).

Currently, control of *S. madagascariensis* involves chemical and mechanical control and pasture management (Sindel *et al*. 2012; Sheppard *et al*. 2013). Chemical control is a short-term solution as plant populations resurge quickly due to seed germination throughout most of the year (Sindel *et al*. 2008). Chemicals need to be re-applied multiple times, resulting in very high costs of chemical control (Le Roux *et al*. 2006; Sindel *et al*. 2008; DEEDI 2011) and the potential for herbicide resistance, which has been recorded in some species of *Senecio* (Park & Mallory-Smith 2006). For example, *Senecio vulgaris* L. has become resistant to the herbicides bromoxynil and terbacil in Oregon, USA (Park & Mallory-Smith 2006). Mechanical

control involves bagging, removing and burning the plants (DEEDI 2011) or alternatively, hand weeding (Sindel *et al*. 2012). Although effective, this is very time consuming and therefore more suited to areas that have light infestations (DEEDI 2011; Sindel *et al*. 2012). Slashing is not recommended as it increases the likelihood of livestock poisoning and may exacerbate the problem by spreading seeds and creating conditions more favourable to reinvasion (DEEDI 2011; Sindel *et al*. 2012). Sheep and goats have been used to control *S. madagascariensis* as they can tolerate much higher levels of the plant in their diet than can horses or cattle (Thorne *et al*. 2005; Kellner *et al*. 2011; Sindel *et al*. 2012). This can be effective but would involve substantial investment by cattle farmers (Sindel *et al*. 2012). Pasture management can also restrict the invasion of *S. madagascariensis* and if there is a dense cover of pasture species, the weed should not be able to dominate (Motooka *et al*. 2004; Sindel *et al*. 2008; Csurhes & Navie 2010; DEEDI 2011). However, there is some debate as to how effective this is, and the large areas already infested do require other control measures (Sindel *et al*. 2008).

Costs of controlling *S. madagascariensis* in Australia include an estimated A$ 9 000 per annum per farm (Sheppard *et al*. 2013), with another estimate of A$ 1 000 and 50 hrs per annum per farmer (Sindel *et al*. 2012). In Hawaii, over 162 000 hectares have been invaded by *S. madagascariensis* which would cost an estimated US$ 11 million annually to control (Ramadan *et al*. 2011). Though estimates vary, it is clear that chemical and mechanical control efforts are expensive and economically unsustainable (Sindel *et al*. 2012). Due to its negative impacts and problematic control, *S. madagascariensis* was recently declared a Weed of National Significance in Australia (Sheppard *et al*. 2013).

Biological control of *S. madagascariensis*

Biological control efforts against *S. madagascariensis* began in Hawaii in the late 1980s and surveys of the plant's insect herbivore fauna were undertaken in Madagascar in order to identify potential biological control agents (McFadyen & Sparks 1996; McFadyen & Morin 2012). Eleven insects and a rust fungus from Madagascar were imported in the late 1990s (McFadyen & Morin 2012). The rust attacked two Hawaiian endemics and was therefore not suitable for release (Ramadan *et al*. 2011). Of the 11 insect species, the defoliating moth, *Secusio extensa* (Butler) (Lepidoptera: Arctiidae) seemed promising, and host-range tests were carried out (Ramadan *et al*. 2011). Although the moth fed on other Asteraceae, all of these Asteraceae are exotic to Hawaii (Ramadan *et al*. 2011) and the moth has since been

released (Osher 2013). This moth is not suitable for release in Australia due to its large native *Senecio* flora (McFadyen & Morin 2012).

In Australia, funding for research into biological control of *S. madagascariensis* was granted in 1989 (McFadyen & Sparks 1996). In 1990, two lepidopterans (*Phycitodes* new sp. (Pyralidae), a flower-head feeder, and *Lobesia* new sp. (Tortricidae), a stem borer) were imported from Madagascar for host-range testing (McFadyen & Sparks 1996; McFadyen & Morin 2012). In 1991, surveys for insects were carried out in KwaZulu-Natal, South Africa and two more potential agents were identified, the flower-head feeder, *Homoeosoma stenotea* Hampson (Lepidoptera: Pyralidae) and the stem borer, *Melanagromyza* sp. (Diptera: Agromyzidae) (McFadyen & Sparks 1996; McFadyen & Morin 2012). However, these were not imported due to difficulties in obtaining good founder populations (McFadyen & Sparks 1996; McFadyen & Morin 2012). The Australian programme was discontinued in 1995 after it was revealed that the two imported candidate agents from Madagascar were not host specific (McFadyen & Morin 2012).

A rust fungus from South Africa, *Puccinia lagenophorae* Cooke (and hybrids thereof) was considered as a potential biocontrol agent for *S. madagascariensis* (Morin *et al*. 2009; McFadyen & Morin 2012). However, *P. lagenophorae* already occurs on *S. madagascariensis* and other Australian *Senecio* species in Australia (Morin *et al*. 2009; McFadyen & Morin 2012). The South African strains and hybrids were not host specific, nor were they more damaging than the strain already present in Australia, and the rust was therefore not considered any further (Morin *et al*. 2009; McFadyen & Morin 2012).

Studies comparing the ITS 1 sequences, morphology and isozymes of *S. madagascariensis* from Australia with *S. madagascariensis* from Madagascar and across South Africa determined that Australian *S. madagascariensis* is most closely related to *S. madagascariensis* from KwaZulu-Natal, South Africa (Scott *et al*. 1998; Radford *et al*. 2000). KwaZulu-Natal was thus deemed to be the best place to find host specific biological control agents for *S. madagascariensis* in Australia (Scott *et al*. 1998; Radford *et al*. 2000). A similar study compared *S. madagascariensis* from Hawaii, Madagascar, Swaziland and South Africa and also found that the centre of origin of Hawaiian *S. madagascariensis* is KwaZulu-Natal (Le Roux *et al*. 2006).

Funding for the biological control of *S. madagascariensis* in Australia has been sporadic in the past but there is renewed interest now that the plant's centre of origin has

been determined and it has been declared a Weed of National Significance in Australia. Consequently, this study forms part of a collaborative research agreement between Australia's Commonwealth Scientific and Industrial Research Organisation (CSIRO) and the University of KwaZulu-Natal (UKZN) that has continued since 2011.

Native range studies are an important first step in a biological control programme and can contribute significantly by helping to select host specific and potentially successful agents (Goolsby *et al.* 2006; van Klinken & Raghu 2006). Information gained from the native range can give an indication of a selected insect's potential to establish and inflict damage on the target plant (Harris 1973; Sheppard 2002). It can also reduce the number of polyphagous insects tested and the number of ineffective agents released (Harris 1973; Sheppard 2002). Factors such as an insect's relative abundance, distribution (Harris 1973; Sheppard 2002; Goolsby *et al*. 2006), seasonal abundance (Ireson *et al*. 2000; Sheppard & Smyth 2012), and response to changes in its host plant's alkaloid concentration (Muller-Scharer *et al*. 2004) can all affect the potential efficacy of an agent. Their field host range can give an indication of which insects are most likely to be monophagous and therefore safe for release (van Klinken 1999; Goolsby *et al* 2006). Finally, native range studies can also provide an indication of the factors (e.g. parasitism, host-plant phenology and climatic factors) that may prevent an insect from feeding on potential host plants.

Aims of the study

The aims of this study were to examine several aspects of the native insect fauna associated with *S. madagascariensis* in KwaZulu-Natal, South Africa in order to prioritise potential insect agents for introduction into Australia. As part of this process, the following were undertaken.

(1) The insect herbivore community associated with *S. madagascariensis* in its native range in KwaZulu-Natal was surveyed and quantified in order to produce a comprehensive list of potential agents. Quantitative surveys were carried out at 21 sites in KwaZulu-Natal to determine the incidence and relative abundance of these herbivores (Chapter 1).

(2) The seasonal abundance of the most important insect herbivores was investigated to determine which were present throughout the year and would be able to inflict sustained damage. Two sites in Pietermaritzburg were sampled four times during 2011 (i.e. once per

season) in order to determine variation in the seasonal abundance of these insects (Chapter 2).

(3) Biological control programmes against invasive Asteraceae worldwide were reviewed in terms of the different agent taxa and feeding guilds that were deployed across countries, their establishment success and their impact on the target weeds. This information, in combination with the results from the insect surveys, was used to prioritise potential agents for Australia (Chapter 3).

(4) The native field host range of those insects with endophagous immature stages was assessed to determine which are most likely to be host specific. Eighteen native species of *Senecio* that coexist with *S. madagascariensis* were surveyed and the larvae of stem-boring and capitulum-feeding taxa (Coleoptera, Diptera and Lepidoptera) were collected. The COI gene of these larvae were sequenced to allow the separation of species between host plants and thereby obtain an indication of host range (Chapter 4).

(5) The implications of increases in concentrations of pyrrolizidine alkaloids (herbivore defence compounds) in Australian plants was examined, to determine whether they have remained susceptible or become more resistant to native insect communities. Plants from the native and invaded range were grown in an outdoor field trial and the impact of the insects recuited in the field on plant biomass was examined, as well as the insect communities, alkaloids and relationship between them (Chapter 5).

This information was used to determine which insect agents are most likely to be effective and host specific and should thus be prioritized for importation into quarantine in Australia and tested first. All of the chapters in this thesis are presented in the format of publications, since some have already been published. As a result, some repetition of introductory information and references has been unavoidable.

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CHAPTER 1

Insect herbivore taxa associated with the invasive *Senecio madagascariensis* (Asteraceae) in its native range in KwaZulu-Natal, South Africa and their potential as biological control agents for Australia and Hawaii

Abstract *Senecio madagascariensis* Poir. (Asteraceae), which has invaded agricultural lands in several countries, has been targeted for biological control in Australia and Hawaii. Quantitative surveys in KwaZulu-Natal, South Africa, considered to be the origin of the Australian and Hawaiian populations, revealed at least 17 insect herbivore taxa associated with the plant's floral and structural tissues that could be considered as candidate biocontrol agents. Many of these taxa were encountered only occasionally or rarely during sampling, with some displaying low to very low abundance. Based on these surveys and earlier precedents in other biocontrol programmes against invasive Asteraceae, I propose that five candidate agents, comprising one capitulum feeder (Lepidoptera: Pyralidae), three stem borers (Coleoptera: Curculionidae; Diptera: Tephritidae; Lepidoptera: Tortricidae) and one root feeder (Coleoptera: Chrysomelidae: Alticinae) be prioritized for further studies. While prospects for the biological control of *S. madagascariensis* in Australia will be constrained by the diversity of native Australian *Senecio* species, they are highly promising in Hawaii where *Senecio* species are not represented in the native flora.

Key words: agent selection, fireweed, invasive plants, phytophagous insects, weed biological control

Introduction

Senecio madagascariensis Poir. (Asteraceae) is an annual or short-lived perennial herb that is native to southern Africa (Hillard 1977), but has become invasive in several countries following accidental importation in contaminated agricultural produce (McFadyen & Morin 2012). The plant is commonly known as fireweed in invaded countries because of its conspicuous bright yellow flowers and has become a major weed of pastures and degraded areas in eastern Australia (McFadyen & Morin 2012), Hawaii (Le Roux *et al.* 2006, 2010), and parts of South America (Lopez *et al*. 2008; Cruz *et al*. 2010; Le Roux *et al*. 2010). Besides replacing desirable pasture species and reducing pastoral productivity, the plant contains pyrrolizidine alkaloids that are toxic to livestock (McFadyen & Morin 2012 and references therein). Since conventional control methods were deemed to provide only short-term

solutions in Australia and Hawaii, both have considered biological control as a long-term strategy (Ramadan *et al.* 2010; McFadyen & Morin 2012; Sheppard *et al*. 2013).

The native range of *S. madagascariensis* includes Madagascar, much of South Africa and several neighbouring southern African countries (Hillard 1977; Lafuma *et al.* 2003). In South Africa, the plant is typically located in disturbed or degraded areas that are below an altitude of 1500 m (Hillard 1977; Lafuma *et al.* 2003). Populations produce flowers throughout the year and thus display a high reproductive output in both native and invaded ranges (Hillard 1977; McFadyen & Morin 2012). In Australia, individual plants can produce 160-200 capitula and 25 000-30 000 seeds annually (Radford & Cousens 2000; Prentis *et al.* 2007). Genetic studies have confirmed that the *S. madagascariensis* plants in Australia and Hawaii are most closely matched with those in KwaZulu-Natal Province, South Africa, where the plant is widespread (Scott *et al.* 1998; Radford *et al.* 2000; Le Roux *et al.* 2006). Although earlier biocontrol efforts were focussed in Madagascar (see Ramadan *et al.* 2010; McFadyen & Morin 2012), it was proposed that future efforts be focussed in KwaZulu-Natal (Sheppard *et al*. 2013).

Earlier surveys for biocontrol agents in South Africa during the 1990s were opportunistic and revealed several potential agents (McFadyen & Morin 2012; Sheppard *et al.* 2013). Since the recent declaration of *S. madagascariensis* as a "Weed of National Significance" in Australia and the availability of increased funding, biocontrol efforts have been renewed in South Africa. A collaborative research agreement between Australia's Commonwealth Scientific and Industrial Research Organisation (CSIRO) and the University of KwaZulu-Natal was thus initiated to facilitate progress with the biocontrol programme (Egli & Olckers 2015). As part of this initiative, a quantitative survey of the insect herbivores associated with *S. madagascariensis* in KwaZulu-Natal was undertaken. In particular, information on the distribution, incidence and abundance of insects in their native range can provide some insight into which taxa might constitute good agents (Goolsby *et al.* 2006). Insects that are present across several seasons and are able to reach high population densities are more likely to inflict sufficient damage to control the target weed in its introduced range (Sheppard 2002).

The aim of this study was to quantitatively survey the herbivorous insects associated with *S. madagascariensis* in KwaZulu-Natal, South Africa to confirm the major taxa and feeding guilds that are associated with this plant. In addition, an assessment of their

incidence, abundance and damage potential was conducted to prioritize specific taxa for consideration as biocontrol agents for Australia and Hawaii.

Materials and methods

Sites surveyed

The surveys, which ranged from the inland to the coastal region of KwaZulu-Natal, commenced in February 2011 and ended in November 2012. Most of the sampling took place during spring, summer and autumn when insect abundances are highest (Chapter2; Egli & Olckers 2015). To avoid sampling of the closely related (and morphologically very similar) *Senecio inaequidens* DC., surveys were conducted in areas below an altitude of 1000 m, as *S. inaequidens* grows at altitudes above 1 400 m. *Senecio madagascariensis* was collected at 21 sites, including five coastal sites and 16 inland sites. At two sites, samples were collected across all seasons, ensuring a total of 29 sampling occasions.

Sampling procedure

Field sites that supported healthy populations of *S. madagascariensis* were mostly visited during the spring and summer months when insect populations were expected to be at their peak. At each site, five randomly-selected *S. madagascariensis* plants that were of a mature size were uprooted and placed in brown paper packets. These samples were frozen for later inspection. At each collection site, two additional samples were collected. One was pressed as a voucher specimen and lodged in the University's John Bews Herbarium (Life Sciences Campus, Pietermaritzburg) (NU) while the other was used to rear immature stages to adulthood for identification purposes. On a few occasions 10 plants were sampled per site, ensuring a total of 185 plants that were sampled.

The frozen plant tissues were examined/dissected while still fresh to expose the immature stages and rear them to adulthood. Leaves with ectophagous and leaf-mining immature stages were placed in Petri dishes, with fresh leaves provided as required. Stems and stalks with stem-boring larvae were resealed and placed in emergence containers, while capitula with endophagous larvae were placed in glass vials. Emerging adults were pinned for later confirmation of identity. During inspections of the frozen material, plants were first searched for any externally-feeding insects and the packets were emptied into a sorting tray to detect any insects that were dislodged. The material was then separated into flowers,

stalks and roots and the material was dissected under a light microscope to detect endophagous species. Leaves were also examined under a light microscope to detect small foliage feeders and leaf miners. The insects were identified to order and, where possible (e.g. immature stages), family and were sorted into morphospecies and assigned to an appropriate guild (e.g. stem borer etc.). Adults were pinned and all immature stages were preserved in vials containing 70% alcohol. The numbers of individuals of each taxon, including both adults and immature stages, were recorded per sampled plant. Adults were sent to the Agricultural Research Council - Plant Protection Research Institute (ARC-PPRI) Biosystematics Division in Pretoria for identification.

Data analysis

EstimateS Version 9 (Colwell 2013) was used to generate mean species accumulation curves after 100 randomisations without replacement. Chao 2 values, which estimate species richness, were calculated using the bias-corrected formula. Sites where insects were collected more than once were considered as separate sampling occasions.

The most important insect herbivores were largely represented by endophagous immature stages that could, at best, only be identified to family level. Consequently, the data were analysed at the levels of families and feeding guilds rather than species. The incidence (i.e. rate of occurrence) of each of the herbivorous taxa was calculated as the percentage of sites where each taxon was present (n = 21), as well as the percentage of plants on which each taxon was present (n = 185). The relative abundance of each insect herbivore taxon was calculated, firstly as the mean number of individuals per plant sampled and secondly as the mean number of individuals per plant where the taxon was present. Taxa present on less than 1% of the sampled plants were deemed to be occasional insects and were excluded from these assessments. Data for sites that were visited more than once were pooled.

Results

Species accumulation curves

A total of 63 insect herbivore taxa were collected on *S. madagascariensis* over 29 sampling occasions during 2011 and 2012. Capitulum feeders reached an asymptote of eight taxa, with 100% of the estimated species richness sampled (Figure 1). Stem-boring taxa did not reach an asymptote, but the 14 taxa collected made up 83% of the estimated total species

richness (Chao 2 = 16.9). The extrapolation indicates that doubling the sampling effort would only yield three additional species (Figure 1). Likewise, foliage feeders did not reach an asymptote and the 41 taxa represented 79% of the estimated species richness (Chao 2 = 52.0). As external feeders were considered to be of lesser importance than endophagous taxa, and most endophages were collected, additional sampling effort for more ectophagous taxa was deemed unnecessary.

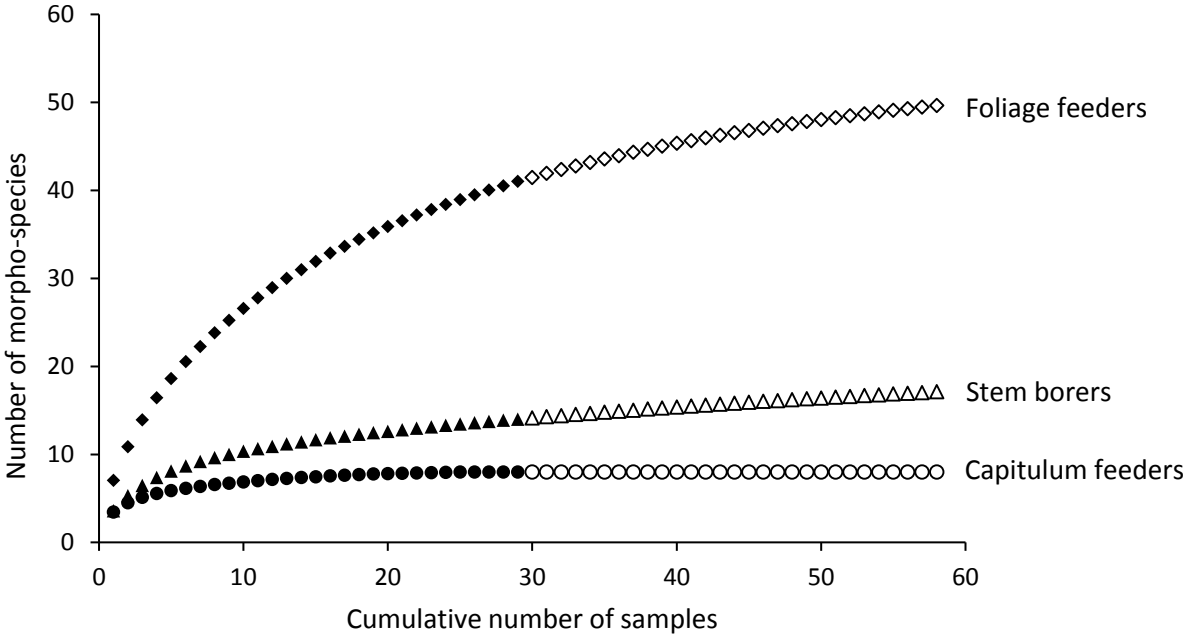

Figure 1: Species accumulation curves of capitulum feeders (circles), stem borers (triangles) and foliage feeders (diamonds) on *Senecio madagascariensis* in KwaZulu-Natal, South Africa. Closed symbols indicate mean values from 100 randomisations and open symbols indicate extrapolated values. Data were extrapolated for twice the sampling effort.

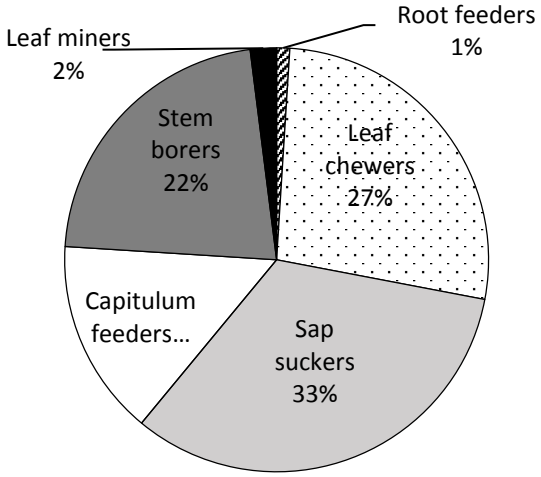

Figure 2: Percentage of insect herbivore taxa in different feeding guilds which were associated with *Senecio madagascariensis* in KwaZulu-Natal, South Africa.

Insect herbivores on Senecio madagascariensis

Of the 63 insect herbivore taxa recorded on *S. madagascariensis*, 37 were consistently associated with the plant (Table 1) and comprised four orders, namely Coleoptera, Diptera, Hemiptera and Lepidoptera. The majority of these taxa comprised ectophagous chewers (27%) and sap suckers (33%), while the endophagous guilds were largely comprised of stem borers (22%) and capitulum feeders (15%), with only one leaf miner (2%) (Figure 2). No gall-forming taxa were recorded. No endophagous root feeders were recovered but an adult flea beetle, the larvae of which feed externally on the roots (see below), was the only designated root feeder (1%).

Table 1: Incidence and relative abundance of the major insect herbivore taxa associated with *Senecio madagascariensis* in KwaZulu-Natal, South Africa.

Feeding guild	Taxon	Reference number	Incidence (%)[a]		Abundance (mean ±SE)[b]	
			Sites	Plants	All plants	Where present
Capitulum feeders	**Coleoptera**					
	Nitidulidae	F.Col01	52.4	17.8	0.99 ± 0.25	5.41 ± 0.46
	Diptera					
	Agromyzidae	F.Dip01/02	90.5	49.7	6.12 ± 1.02	11.92 ± 1.28
	Tephritidae	F.Dip03	52.4	14.1	0.34 ± 0.07	2.48 ± 0.11
	Lepidoptera					
	Pyralidae	F.Lep01-05	38.1	5.4	0.16 ± 0.04	1.50 ± 0.08
Stem borers	**Coleoptera**					
	Cerambycidae	S.Col04	9.5	1.1	0.02 ± 0.01	1.50 ± 0.05
	Curculionidae	S.Col02	33.3	11.4	0.29 ± 0.11	2.94 ± 0.29
	Unidentified[c]	S.Col01	33.3	5.9	0.07 ± 0.02	1.18 ± 0.03
	Diptera					
	Agromyzidae	S.Dip01	85.7	42.2	0.89 ± 0.15	2.70 ± 0.20
	Cecidomyiidae	S.Dip02	33.3	9.7	0.26 ± 0.08	2.67 ± 0.17
	Tephritidae	S.Dip03	90.5	48.1	1.11 ± 0.21	3.60 ± 0.31
	Lepidoptera					
	Tortricidae	S.Lep01	42.9	10.3	0.30 ± 0.05	2.29 ± 0.08
Leaf miners	**Diptera**					
	Agromyzidae	L.Dip1	52.4	17.8	0.36 ± 0.08	2.13 ± 0.13
Root feeders	**Coleoptera**					
	Chrysomelidae	Col02	23.8	2.7	0.03 ± 0.02	1.20 ± 0.03

Table 1: Continued

Feeding guild	Taxon	Reference number	Incidence (%)[a]		Abundance (mean ±SE)[b]	
			Sites	Plants	All plants	Where present
Foliage feeders	**Hemiptera**					
	Aphididae	Aphid	100.0	69.7	38.41 ± 8.92	55.09 ± 10.46
	Cicadellidae	Hem11	9.5	1.6	0.02 ± 0.01	1.33 ± 0.04
		Hem28	9.5	1.6	0.03 ± 0.02	1.67 ± 0.09
	Coreidae	Hem03	33.3	8.6	0.25 ± 0.09	2.88 ± 0.25
		Hem13	23.8	3.8	0.06 ± 0.02	1.57 ± 0.06
	Miridae	Hem14	9.5	1.1	0.01 ± 0.01	1.00 ± 0.00
		Hem01	4.8	1.1	0.01 ± 0.01	1.00 ± 0.00
		Hem15	28.6	5.4	0.09 ± 0.03	1.60 ± 0.07
		Hem25	19.0	4.3	0.10 ± 0.04	2.38 ± 0.13
	Tettigometridae	*Hilda*	47.6	24.9	3.61 ± 0.88	14.50 ± 1.51
	Tingidae	Hem17/18	14.3	3.2	0.12 ± 0.07	3.67 ± 0.35
	Unknown[c]	Hem04	47.6	10.8	0.18 ± 0.04	1.65 ± 0.07
		Hem05	23.8	4.3	0.07 ± 0.03	1.63 ± 0.06
		Hem21	33.3	6.5	0.09 ± 0.03	1.33 ± 0.06
		Hem24	14.3	2.7	0.03 ± 0.02	1.50 ± 0.07
	Coleoptera					
	Curculionidae	Col10	9.5	3.2	0.03 ± 0.01	1.00 ± 0.00
		Col11	14.3	2.2	0.08 ± 0.05	3.50 ± 0.20
		Col12	9.5	1.6	0.02 ± 0.01	1.00 ± 0.00
		Col14	4.8	1.1	0.02 ± 0.02	2.00 ± 0.10
		Col17	14.3	1.6	0.02 ± 0.01	1.33 ± 0.04
	Leipdoptera					
	Erebidae (Arctiidae)	Lep10	4.8	1.1	0.01 ± 0.01	1.00 ± 0.00
	Tortricidae	Lep02	14.3	1.6	0.02 ± 0.01	1.00 ± 0.00
		Lep08	9.5	1.1	0.01 ± 0.01	1.00 ± 0.00
	Unknown[c]	Lep12	4.8	1.1	0.08 ± 0.07	7.50 ± 0.47

[a] Percentage of sites and of sampled plants (n = 185) where recorded.
[b] Expressed as means of all plants sampled (n = 185) and of those where the taxon was present.
[c] Immature stages could not be identified to family level.

The most commonly encountered capitulum feeders were the larvae of Agromyzidae (Diptera), occurring at 91% of sites sampled, with the larvae of Tephritidae (Diptera) and Nitidulidae (Coleoptera) both recorded at 52% of sites (Table 1). Larvae of Pyralidae (Lepidoptera) were recorded in the capitula at 38% of sites. The most abundant capitulum feeders were the agromyzid and nitidulid larvae, with the tephritid and pyralid larvae considerably less abundant (Table 1).

Seven taxa with stem-boring larvae were frequently associated with *S. madagascariensis* and included three coleopterans (Curculionidae, Cerambycidae and an unidentified family), three dipterans (Agromyzidae, Tephritidae, and Cecidomyiidae) and one lepidopteran (Tortricidae). The most commonly encountered stem borers were the larvae of Tephritidae (91% of sites), Agromyzidae (86%) and Tortricidae (43%), while those of Curculionidae, Cecidomyiidae and the unidentified coleopteran displayed a similar incidence (33%) (Table 1). The larvae of Cerambycidae were rarely encountered (10%). The most abundant stem borers included the larvae of Tephritidae, Agromyzidae, Curculionidae, Cecidomyiidae and Tortricidae (Table 1), while the larvae of the unidentified coleopteran and cerambycid were scarce.

Larvae of a dipteran leaf miner (Agromyzidae) were recovered at 52% of the sites, although in relatively low numbers (Table 1). Many of the 41 foliage-feeding taxa recorded on *S. madagascariensis* (Figure 1) were rarely encountered and only 26 (including the leaf miner) were collected on >1% of the sampled plants. Most foliage feeders were sap-suckers from the order Hemiptera (15 taxa), although leaf-chewing Coleoptera (6 taxa) and lepidopteran larvae (4 taxa) were also collected (Table 1). Of the sap suckers, the most abundant and commonly encountered taxa were Aphididae (100% of sites), Tettigometridae (48%) and unknown heteropteran nymphs (48%). Adults of the flea beetle, *Longitarsus basutoensis* Bechyné (Chrysomelidae: Alticinae), whose larvae were later confirmed to feed externally on the roots, were recorded in low numbers at 24% of sites (Table 1). Five weevil taxa (Curculionidae) were collected as adults, also in low numbers and at relatively few sites (5-14%) with one demonstrated to have stem-boring larvae. The lepidopteran larvae, comprising Erebidae (Arctiidae), Tortricidae and an unknown family were uncommon (5-14% of sites) and were recorded in low numbers (Table 1).

Potential biological control agents

Several herbivorous taxa from each of the major feeding guilds were deemed to warrant further investigation as biological control agents for *S. madagascariensis* in Australia and Hawaii (Table 2). Since many of these were endophagous taxa which were recovered as immature stages, identifications to species level have yet to be confirmed. However, provisional identifications have been proposed for some taxa, based on earlier identifications of species recorded on *S. madagascariensis* in South Africa (Table 2). In several cases,

previously identified species could not be consistently matched to species collected in this study, largely because immature stages were not conclusively matched with adults. For example, the larvae of capitulum-feeding Tephritidae could be any of two previously-recorded species or a new species. However, the stem-boring Curculionidae, Agromyzidae and Tephritidae are likely to be *Gasteroclisus tricostalis* (Thunberg), a species of *Melanagromyza* and *Coelopacidia strigata* Bezzi, respectively. In addition, the sap-sucking Tettigometridae are almost certainly *Hilda patruelis* Stal. The flea beetle has been identified as *Longitarsus basutoensis* Bechyné.

Based on this research, the potential of each candidate taxon (i.e. family and feeding guild) to be a successful biocontrol agent was assessed on the basis of the damage that it inflicted on specific plant tissues and whether other species in that taxon had previously been deployed for the biocontrol of other invasive Asteraceae (Table 2). From the Australian (but not Hawaiian) perspective, the recovery of similar native taxa on *S. madagascariensis* in Australia (see Table 2) was considered to reduce the potential of some taxa. Although the agromyzid was the most common and abundant of the capitulum-feeding taxa, it was not deemed to be sufficiently damaging. In contrast, the less frequently encountered Tephritidae inflicted more damage, but similar native tephritids have already been reported from *S. madagascariensis* in Australia. Despite the recovery of a native capitulum-feeding pyralid moth in Australia, the most promising capitulum-feeding taxon was the pyralid, presumably *Homeosoma stenotea* Hampson, whose larvae were able to destroy entire capitula. Several stem-boring taxa were considered to be sufficiently damaging for further assessment and included a curculionid (presumably *G. tricostalis*), agromyzid (presumably *Melanagromyza* sp.), tephritid (presumably *C. strigata*) and an unknown tortricid. Similar native stem-boring agromyzids and tortricids have been reared from *S. madagascariensis* in Australia, suggesting that *G. tricostalis* and *C. strigata* should be ranked higher. Although a few foliage-feeding taxa may warrant further consideration (e.g. sap-sucking tingids), most were considered to have limited potential. In contrast, *L. basutoensis* was considered to be the most promising foliage-feeder, largely because of its root-feeding larvae and because flea beetles have not been reported from *S. madagascariensis* in Australia. This represents an unoccupied niche in Australia.

Table 2. Insect herbivore taxa associated with *Senecio madagascariensis* in KwaZulu-Natal, South Africa that may have potential as biological control agents in Australia and Hawaii. Ratings were based on an overall assessment of their damage and the information presented below.

Feeding guild	Taxon	Possible identity [a]	Incidence [b]	Abundance [c]	Biocontrol potential	
					Rating	Previous use [d]
Capitulum feeders	**Coleoptera**					
	Nitidulidae	Indeterminate genus and species	Common/Occasional	Scarce	Low	No
	Diptera					
	Agromyzidae	Indeterminate genus and species	Very common	Very abundant	Low	No
	Tephritidae	At least two species	Common/Occasional	Very scarce	Low*	Yes (19 spp.)
	Lepidoptera					
	Pyralidae	*Homeosoma stenotea* Hampson	Occasional/Rare	Very scarce	High*	No
Stem borers	**Coleoptera**					
	Cerambycidae	Indeterminate genus and species	Rare	Very scarce	Low	Yes (3 spp.)
	Curculionidae	*Gasteroclisus tricostalis* (Thunberg)	Occasional	Very scarce	High	Yes (8 spp.)
	Unidentified	Indeterminate genus and species	Occasional/Rare	Very scarce	Low	?
	Diptera					
	Agromyzidae	*Melanagromyza* sp.	Very common	Very scarce	High*	No
	Cecidomyiidae	Indeterminate genus and species	Occasional	Very scarce	Low*	Yes (4 spp.)
	Tephritidae	*Coelopacidia strigata* Bezzi	Very common	Scarce	High	Yes (4 spp.)
	Lepidoptera					
	Tortricidae	Indeterminate genus and species	Common/Occasional	Very scarce	High*	Yes (5 spp.)
Leaf miners	**Diptera**					
	Agromyzidae	Indeterminate genus and species	Common/Occasional	Very scarce	Low*	Yes (2 spp.)
Root feeders	**Coleoptera**					
	Chrysomelidae	*Longitarsus basutoensis* Bechyné	Occasional/Rare	Very scarce	High	Yes (2 spp.)

Table 2. Continued

Feeding guild	Taxon	Possible identity [a]	Incidence [b]	Abundance [c]	Biocontrol potential	
					Rating	Previous use [d]
Foliage feeders	**Hemiptera**					
	Tettigometridae	*Hilda patruelis* Stal	Common/Occasional	Abundant/Very abundant	Low	No
	Tingidae	Indeterminate genus and species	Rare	Very scarce	Low	No
	Lepidoptera					
	Erebidae (Arctiidae)	Indeterminate genus and species	Rare	Very scarce	Low*	Yes (1 spp.)
	Tortricidae	*Epichorestoides acerbella* Walker	Rare	Very scarce	Low*	Yes (2 spp.)

[a] Based on previous identifications (McFadyen & Morin 2012; Sheppard *et al*. 2013).

[b] Incidence: Very common = ≥60% of sites and ≥40% of plants; Common = 40-59% of sites and 30-39% of plants; Occasional = 20-39% of sites and 10-29% of plants; Rare = <20% of sites and <10% of plants.

[c] Abundance: Very abundant = ≥5 individuals per plant and ≥10 individuals per plant when present; Abundant = 3-4 individuals per plant and 7-9 individuals per plant when present; Scarce = 1-2 individuals per plant and 4-6 individuals per plant when present; Very scarce = <1 individual per plant and 1-3 individuals per plant when present.

[d] Number of species from that feeding guild within each family that were released for the biological control of Asteraceae (Winston *et al*. 2014).

* Similar native taxa were recovered from *S. madagascariensis* in Australia (see Holtkamp & Hosking 1993; Harvey *et al*. 2015), thereby reducing their potential as biocontrol agents in that country.

Discussion

The dominant *S. madagascariensis* herbivores were similar to those recorded in earlier opportunistic surveys in South Africa that included eight capitulum feeders, five stem borers, two root feeders, and 14 foliage feeders (McFadyen & Morin 2012; Sheppard *et al.* 2013). The insect families and feeding guilds that were previously reported were very similar to those recorded in this study. Several sap-sucking species, notably Aphididae, that were previously identified to species level were disregarded due to their confirmed pest status (McFadyen & Morin 2012) and were thus grouped at family level in this study. The only previously-reported taxa of importance that were not recorded during this study were the capitulum-feeding Cecidomyiidae and the stem- and root-boring Sciaridae (Diptera) (McFadyen & Morin 2012). However, it is possible that the latter taxon may be the same as the stem-boring Cecidomyiidae recorded in this study. Considering that the *S. madagascariensis* fauna was assessed at a broader taxonomic level, with some taxa (e.g. capitulum-feeding Tephritidae) comprising more than one species, the associated herbivore assemblage reported here is an underestimation in terms of actual species richness.

The insect orders and families recovered on *S. madagascariensis* were very similar to those typically associated with plant species from different tribes of Asteraceae (e.g. Hilgendorf & Goeden 1983; Goeden & Ricker 1976, 1987; Boldt & Robbins 1994; Briese *et al.* 1994), with none unique to *S. madagascariensis*. Of the 17 families recorded on *S. madagascariensis*, 10 (=58.8%) have featured in the biological control of invasive Asteraceae (Winston *et al.* 2014). Coleoptera, largely from the families Curculionidae (22 species) and Chrysomelidae (19 species), have featured the most in the biocontrol of asteraceous weeds, followed by Diptera, largely from the family Tephritidae (23 species). Some 32 species of Lepidoptera from 13 families have also featured as agents, while only one agent from the Hemiptera (Delphacidae) has been deployed (Winston *et al.* 2014). These precedents suggest that the insect herbivore fauna of *S. madagascariensis* in KwaZulu-Natal is likely to incorporate several potential biocontrol agents.

Although 17 taxa were highlighted as potential biocontrol agents (Table 2) some are unlikely to be investigated further. Many of these taxa were only occasionally or rarely encountered during sampling with some displaying low to very low abundance. However, low levels of incidence and abundance in the native range do not necessarily imply poor potential as agents, depending on the factors responsible for their scarcity. For example, low

population densities based on regulation by natural enemies can be regarded as a positive sign, given that escape from these can promote high densities in the introduced range (Harris 1973). High numbers of parasitoids have been collected from the natural enemies of fireweed (Chapter 5). However, since there are 87 native *Senecio* species in Australia (Thompson 2006), introduced biocontrol agents seem likely to acquire native parasitoids from closely-related and ecologically-similar native insect taxa that are associated with them (i.e. "ecological analogues"), which is a typical pattern in weed biocontrol (Paynter *et al.* 2010). Also, several native insect herbivores, that are normally associated with closely-related native Australian *Senecio* species, have colonized *S. madagascariensis* in Australia (Holtkamp & Hosking 1993; McFadyen & Morin 2012; Harvey *et al.* 2015). Some of these taxa (e.g. capitulum-feeding tephritids and stem-boring-agromyzids) are the same as those highlighed as candidate agents, suggesting lesser importance as these taxa are already present on the weed (McFadyen & Morin 2012). In addition, there were no significant differences between *S. madagascariensis* and other native Australian *Senecio* species in relation to the species richness and abundance of insect herbivores (Harvey *et al.* 2015), further suggesting that taxa already represented on the weed in Australia should not be considered as agents. However, none of these restrictions apply in Hawaii where the genus *Senecio* is not represented in the native flora (Ramadan *et al.* 2010) and none of the potential agents should thus be disregarded at present. South America, like Australia, has a diverse native *Senecio* fauna and is therefore likely to face similar challenges to Australia in implementing biological control.

The similarity in insect herbivore faunas between *S. madagascariensis* and some native *Senecio* species in Australia (Harvey *et al*. 2015) raises questions about the main driver of invasion and whether biocontrol has a role to play in the weed's management. In Australia, however, endophages represented less than 10% of species richness (Harvey *et al*. 2015) while in this study, endophages made up 35% of morphospecies. Fireweed in Australia appears to have escaped from specialist natural enemies. The contention that biocontrol is an appropriate intervention for fireweed was supported by enemy-exclusion trials in South Africa which recorded higher biomass of plants where insect herbivores were chemically excluded (Harvey *et al*. unpublished data).

Given the above considerations, I propose that five candidate agents comprising one capitulum feeder, three stem borers and one root feeder be prioritized for further studies. Larvae of the pyralid moth (probably *H. stenotea*) caused the most damage to infested

capitula, despite it being uncommon and not recorded across all seasons (see Egli & Olckers 2015). Larvae of the stem-boring tephritid (probably *C. strigata*) were abundant and recorded throughout the year, while those of the curculionid (probably *G. tricostalis*) and tortricid were rarely encountered and not consistently recorded across all seasons (see Egli & Olckers 2015). All three of these stem-boring taxa have featured in the biocontrol of invasive Asteraceae (Winston *et al.* 2014). Despite being recorded in low numbers and not consistently across all seasons (see Egli & Olckers 2015), the flea beetle, *L. basutoensis*, is currently the candidate with the highest priority following confirmation that its larvae are external root feeders (KJ Harvey, pers. comm.). In particular, root-feeding larvae of the flea beetle *Longitarsus flavicornis* (Stephens) were able to damage the related weed *Jacobaea vulgaris* Gaert. (previously *Senecio jacobaea* L.) throughout much of the year in Australia and contribute to its biological control (Ireson *et al*. 1991; Ireson & McLaren 2012).

Releases of South African insects in Australia will be difficult to justify, considering the diverse native *Senecio* flora in Australia (Thompson 2006) and the acquisition of native *Senecio* insects by *S. madagascariensis* (Holtkamp & Hosking 1993; McFadyen & Morin 2012; Harvey *et al.* 2015), which may suggest that introduced biocontrol agents could similarly colonize native Australian *Senecio* species. However, assessments of field host range have indicated restriction to plants in the *S. madagascariensis* species complex, in all taxa examined except stem-boring Diptera (Chapter 4). Two candidate agents from Madagascar, that belong to the same taxonomic groups and feeding guilds as candidates identified from South Africa (see above), were previously introduced into quarantine in Australia but were rejected due to a lack of host specificity. These include the capitulum-feeding moth *Phycitodes* new sp. (Pyralidae) and the stem- and root-boring moth *Lobesia* new sp. (Tortricidae) (McFadyen & Morin 2012). Given that most of the highlighted taxa in the current study were recovered as endophagous immature stages, few of which were reared to adulthood, it was very difficult to determine whether these are likely to be specific to *S. madagascariensis*. The genetic sequencing of endophagous coleopteran, dipteran and lepidopteran larvae that were collected from a range of native *Senecio* species in KwaZulu-Natal was thus conducted to provide further insight into the host range of candidate agents for *S. madagascariensis* (Chapter 4).

In conclusion, there are several insect herbivore taxa that exploit different host tissues of *S. madagascariensis* in KwaZulu-Natal, South Africa and could be considered as candidate

biocontrol agents. While releases of these taxa in Australia are likely to prove challenging, prospects for their deployment in Hawaii are highly promising.

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CHAPTER 2

Seasonal abundance of insect herbivore taxa associated with the invasive *Senecio madagascariensis* (Asteraceae) in its native range in KwaZulu-Natal, South Africa

Abstract *Senecio madagascariensis* Poir. (Asteraceae), which is native to southern Africa, has invaded agricultural lands in several countries worldwide, reducing the productivity of pastures and poisoning livestock. Severe infestations in Australia and Hawaii have prompted investigations into the feasibility of biological control. Surveys in KwaZulu-Natal, South Africa, considered to be the origin of the plants that have invaded Australia and Hawaii, revealed several insect herbivore taxa that attack the plant's floral and structural tissues. However, their potential as biological control agents may be influenced by their seasonal abundance. Populations of *S. madagascariensis* around Pietermaritzburg were sampled seasonally over one year to determine the presence and abundance of the major herbivore taxa in relation to the plant's phenology. Similar amounts of foliar and floral material were available to the various guilds of herbivorous insects throughout the year. The sampled plant populations supported 84% of the insect herbivore taxa known to be associated with *S. madagascariensis* in KwaZulu-Natal. Nine of the 10 taxa that were deemed promising as candidate agents were recovered and included three capitulum feeders, four stem borers and two foliage feeders. Of these, four were recovered all year round, two during three seasons, two during two seasons (summer and autumn) and one during one season (summer) only. There were significant seasonal differences in the abundance of these candidate agents, possibly due to differences in their life cycles. The release of combinations of agents, that attack the same or different tissues, may be required to compensate for differences in seasonal abundance and ensure that herbivore pressure is sustained throughout the weed's phenology.

Key words: agent selection, fireweed, invasive plants, phytophagous insects, weed biological control

Introduction

Native to southern Africa, *Senecio madagascariensis* Poir. (Asteraceae) is an annual or short-lived perennial herb that has been introduced to several countries worldwide via contaminated agricultural produce (Hilliard 1977; Csurhes & Navie 2010; McFadyen & Morin 2012). Commonly known as fireweed because of its profusion of yellow flowers, the plant has become invasive in Australia (Sindel & Michael 1992; McFadyen & Morin 2012), Hawaii (Le

Roux *et al.* 2006), Japan (Tsutsumi 2011), Brazil (Cruz *et al.* 2010), Uruguay, Columbia (Le Roux *et al.* 2010) and northern Argentina (Lopez *et al*. 2008). It invades pastures and reduces their carrying capacity (McFadyen & Sparks 1996; Thorne *et al.* 2005; Le Roux *et al.* 2006; Prentis *et al.* 2010) but also contains pyrrolizidine alkaloids that are toxic to livestock (Thorne *et al.* 2005; Gardner *et al.* 2006; Csurhes & Navie 2010). Since chemical and mechanical control methods are costly and provide only short term solutions, Australia and Hawaii have expressed an interest in biological control (Ramadan *et al.* 2011; Sindel *et al.* 2012; Sheppard *et al.* 2013).

In South Africa, *S. madagascariensis* typically reaches 50 cm in height and occurs predominantly in disturbed or degraded areas and along roadsides (Hilliard 1977; Csurhes & Navie 2010). The plant displays considerable reproductive output in both its native and introduced ranges, with flowering commencing as early as 4-6 weeks after germination and continuing throughout the year (Hilliard 1977; Csurhes & Navie 2010). Single plants are able to produce around 100 capitula in the invaded range, each containing up to 150 achenes (Radford & Cousens 2000; Prentis *et al.* 2007; Csurhes & Navie 2010). Seeds are dispersed in agricultural produce and by wind, animals and vehicles, and are able to germinate throughout the year (Csurhes & Navie 2010). Genetic studies have confirmed that the populations in Australia and Hawaii are closest matched to *S. madagascariensis* in KwaZulu-Natal, South Africa (Scott *et al.* 1998; Radford *et al.* 2000; Le Roux *et al.* 2006), necessitating that surveys for potential biological control agents be focused in this region (Sheppard *et al.* 2013).

Given the sporadic nature of funding for the biological control of *S. madagascariensis* in both Australia and Hawaii, a few rapid and opportunistic surveys were carried out in South Africa during the early 1990s (Sheppard *et al.* 2013). Following confirmation of the plant's centre of origin and its recent declaration as a "Weed of National Significance" in Australia, interest in biological control has been rekindled. As part of a collaborative research agreement between Australia's Commonwealth Scientific and Industrial Research Organisation and the University of KwaZulu-Natal, a quantitative survey of the insect herbivores associated with *S. madagascariensis* in KwaZulu-Natal was carried out during 2011-2012 (Chapter 1; Egli 2013). Several insect herbivore taxa that attack the floral and structural tissues of the plant were proposed as candidate agents (Chapter 1; Egli 2013). However, following their release and establishment, insect agents may fail to control their target weeds for a number of reasons that include climatic incompatibility, recruitment of

natural enemies and poor synchrony between the agents and their host plant (*e.g.* Julien & Griffiths 1998; Julien *et al.* 2012).

Phytophagous insects need to inflict sustained damage throughout the year in order to influence the persistence and reproductive output of short-lived plant species like *S. madagascariensis* that reproduce at an early stage. In particular, agents that directly reduce reproductive output should be present throughout the plant's flowering period in order to destroy sufficient floral material to have an impact on its population dynamics (Briese *et al.* 1994; Woodburn & Cullen 1995). Similarly, insects feeding on photosynthetic, structural or underground tissues also need to inflict sufficient and sustained damage to limit plant recovery and cause mortality (Ireson *et al.* 2000; Sheppard & Smyth 2012). The biological control of *S. madagascariensis* may thus be dependent on the establishment of a suite of agents that are capable of inflicting complementary damage throughout the plant's phenology. Natural enemies that are present throughout the year are thus best suited for this purpose (Briese *et al.* 1994).

The aim of this study was to determine the seasonal abundance of the more important phytophagous insect taxa associated with *S. madagascariensis* in the KwaZulu-Natal midlands, where the plant is particularly abundant, in order to assess their ability to inflict sustained damage in relation to the plant's phenology.

Materials and methods

Data collection

Seasonal surveys on the abundance of insect herbivores associated with *S. madagascariensis* were carried out at the Ukulinga Research Farm (University of KwaZulu-Natal) in Pietermaritzburg, from February to November 2011. Two populations in close proximity to each other were sampled, one in an orchard (29°40'09.72"S 30°24'44.24"E) and the other in a paddock (29°39'46.54"S 30°24'11.91"E), neither of which were sprayed with pesticide. Ten randomly selected flowering plants were sampled from each population during summer (mid-February), autumn (late May), winter (mid-August) and spring (mid-November), totalling 20 plants per season and 80 plants overall. The plants were uprooted, placed in brown paper packets, and taken back to the laboratory where they were placed in a freezer

for later inspection. The height of each plant was recorded, as were the numbers of leaves and capitula (both mature and immature).

During inspections of the collected material, plants were first searched for any externally feeding insects and then separated into flowers, stems and roots. The material was then dissected under a light microscope to record the immature stages of endophagous species. Leaves were also examined to detect small foliage feeders and leaf miners. The brown paper packets were finally emptied into a sorting tray to detect any insects that had been dislodged from the plants. The insects were identified to order and where possible (*e.g.* immature stages) family and were assigned to an appropriate guild (*e.g.* capitulum feeder, stem borer *etc.*). The numbers of individuals of each taxon (adults and immatures) were recorded per plant. Adults were pinned and all immature stages were preserved in vials containing 70% alcohol for comparison with previously collected taxa (Chapter 1; Egli 2013).

Data analysis

Because the most important insect species were largely represented by endophagous immature stages that could, at best, only be identified to family level, the data were analysed at the level of families and feeding guilds rather than species. The total number of herbivore taxa and that for each of the major herbivore guilds was compared between the seasons. Taxa found only once overall were excluded, while those found only once during these surveys but which were collected during earlier surveys at other locations (Chapter 1; Egli 2013) were included. The seasonal abundance of the most important taxa (Chapter 1; Egli 2013) was calculated as the mean number of individuals per plant, with data from the two populations pooled.

Using SPSS Statistics 21, statistical analyses were carried out on plant features and on insect taxa that were present in at least three seasons. Data on plant height met the assumptions of normality and homogeneity of variances and were compared between seasons using one-way ANOVA and Tukey HSD post hoc tests. As the other plant data (i.e. numbers of leaves and capitula) and insect abundance data did not meet the assumptions of normality, generalized linear modelling was used to compare these between seasons. These models incorporated a Poisson distribution (corrected for over-dispersion) with a log link function. Significance ($P < 0.05$) was assessed using Wald Chi-square statistics and post hoc paired comparisons (Bonferroni adjusted) were performed to indicate differences between

the seasons. Models that analyzed binary data, notably the numbers of mature capitula that were infested, versus uninfested, incorporated a binomial distribution and logit link function.

Results

Plant phenology and utilization

There were significant differences in the sizes of the *S. madagascariensis* plants sampled over the four seasons ($F = 5.788$, $df = 3$, $P = 0.001$), with taller plants sampled in summer (mean ± SE = 59.8 ± 2.8 cm) than in spring (46.2 ± 2.4 cm) and winter (46.6 ± 3.0 cm). Plants sampled in autumn were of intermediate height (54.4 ± 2.7 cm) and not significantly different to those sampled in the other seasons. Despite these size differences, there were no significant seasonal differences in either the numbers of leaves ($Chi^2 = 6.162$, $df = 3$, $P = 0.104$) or mature capitula ($Chi^2 = 7.393$, $df = 3$, $P = 0.06$; Figure 1) available on the plants. Plant height was thus a poor indicator of leaf availability ($y = 3.3579x + 148.98$, $r^2 = 0.0471$) or flowering ($y = 0.3293x + 12.062$, $r^2 = 0.0168$) on the plants. Equivalent amounts of foliar and floral material were thus available to the various guilds of herbivorous insects throughout the year.

In general, the plants were healthy and did not appear to suffer extensive levels of exploitation by the various insect herbivores. While levels of leaf damage were not quantified, some 60% of plants displayed evidence of stem-borer activity. Stem-boring larvae were present in 70% of plants sampled in summer, 85% in autumn, 40% in winter and 45% in spring. In contrast, some 87% of plants were infested by capitulum feeders. Capitulum-boring larvae were present in 89% of plants sampled in summer and 80% in both autumn and winter, while all plants were infested in spring. However, in relation to the numbers of mature capitula available per plant, some 32% were infested by insect larvae in summer, compared with only 12% in autumn and 22% in winter and then 64% in spring (Figure 1). The seasonal differences in capitulum exploitation were significant ($Chi^2 = 76.076$, $df = 3$, $P < 0.0005$) with considerably higher percentages of capitula infested in spring.

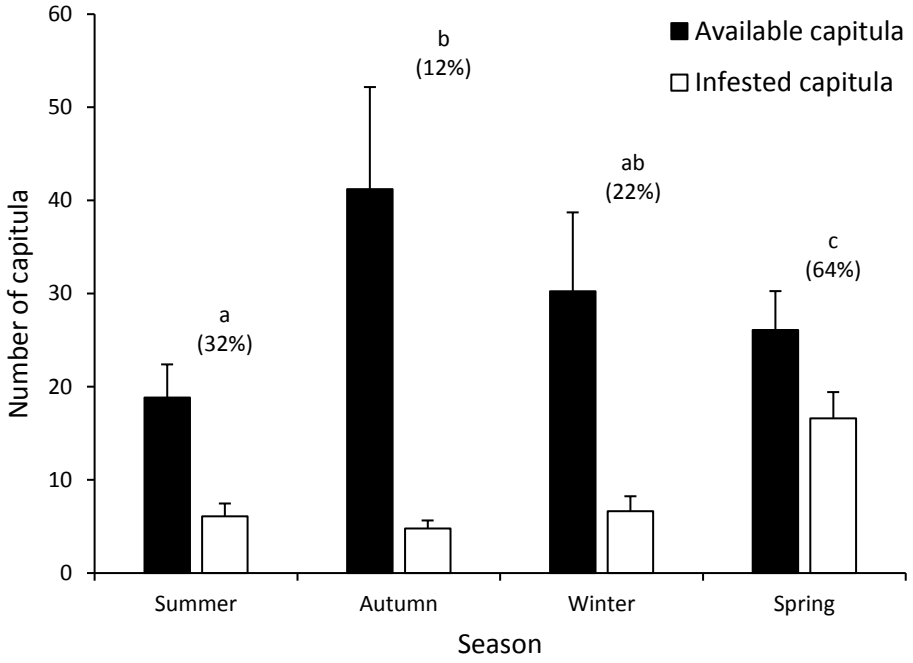

Figure 1: Mean (+SE) numbers of mature capitula that were available to, and infested by, capitulum-feeding taxa on individual plants of *Senecio madagascariensis* during each season. The mean percentages of infested capitula are indicated in brackets and compared using generalized linear modelling; means with different letters are significantly different ($P < 0.05$).

Seasonal diversity of herbivore taxa

Of the 37 insect herbivore taxa that were recorded on *S. madagascariensis* at different localities in KwaZulu-Natal (see Chapter 1; Egli 2013), some 31 (84%) were recovered from the sampled populations. In particular, the most important endophagous taxa (see below) including capitulum feeders, stem borers and leaf miners were present, indicating that the plant populations were well representative of the *S. madagascariensis* insect herbivore fauna in KwaZulu-Natal. As before, no endophagous larvae were recovered from the roots. All 31 taxa were recorded in summer, while 81% and 71% of them were recorded in autumn and spring, respectively, and only 35% of them in winter (Figure 2). These patterns were consistent with the capitulum feeders, stem borers and foliage feeders, while the leaf miners remained the same throughout (Figure 2). Summer was thus confirmed as the best time for the sampling of insects on *S. madagascariensis* populations.

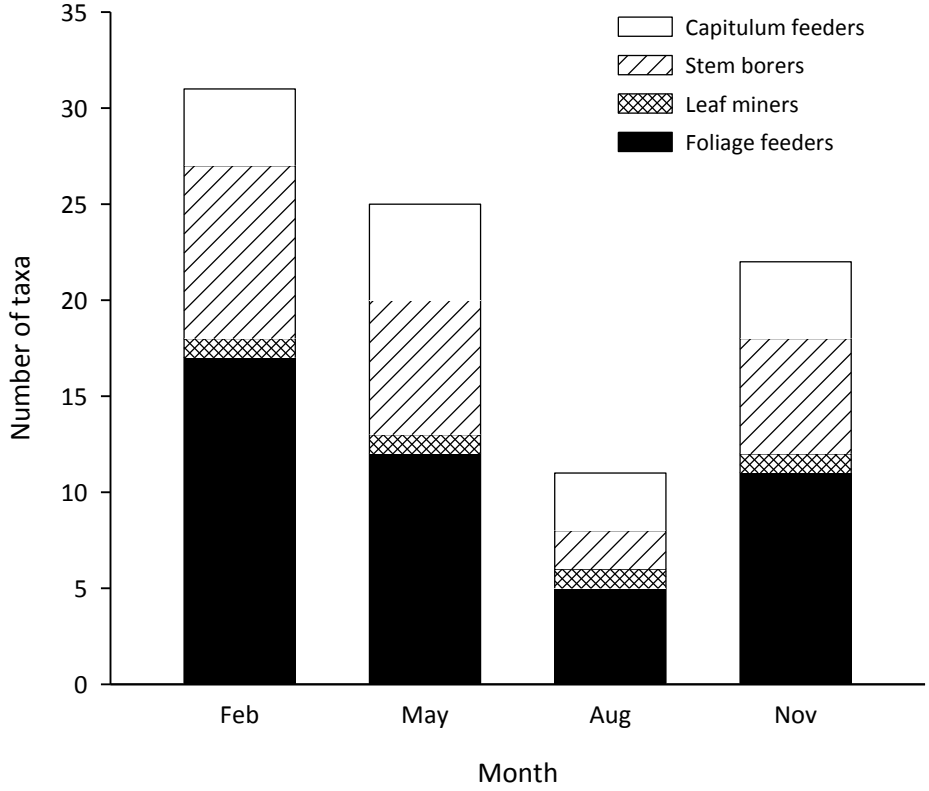

Figure 2: Total numbers of herbivorous insect taxa, from different feeding guilds, associated with populations of *Senecio madagascariensis* during each season.

Seasonal abundance of major herbivore taxa

Of the 10 taxa that were highlighted as potential biological control agents (see Chapter 1; Egli 2013), nine were recovered during this study and included three capitulum feeders, four stem borers and two foliage feeders (Table 1). Dipteran (Agromyzidae and Tephritidae) and lepidopteran (Pyralidae) larvae were the primary capitulum feeders, while coleopteran (Curculionidae), dipteran (Agromyzidae and Tephritidae) and lepidopteran (Tortricidae) larvae were the main stem borers. The foliage feeders included adults and nymphs of the sap-sucking bug *Hilda patruelis* Stal (Tettigometridae) and adults of *Longitarsus basutoensis* Bechnyé (Chrysomelidae: Alticinae) which has root-feeding larvae.

Four of the nine taxa were recovered in all four seasons (Table 1), namely the capitulum-feeding Agromyzidae (Figure 3a), stem-boring Agromyzidae and Tephritidae (Figure 3d, 3e) and foliage-feeding Tettigometridae (Figure 3f). Two taxa were recovered in three seasons (Table 1), namely the capitulum-feeding Tephritidae (Figure 3b), which were absent in summer, and the stem-boring Curculionidae (Figure 3c), which were absent in

winter. Three taxa were recorded in fewer than three seasons (Table 1, 2) and included the stem-boring Tortricidae (summer and autumn), foliage-feeding Chrysomelidae (summer and autumn) and capitulum-feeding Pyralidae (summer only).

Of the capitulum-feeders, the Agromyzidae were the most commonly encountered and were recorded in the highest numbers, while the Tephritidae and Pyralidae were less frequently encountered and were recorded in considerably lower numbers (Table 1; Figure 3). There were significant differences in the abundance of the Agromyzidae between the seasons ($Chi^2 = 27.968$, df = 3, $P < 0.0005$), with the highest numbers recorded in spring and summer and considerably fewer recorded in autumn and winter (Figure 3a). The Tephritidae were absent in summer but occurred in similar numbers from autumn to spring (Figure 3b) and the differences were not significant ($Chi^2 = 0.071$, df = 2, $P = 0.965$), while the Pyralidae were rare and recovered in low numbers in summer only (Table 2).

Of the stem-borers, the Agromyzidae and Tephritidae were the most commonly encountered and were recorded in the highest numbers, while the Curculionidae and Tortricidae were less frequently encountered and were recorded in lower numbers (Table 1; Figure 3). Although present in the stems of *S. madagascariensis* throughout the year, there were significant differences in the abundance of both the Agromyzidae ($Chi^2 = 15.893$, df = 3, $P = 0.001$) and Tephritidae ($Chi^2 = 18.253$, df = 3, $P < 0.0005$) between seasons, with numbers peaking in autumn (Figures 3d, e). There were also significant differences in the abundance of the Curculionidae ($Chi^2 = 8.820$, df = 2, $P = 0.012$) with numbers peaking in summer, when the abundance of the two dipteran taxa was relatively low. However, the numbers of weevil larvae in the stems were not significantly different between the three seasons in which they were recorded (Figure 3c). The larvae of the Tortricidae were recorded in summer and autumn only and in similarly low numbers (Table 2).

Of the foliage-feeders, adults and nymphs of the sap-sucking *H. patruelis* were recovered throughout the year (Table 1) and in fairly high numbers (Figure 3f). There were significant differences between the seasons ($Chi^2 = 10.250$, df = 3, $P = 0.017$), with fewer individuals recorded during winter. Adults of the flea beetle *L. basotuensis* were recorded in summer and autumn only (Table 1) and in very low numbers (Table 2).

Table 1: Seasonal incidence of insect herbivore taxa commonly associated with *Senecio madagascariensis* in KwaZulu-Natal, South Africa that may have potential as biological control agents.

Guild	Taxon	Possible identity[a]	Seasonal incidence[b]			
			Sum	Aut	Win	Spr
Capitulum feeders	Diptera					
	Agromyzidae	Indeterminate genus and species	85	55	35	90
	Tephritidae	At least two different species	0	10	15	15
	Lepidoptera					
	Pyralidae	*Homeosoma stenotea* Hampson	20	0	0	0
Stem borers	Coleoptera					
	Curculionidae	*Gasteroclisus tricostalis* (Thunberg)	25	5	0	10
	Diptera					
	Agromyzidae	*Melanagromyza* sp.	5	60	35	15
	Tephritidae	*Coelopacidia strigata* Bezzi	10	40	15	5
	Lepidoptera					
	Tortricidae	*Lobesia* sp.	15	15	0	0
Foliage feeders	Hemiptera					
	Tettigometridae	*Hilda patruelis* Gerstaecker	45	45	20	40
	Coleoptera					
	Chrysomelidae	*Longitarsus basutoensis* Bechnyé	5	5	0	0

[a] Based on previous identifications (McFadyen & Morin 2012; Sheppard *et al.* 2013).

[b] Percentage of plants on which each taxon was recorded during summer (Sum), autumn (Aut), winter (Win) and spring (Spr).

Table 2: Mean (±SE) numbers of individuals of important insect herbivore taxa that were associated with *Senecio madagascariensis*, but in fewer than three seasons.

Feeding guild	Taxon	Seasons	Numbers/plant
Capitulum feeders	Lepidoptera		
	Pyralidae	Summer	0.2 ± 0.09
Stem borers	Lepidoptera		
	Tortricidae	Summer	0.3 ± 0.14
		Autumn	0.2 ± 0.12
Foliage feeders	Coleoptera		
	Chrysomelidae	Summer	0.05 ± 0.05
		Autumn	0.1 ± 0.10

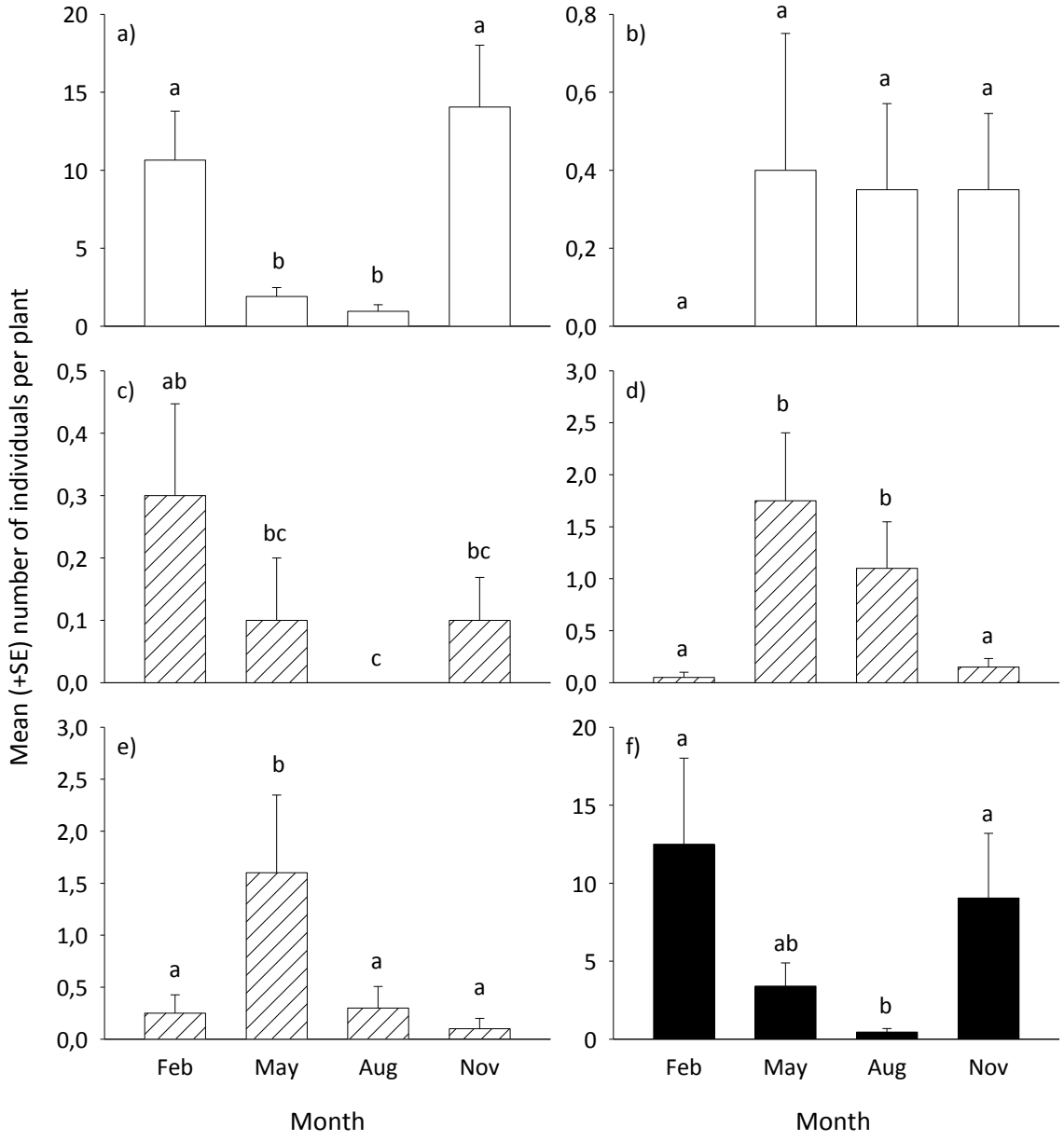

Figure 3: Mean (+SE) numbers of individuals of capitulum-feeding a) Agromyzidae and b) Tephritidae; stem-boring c) Curculionidae, d) Agromyzidae and e) Tephritidae; and foliage-feeding f) Tettigometridae on *Senecio madagascariensis* during each season. Means were compared using generalized linear modelling; those with different letters are significantly different ($P < 0.05$).

Discussion

Senecio madagascariensis populations remain phenologically similar throughout the year (Hilliard 1977; Csurhes & Navie 2010), with individual plants providing equivalent amounts of foliar and floral resources for herbivorous insects. In countries where the plant is invasive, herbivore pressure should thus be sustained throughout the year in order to achieve biological control. Indeed, several insect agents that were deployed against asteraceous weeds have failed to achieve adequate impact because of inconsistent seasonal presence/abundance (Woodburn & Cullen 1995; Julien & Griffiths 1998; Hodson *et al*. 2003; Julien *et al*. 2012). In particular, the plant's ability to flower continuously throughout the year will require the release of capitulum-feeding taxa that can sustain constant pressure on its reproductive tissues. In its native European habitats, the capitulum weevil, *Larinus latus* Herbst (Curculionidae), is present throughout the flowering period of *Onopordum* thistles (Asteraceae), and therefore sustains continual pressure (Briese *et al*. 1994). In Australia, *L. latus* and the stem-boring weevil *Lixus cardui* Olivier are able to control *Onopordum* species by consistently reducing seed production and plant vigour (Briese 2012).

The agromyzid was the most abundant capitulum-feeder on *S. madagascariensis* and was recorded throughout the year. However, the agromyzid is considered to be the least damaging of the capitulum feeders (Chapter 1; Egli 2013) and is thus not a priority candidate. Tephritid larvae, comprising several species (Sheppard *et al*. 2013), are more damaging but were present in much lower numbers and were not recorded in summer. However, this may be an artefact of low and variable abundance since the tephritids were recorded in summer at other sites in the province (Chapter 1; Egli 2013). Larvae of the pyralid (probably *Homeosoma stenotea* Hampson) clearly caused the most damage to infested capitula, but were uncommon and recorded only in summer, probably also due to low and variable abundances. However, combinations of agents from the same guild can offset low densities of a particular agent at specific times. For example, a combination of the capitulum weevil *Rhinocyllus conicus* Froelich, which occurs only at the start of the flowering season, and the capitulum fly *Urophora solstitialis* (L.) (Tephritidae), which occurs throughout the flowering season, provided control of nodding thistle, *Carduus nutans* L. (Asteraceae), in Australia (Woodburn & Cullen 1995; Cullen & Sheppard 2012). Consequently, the pyralid and tephritid larvae, which displayed presence and abundance at different times, may be able to complement each other with less likelihood of negative competitive interactions.

Besides capitulum-feeding agents, those that feed on photosynthetic and structural tissues are also required to consistently reduce the growth rates of *S. madagascariensis* plants as well as indirectly reduce their reproductive output. Indeed, plants are often able to recover from feeding damage when it is not sustained. Larval damage by the stem-boring weevil, *Ceutorhynchus litura* (Fabricius), during spring, is insufficient to control *Cirsium arvense* (Linnaeus) Scopoli (Asteraceae) because the plants are able to recover during the following summer (Peschken & Derby 1992; Hein & Wilson 2004). Of the four stem-boring taxa, the agromyzid (presumably a species of *Melanagromyza*) and the tephritid (probably *Coelopacidia strigata* Bezzi) were the most abundant and occurred throughout the year. In contrast, the weevil (probably *Gasteroclisus tricostalis* (Thunberg)) appeared to be the most damaging, but occurred in lower numbers and was not recorded in winter. The tortricid (presumably a species of *Lobesia*) was similarly uncommon and was recorded only in summer and autumn. Although there were seasonal differences between the stem borers in relation to their presence and abundance, none should be excluded as candidate agents at this stage and additional studies should provide a better indication of which are the most promising.

Only two ectophagous foliage-feeding taxa were recovered in appreciable numbers during this study, namely the tettigometrid (*H. patruelis*) and the flea beetle (*L. basotuensis*). The tettigometrid was present throughout the year and occurred in relatively high numbers in spring and summer. Besides uncertainty about the extent of damage inflicted, this species was also recorded on *Senecio polyanthemoides* Sch. Bip., albeit only in summer and in much lower numbers than on *S. madagascariensis* (Chapter 1; Egli 2013). Although this species is polyphagous and unsuitable for biocontrol, oligophagous species with narrower host ranges might be safe for release in Hawaii where host-specificity requirements may be less stringent due to an absence of native *Senecio* species (Ramadan *et al*. 2011). However, this is not true of Australia which supports a diverse native *Senecio* flora (McFadyen & Morin 2012). Adults of the flea beetle were recovered in very low numbers and only in summer and autumn. Because of the mobility of the adults, these low recoveries may be an artefact of the sampling methods which allowed the adults to escape. Despite no evidence of root-boring immature stages, it is now known that the flea beetle larvae are soil dwelling and feed externally on the roots of *S. madagascariensis*. The uprooting of plants would similarly have precluded the sampling of larvae and procedures that specifically target flea beetles and their larvae would be necessary to accurately determine their seasonal abundance. The potential of the flea

beetle is exemplified by the case of the related weed *Jacobaea vulgaris* Gaertn. (previously *Senecio jacobaea* L.) in Australia, where root-feeding larvae of the flea beetle *Longitarsus flavicornis* (Stephens) were able to damage the plant throughout much of the year and contribute to its control (Ireson *et al*. 1991, 2000; Ireson & McLaren 2012).

In conclusion, there are several insect herbivore taxa that exploit the floral and foliar tissues of *S. madagascariensis* and could be considered as candidate biological control agents for countries like Australia and Hawaii. Although host-specificity tests and impact assessments will ultimately determine the suitability of these, seasonal abundance data have provided some insights into which are likely to cause sustained damage. In particular, some agents that were deemed to be promising from a damage perspective displayed considerable seasonal variation in presence/abundance (*e.g.* the capitulum-feeding pyralid) while others were less variable (*e.g.* most stem-boring taxa). The release of combinations of agents, that attack the same or different tissues, may be required to compensate for differences in seasonal abundance in order to ensure sustained herbivore pressure throughout the weed's phenology.

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CHAPTER 3

Establishment and impact of insect agents deployed for the biological control of invasive Asteraceae: Prospects for the control of *Senecio madagascariensis*

Abstract Several invasive Asteraceae have been targeted for biological control worldwide, with variable success. *Senecio madagascariensis* Poiret, which invades agricultural lands in Australia and Hawaii, is a recent target. Since several potential insect agents were recorded in the plant's native range in South Africa, we assessed biocontrol efforts against asteraceous weeds to determine those most likely to deliver success. Some 108 insect species, from five orders and 23 families, were deployed against 38 weed taxa, mostly in the mainland USA, Canada, Australia and New Zealand. Coleoptera (mainly Curculionidae and Chrysomelidae), Diptera (Tephritidae) and Lepidoptera (Tortricidae) featured the most. Despite high establishment success (73% of releases across countries), only 37% of successful releases achieved meaningful impact. Although root-feeding and stem-feeding insects appeared to be the best candidates, neither insect family nor feeding guild significantly influenced the probability of success. This synthesis of the global contribution of different guilds of specialist herbivores to the management of invasive Asteraceae is guiding the selection of candidate agents for the biocontrol of *S. madagascariensis* in Australia.

Keywords: agent selection, fireweed, insect herbivore guilds, success rates, weed biocontrol

Introduction

The Asteraceae is one of the world's most successful plant families, as indicated by its high species diversity (around 22,000), wide geographical distribution, occupation of diverse habitats, and tolerance of a broad range of environmental conditions (Kadereit 2007). Several common traits, although not necessarily unique to Asteraceae, contribute to this success, notably short life histories that promote rapid evolution, self-compatibility, unspecialized pollinators and high seed set (Kadereit 2007). Such traits are also typical of invasive plants (Culliney 2005) and several species of Asteraceae have become invasive worldwide, with negative impacts on agriculture, forestry, animal/human health, and native biodiversity (Culliney 2005; Julien *et al*. 2012). Many of these were targeted with biological control, with some notable successes (Culliney 2005; Julien *et al*. 2012; Winston *et al*. 2014). These

precedents suggested that biocontrol of the highly invasive *Senecio madagascariensis* Poiret was feasible (Sheppard *et al*. 2013).

Native to southern Africa and Madagascar, *S. madagascariensis* was introduced worldwide through contaminated agricultural produce and became invasive in several countries, notably Australia and Hawaii, USA (Le Roux *et al*. 2010; McFadyen & Morin 2012). Commonly known as fireweed because of its profusion of yellow flowers in invaded landscapes, the plant causes major agricultural losses due to reduced grazing potential and livestock poisoning (McFadyen & Morin 2012). The ineffectiveness of conventional control methods has fostered considerable interest in biological control (Ramadan *et al*. 2010; McFadyen & Morin 2012). Earlier efforts by Australia culminated in the testing of two insect agents from Madagascar and one rust fungus from South Africa. Neither of the insects were released due to a lack of host specificity (McFadyen & Morin 2012), while the rust fungus was deemed to have insufficient impact relative to other rusts already associated with the weed (Morin *et al*. 2009). Efforts by Hawaii resulted in the release of one insect agent from Madagascar (Ramadan *et al*. 2010). The possibility of new agents has recently arisen (McFadyen & Morin 2012; Sheppard *et al*. 2013) because weed populations in both Australia and Hawaii are now known to originate from KwaZulu-Natal, South Africa and not Madagascar (Radford *et al*. 2000).

The insect herbivore fauna of *S. madagascariensis* was quantitatively surveyed around KwaZulu-Natal, South Africa and several taxa were tentatively prioritized as candidate agents on the basis of their distribution, abundance and observed damage (Chapter 1; Chapter 2; Egli 2013; Egli & Olckers 2015). Agent selection may be facilitated by considering the establishment and success of other species in the same family/guild that were released elsewhere for the biocontrol of Asteraceae. Based on an analysis of the outcomes of weed biocontrol programmes some 30 years ago, Crawley (1989) concluded that insect taxa like Curculionidae and Chrysomelidae were more successful as agents than other taxa. Given the substantial increase in the number of biocontrol programmes and agent releases, these trends may not apply across all weed taxa (e.g. Asteraceae).

An assessment of biocontrol programmes launched against asteraceous weeds was thus undertaken to determine the nature of the agents that were deployed and the outcomes of the releases. The hypotheses that the release of certain agent taxa (e.g. Curculionidae) or types of agents (e.g. stem borers) influenced agent establishment and biocontrol success

were tested. Such information could provide some insight into the insect taxa and feeding guilds that are most likely to become established and contribute to the control of *S. madagascariensis*.

Materials and methods

We compiled a database on insect biocontrol agents that were deployed against invasive Asteraceae, focusing on programmes in the six most active countries, namely Australia, Canada, Hawaii (USA), New Zealand, South Africa and the mainland USA (given its remote location, Hawaii was assessed as a separate 'country'). Details on all agents released in these countries were retrieved from the most recent weed biocontrol database (Winston *et al*. 2014). These included "reassociation weeds" (see Paynter *et al*. 2012) where agents were opportunistically released on weeds that are closely related to the main target. Recent releases not recorded in Winston *et al*. (2014) were excluded from our analysis.

The establishment success and impact of each agent was recorded for each country in which it was released. Agents were assigned to one of four guilds: 1) capitulum feeders (i.e. seed feeders, flower head feeders and flower gallers); 2) foliage feeders (i.e. leaf feeders, sap suckers, leaf miners and leaf gallers); 3) stem feeders (i.e. stem borers, crown borers, stem gallers and stolon gallers) and; 4) root feeders (i.e. root borers and external root feeders). In assessing agent impact, we excluded records where the agents did not establish or where there was no information on project outcomes, either because no comprehensive post-release evaluations had been conducted or because the programmes were still in their infancy. Agent impact was assigned to one of three categories: 1) extensive (i.e. reduced the weed's populations or reproductive output over much of the invaded range, rendering it a minor problem); 2) considerable (i.e. reduced the weed's populations or reproductive output over part of the invaded range, rendering it a minor problem in some areas, or a lesser problem overall); or 3) negligible (i.e. caused no reduction in weed populations or reproductive output or only limited localised impacts). Impact categories were defined according to those used by Klein (2011) to categorise agent impacts in South Africa. The outcomes of releases were assigned to these based on the descriptions of impact in Winston *et al*. (2014).

The efficacy of the different insect taxa (i.e. families) and feeding guilds was evaluated (on a countrywide basis) in terms of releases that resulted in establishment and established

agents that contributed to different levels of impact. Releases that achieved extensive or considerable impacts were considered as successful. Since establishment and success were assessed at a binary level, generalized linear modelling, incorporating a binomial distribution, logit link function and an exchangeable working correlation matrix, was used to determine any effects of agent family and feeding guild. Significance ($P < 0.05$) was assessed using Wald chi-square statistics and post-hoc paired comparisons (Bonferroni adjusted) were performed to indicate any differences between the insect families or guilds. To allow computation, only families that incorporated a minimum of three releases ($n = 16$) were included in the statistical analysis.

Results

Biological control targets

Some 38 asteraceous weed taxa (including subspecies and species groups) from 10 tribes have been subjected to biocontrol by the world's most active countries (Table 1). Around 50% of these were targeted by a single country while 26%, 13% and 11% were targeted by two, three and four or more countries, respectively. The most targeted tribes were the Cardueae (20 taxa), Cichorieae (5), Heliantheae (3) and Eupatorieae (3), all of which are distantly related to the Senecioneae and hence *S. madagascariensis* (Funk *et al*. 2009). A single species in the tribe Senecioneae was targeted, namely *Jacobaea vulgaris* Gaertn. (= *Senecio jacobaea* L.).

Some 108 insect species, from 23 families in 5 orders, were deployed against these plants. Weedy asteraceous taxa (Table 1; Figure 1) were mostly targeted by the mainland USA (19), Canada (16), Australia (13) and New Zealand (12). Similarly, most releases of insect agents (Table 1) were conducted by the mainland USA (80 species), Australia (70), Canada (56) and New Zealand (33). Weeds were targeted with most success (i.e. the agents had extensive or considerable impacts) in the mainland USA (12 species; 3 extensive), Australia (9; 4), Canada (6; 2) and New Zealand (3; 1) (Figure 1).

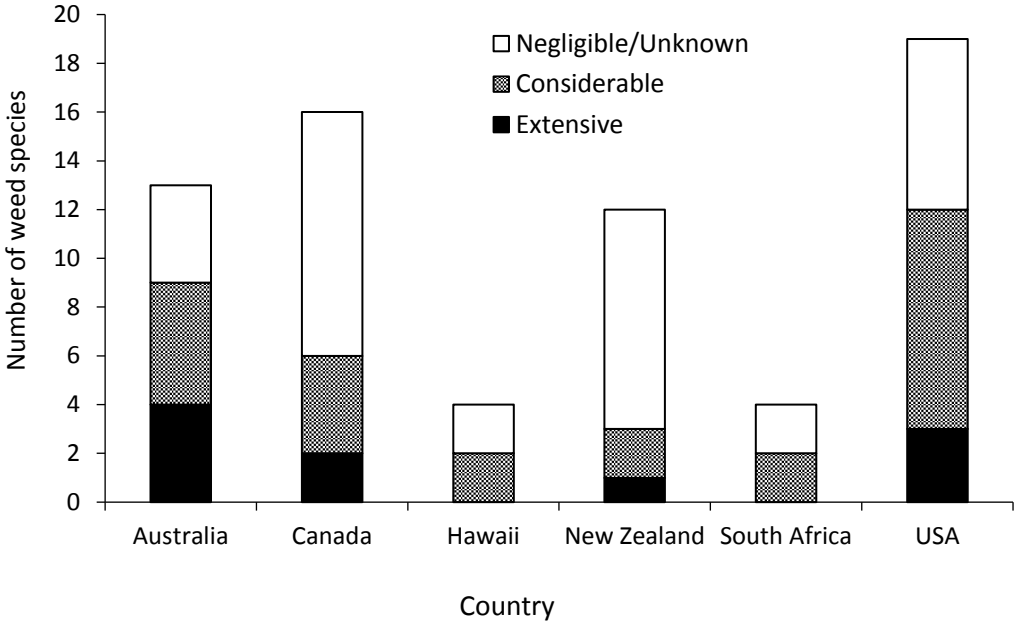

Figure 1: Number of weed species in the family Asteraceae that suffered extensive, considerable and negligible/unknown damage when targeted with biocontrol by the world's six most active countries.

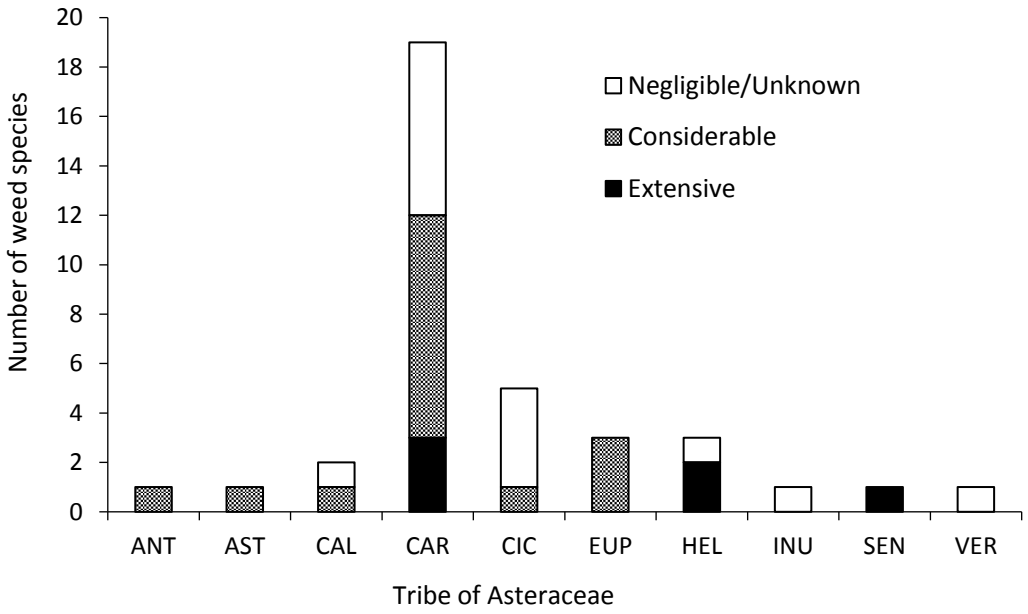

Figure 2: Number of weed species in the different tribes of Asteraceae that were targeted with biocontrol by the world's six most active countries, with extensive, considerable and negligible/unknown outcomes in at least one country. ANT = Anthemideae; AST = Astereae; CAL = Calenduleae; CAR = Cardueae; CIC = Cichorieae; EUP = Eupatorieae; HEL = Heliantheae; INU = Inuleae; SEN = Senecioneae; and VER = Vernonieae.

Overall, 16% of the targeted weeds suffered extensive impacts, 43% considerable impacts and 41% negligible or unknown impacts (Figure 2). Weeds in the tribe Cardueae (thistles) were targeted with the most success (i.e. extensive or considerable impacts in at least one country), with three species (16%) extensively impacted and nine species (47%) considerably impacted by the agents (Figure 2). Extensive impacts were also achieved with weeds in the tribes Heliantheae (two species) and Senecioneae (one species), while considerable impacts were achieved with three species in the Eupatorieae and one species each in the Astereae, Anthemidae, Calenduleae and Cichorieae (Figure 2).

Table 1: Invasive species of Asteraceae against which insect agents were released in the world's six most active countries, namely Australia (AU), Canada (CA), Hawaii (HI), New Zealand (NZ), South Africa (SA) and the mainland United States of America (US). The numbers of agent species that were released, became established and were effective are provided for each country.

Tribe	Countries	Numbers of agent species		
Plant species		Released	Established	Effective [a]
Anthemideae				
Tripleurospermum inodorum (L.) Schip. Bip.	CA	3	3	2
Astereae				
Baccharis halimifolia L.	AU	13	6	2
Calenduleae				
Chrysanthemoides monilifera (L.) Norl. subsp. *monilifera*	AU, NZ	7, 1	0, 1	0, 0
Chrysanthemoides monilifera (L.) Norl. subsp. *rotundata* (DC.) Norl.	AU	7	4	1
Cardueae				
Carduus acanthoides L.	CA, NZ, US	3, 1, 3	3, 1, 2	0, 0, 1
Carduus nutans L.	AU, CA, NZ, US	3, 3, 3, 5	3, 3, 3, 2	3, 3, 3, 2
Carduus pycnocephalus L.	NZ, US	2, 3	2, 3	0, 3
Carduus tenuiflorus Curt.	NZ, US	2, 3	2, 3	0, 2
Centaurea cyanus L.	US	1	1	0
Centaurea diffusa Lam.	CA, US	9, 12	8, 12	3, 3
Centaurea jacea L. nothosubsp. *pratensis* (W.D.J. Koch) Čelak	CA, US	1, 5	1, 2	0, 0
Centaurea jacea L. subsp. *jacea*	US	1	1	1
Centaurea jacea L. subsp. *nigra* (L.) Bonnier & Layens	US	1	1	1
Centaurea solstitialis L.	US	6	5	1
Centaurea stoebe L.	CA, US	12, 12	8, 11	3, 4
Centaurea virgata Lam. subsp. *squarrosa* (Boiss.) Gugler	US	8	4	2
Cirsium arvense (L.) Scopoli	CA, NZ, US	6, 7, 5	4, 4, 4	0, 0, 0

Table 1: Continued

Tribe	Countries	Numbers of agent species		
Plant species		Released	Established	Effective [a]
Cardueae				
Cirsium palustre (L.)	CA, NZ	2, 2	2, 2	0, 0
Cirsium vulgare (Savi) Ten.	AU, CA, NZ, SA, US	3, 3, 2, 2, 4	3, 3, 2, 1, 4	1, 1, 0, 0, 1
Onopordum acanthium L.	CA, US	2, 2	2, 0	0, 0
Onopordum acaulon L.	AU	2	0	0
Onopordum spp. (parent & hybrid forms of *O. acanthium* L. and *O. illyricum* L.)	AU	7	4	4
Rhaponticum repens (L.) Hidalgo	CA, US	2, 2	2, 2	0, 0
Silybum marianum (L.) Gaertn.	AU, SA, US	1, 1, 1	1, 0, 1	0, 0, 0
Cichorieae				
Chondrilla juncea L.	AU, CA, US	2, 1, 2	1, 1, 2	1, 0, 0
Pilosella aurantiaca (L.) F.W. Schultz & Sch. Bip.	CA, US	1, 1	0, 0	0, 0
Pilosella flagellaris (Willd.) Arv.-Touv.	CA	1	0	0
Pilosella officinarum Vaill.	NZ	5	2	0
Sonchus arvensis L.	CA	3	2	0
Eupatorieae				
Ageratina adenophora (Spreng,) R.M. King & H. Rob	AU, HI, NZ, SA	1, 2, 1, 1	1, 1, 1, 1	0, 1, 0, 1
Ageratina riparia (Regel) R.M. King & H. Rob.	AU, HI, NZ	1, 3, 1	1, 2, 1	0, 2, 1
Chromolaena odorata (L.) R.M. King & H. Rob	SA	5	2	1
Heliantheae				
Ambrosia artemisiifolia L.	AU	4	3	2
Parthenium hysterophorus (L.)	AU	9	9	4
Xanthium strumarium L. sens. lat. (several species)	AU	4	3	0
Inuleae				
Pluchea carolinensis (Jacq.) G. Don	HI	2	2	0
Senecioneae				
Jacobaea vulgaris Gaertn.	AU, CA, NZ, US	6, 4, 6, 3	5, 4, 4, 3	3, 2, 3, 2
Vernonieae				
Elephantopus mollis Kunth	HI	1	1	0

[a] Effective agents include those that resulted in extensive and considerable impacts (see Methods for description)

Agent establishment and impact by taxon

Five insect orders have provided agents for asteraceous weeds (Table 2), with most sourced from the Coleoptera (45 species), Diptera (35) and Lepidoptera (25) and few from the Hymenoptera (2) and Hemiptera (1). Agents were sourced from 23 insect families, six in the Coleoptera, five in the Diptera and 10 in the Lepidoptera (Table 2). Coleopteran agents

mostly comprised Curculionidae (21 species) and Chrysomelidae (18), while dipteran agents mostly comprised Tephritidae (21 species). Lepidopteran agents largely comprised Tortricidae (7 species), Pterophoridae (4), Erebidae (4) and Gelechiidae (3).

Some 256 releases of these agents were carried out by world's most active countries, of which 188 (73%) achieved establishment (Table 1). Establishment success was highest in Canada (82%), the mainland USA (79%), New Zealand (76%) and Hawaii (75%), with lower success in Australia (63%) and South Africa (44%). Of the five insect orders deployed across these countries (Table 2), two of two releases (albeit a single species) of Hemiptera established, compared with 88 of 118 releases of Coleoptera (75%), 38 of 53 releases of Lepidoptera (72%), 52 of 73 releases of Diptera (71%), and three of six releases (albeit only two species) of Hymenoptera (50%).

In relation to establishment success by insect family, there were cases where a single agent species was released in one or more countries, resulting in either 100% success (e.g. Arctiidae (four of four releases), Brentidae (one of one), Delphacidae (two of two), Sesiidae (one of one)) or failure to establish (e.g. Apionidae (one of one)). Besides these, the families that established best (Table 2) were Cecidomyiidae (eight of eight releases (100%); 6 species), Gelechiidae (six of six (100%); 3), Curculionidae (69 of 80 (86%); 21), Pterophoridae (four of five (80%); 4), Tephritidae (37 of 51 (73%); 21), Tortricidae (14 of 20 (70%); 7) and Cerambycidae (two of three (67%); 3). Although Chrysomelidae (18 species) featured in several programmes, only 11 of 27 releases (41%) achieved establishment. Overall, there were significant differences between families in relation to their probability of establishment ($\chi^2 = 27.304$, df = 15, P = 0.026).

Table 2: Numbers of insect species from different orders and families that were released in the world's six most active countries for the biological control of weeds in the family Asteraceae, numbers of releases undertaken and the numbers of these that achieved establishment and different levels of impact.

Order Family	No. of species	No. of releases [a]	No. releases established [a]	No. releases with impact on weed [a]		
				Extensive	Considerable	Negligible/ Unknown
Hemiptera	**1**	**2**	**2**	**0**	**0**	**2**
Delphacidae	1	2	2	0	0	2
Coleoptera	**45**	**118**	**88**	**8**	**30**	**50**
Apionidae	1	1	0	–	–	–
Brentidae	1	1	1	0	1	0
Buprestidae	1	6	5	0	3	2
Cerambycidae	3	3	2	0	1	1
Chrysomelidae	18	27	11	2	4	5
Curculionidae	21	80	69	6	21	42
Diptera	**35**	**73**	**52**	**0**	**16**	**36**
Agromyzidae	2	2	1	0	0	1
Anthomyiidae	3	6	3	0	0	3
Cecidomyiidae	6	8	8	0	3	5
Syrphidae	3	6	3	0	1	2
Tephritidae	21	51	37	0	12	25
Hymenoptera	**2**	**6**	**3**	**0**	**0**	**3**
Cynipidae	2	6	3	0	0	3
Lepidoptera	**25**	**53**	**38**	**4**	**9**	**25**
Arctiidae	1	4	4	0	2	2
Bucculatricidae	2	2	2	0	0	2
Erebidae	4	5	2	0	2	0
Gelechiidae	3	6	6	0	0	6
Geometridae	1	2	1	0	1	0
Pyralidae	1	3	2	0	0	2
Pterolonchidae	1	5	2	0	0	2
Pterophoridae	4	5	4	1	2	1
Sesiidae	1	1	1	0	0	1
Tortricidae	7	20	14	3	2	9

[a] In this context, release implies deployment in a particular country and consequently whether this achieved establishment and impact on the target

Of the 188 releases that achieved establishment in the six countries, only 70 (37%) achieved a measure of success (i.e. either extensive or considerable impact) (Table 1). Successes were lowest in New Zealand (28%) and Canada (30%) and higher in the mainland USA (37%) and Australia (43%). Higher successes in Hawaii and South Africa (both 50%) were skewed by fewer programmes against Asteraceae. Coleopteran agents delivered more

successes (38 of 88 releases (43%)) than lepidopteran (13 of 38 (34%)) and dipteran (16 of 52 (31%)) agents (Table 2). Only four insect families (Table 2) provided agents that inflicted extensive impact, namely Pterophoridae (one of four releases (25%)), Tortricidae (three of 14 (21%)), Chrysomelidae (two of 11 (18%)) and Curculionidae (six of 69 (9%)). In contrast, 13 families (Table 2) provided agents that inflicted considerable impact, notably Buprestidae (three of five releases (60%)), Arctiidae (two of four (50%)), Pterophoridae (two of four (50%)), Cecidomyiidae (three of eight (38%)), Chrysomelidae (four of 11 (36%)), Tephritidae (12 of 37 (32%)), Curculionidae (21 of 69 (30%)) and Tortricidae (two of 14 (14%)). However, the differences between families, in relation to the probability of either extensive or considerable impacts being achieved, were not significant ($\chi^2 = 4.640$, df = 15, P = 0.995).

Agent establishment and impact by guild

Insect agents released against Asteraceae comprised mainly foliage feeders (39 species), capitulum feeders (32) and stem feeders (27), with relatively few root feeders (10). Foliage feeders comprised mainly Coleoptera (18 species) and Lepidoptera (14), with fewer Diptera (5) and one species each of Hemiptera and Hymenoptera (Figure 3). Capitulum feeders comprised mainly Diptera (20 species) and Coleoptera (11), with one species of Lepidoptera (Figure 3). Stem feeders comprised mainly Coleoptera (12 species) with fewer Diptera (9) and Lepidoptera (5) and a single species of Hymenoptera, while root feeders comprised similar numbers of Lepidoptera (5) and Coleoptera (4) and one species of Diptera (Figure 3).

Although agent establishment appeared lower in foliage feeders than in capitulum feeders, stem feeders and root feeders (57% vs. 77%, 79% and 79% of releases, respectively; Figure 4), the differences between guilds were not significant ($\chi^2 = 6.449$, df = 3, P = 0.092). Agents from all four guilds delivered a measure of success (i.e. extensive or considerable impacts). Extensive impacts occurred more frequently in root feeders than in stem feeders, capitulum feeders and foliage feeders (12% vs. 9%, 6% and 4% of establishments, respectively; Figure 5). Considerable impacts occurred more frequently in root feeders, stem feeders and foliage feeders, than in capitulum feeders (44%, 41% and 41% vs. 34% of establishments, respectively; Figure 5). Although root feeders and stem feeders appeared more successful than foliage feeders and capitulum feeders (56% and 50% vs. 45% and 40%, respectively; Figure 5), there were no significant differences between these guilds in relation

to the probability of success being achieved through either extensive or considerable impacts ($\chi^2 = 1.635$, df = 3, P = 0.651).

Ten insect taxa that include capitulum feeders, stem borers, foliage feeders and root feeders were prioritized (Cahpter 1; Chapter 2; Egli 2013; Egli & Olckers 2015) as candidate agents for *S. madagascariensis* (Table 3). Five equivalent taxa, notably capitulum-feeding Tephritidae, stem-feeding Curculionidae, Tephritidae and Tortricidae, and root-feeding Chrysomelidae (Alticinae) were deployed against other invasive Asteraceae. In terms of agent species released, capitulum-feeding Tephritidae (18 species in 42 releases) and stem-boring Curculionidae (8, 10) were deployed the most, with stem-feeding Tephritidae (3, 10), stem-boring Tortricidae (3, 7) and root-feeding Chrysomelidae (2, 5) less utilized (Table 3). Taxa not yet deployed against asteraceous weeds include capitulum-feeding or stem-boring Agromyzidae, capitulum-feeding Pyralidae (although other capitulum-feeding Lepidoptera were used) and foliage-feeding Tettigometridae or Tingidae (Table 3).

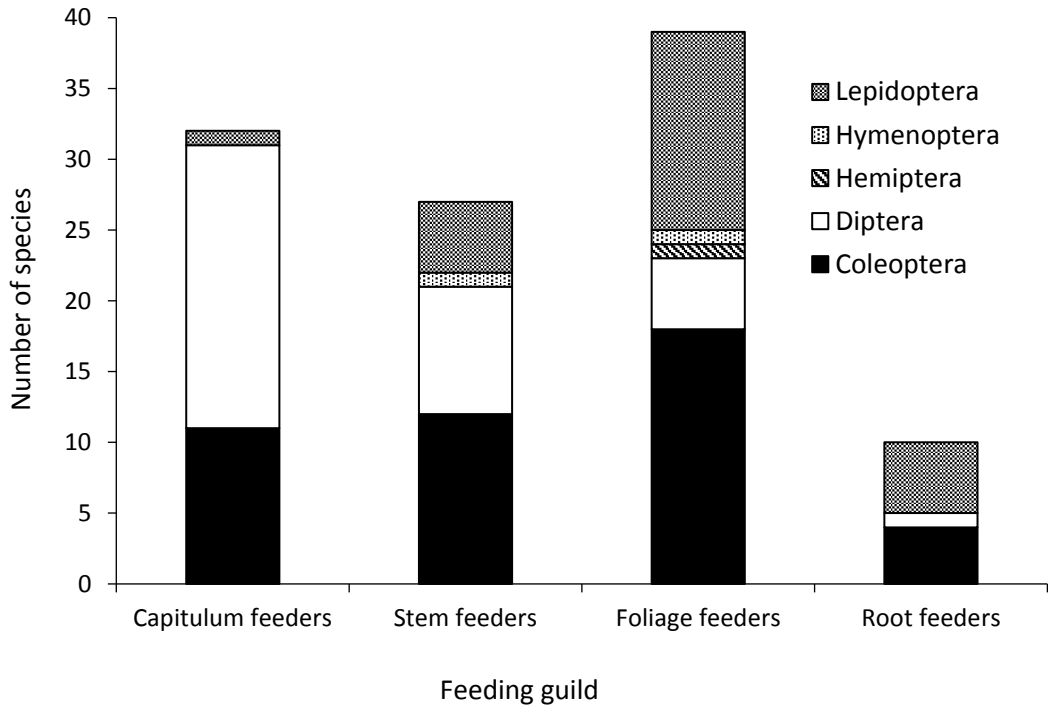

Figure 3: Number of insect species from different feeding guilds and orders that were released for the biocontrol of weeds in the family Asteraceae, in the world's six most active countries.

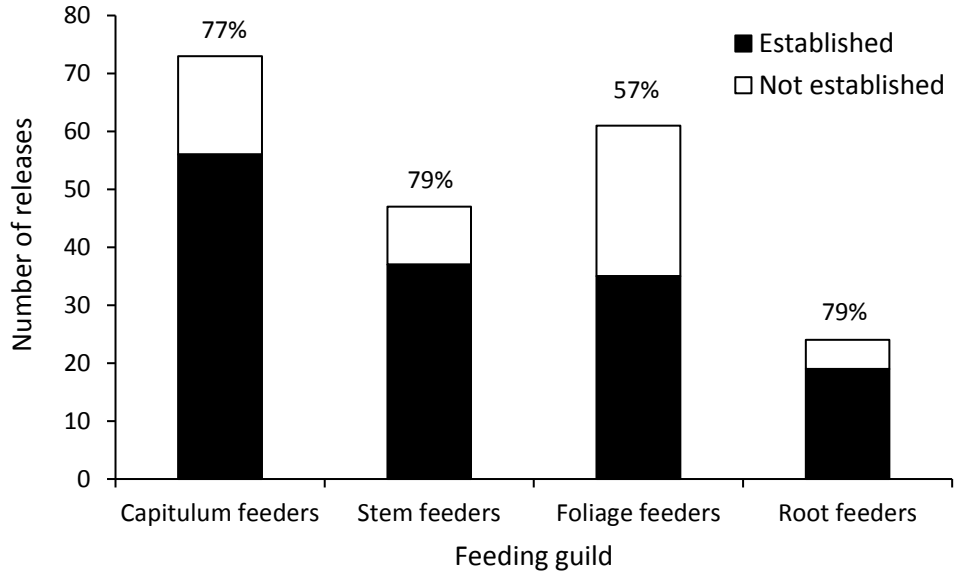

Figure 4: Number of releases of insect agents in each feeding guild that resulted in establishment or failure to establish on weeds in the family Asteraceae, in the world's six most active countries. Percentages on the bars indicate releases that achieved establishment.

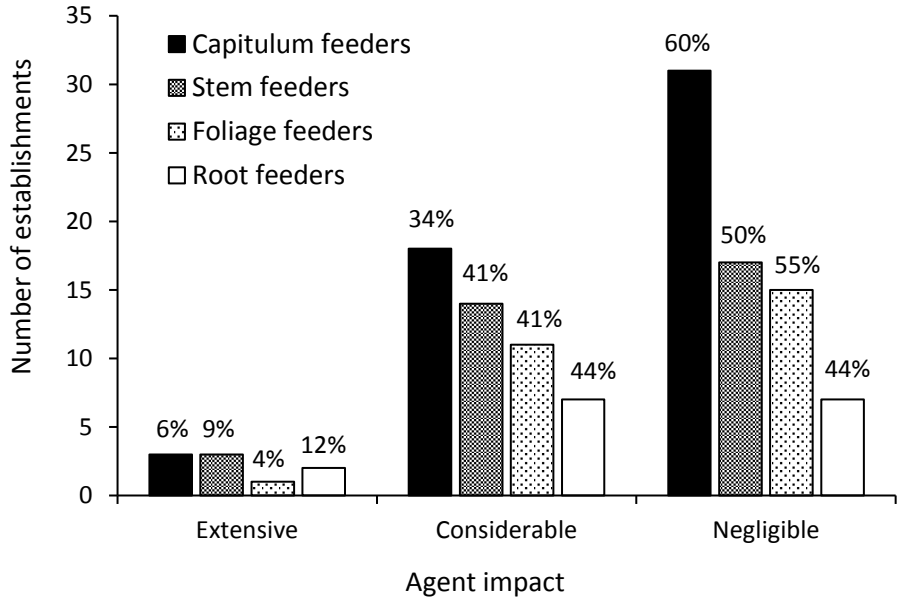

Figure 5: Number of agent establishments in each feeding guild that resulted in extensive, considerable and negligible/unknown impact on weeds in the family Asteraceae, in the world's six most active countries. Percentages represent establishments in each guild that achieved extensive, considerable and negligible/unknown impacts.

Selecting agents for S. madagascariensis

Table 3: Comparison of candidate agents for *S. madagascariensis* with similar agents (i.e. by taxon and guild) that were used in other biocontrol programmes against Asteraceae.

Guild	Taxon	Possible identity [a]	Previous use in biocontrol [b]				
			Spp	Rel	Est	Con	Ext
Capitulum feeders	Diptera						
	Agromyzidae	Indeterminate genus and species	0	-	-	-	-
	Tephritidae	At least three different species	18	42	25	8	0
	Lepidoptera						
	Pyralidae	*Homeosoma stenotea* Hampson	0	-	-	-	-
Stem borers	Coleoptera						
	Curculionidae	*Gasteroclisus tricostalis* (Thunberg)	8	10	7	3	0
	Diptera						
	Agromyzidae	*Melanagromyza* sp.	0	-	-	-	-
	Tephritidae	*Coelopacidia strigata* Bezzi	3	10	10	4	0
	Lepidoptera						
	Tortricidae	Indeterminate genus and species	3	7	6	1	3
Foliage feeders	Hemiptera						
	Tettigometridae	*Hilda patruelis* Stal	0	-	-	-	-
	Tingidae	Indeterminate genus and species	0	-	-	-	-
Root feeders	Coleoptera						
	Chrysomelidae	*Longitarsus basutoensis* Bechnyé	2	5	5	2	2

[a] Based on previous identifications (McFadyen and Morin 2012; Sheppard *et al*. 2013)

[b] Where: Spp = number of agent species; Rel = number of releases in all countries; Est = number of releases that achieved establishment; Con/Ext = number of releases that achieved a considerable (Con) or extensive (Ext) impact

All 10 releases of stem-feeding Tephritidae and five releases of root-feeding Chrysomelidae achieved establishment, compared with six of seven releases of stem-boring Tortricidae (86%), seven of 10 releases of stem-boring Curculionidae (70%) and 25 of 42 releases of capitulum-feeding Tephritidae (60%) (Table 3). However, only four of 10 releases of stem-boring Tephritidae achieved considerable impacts (40%) and none extensive impacts. In contrast, two of five releases of root-feeding Chrysomelidae achieved extensive impacts (40%) while two achieved considerable impacts (40%). Of those that achieved establishment (Table 3), three of six releases of stem-boring Tortricidae achieved extensive impacts (50%) and one a considerable impact (17%), while three of seven releases of stem-boring Curculionidae achieved considerable impacts (43%) and none extensive impacts. Similarly, eight of 25 releases of capitulum-feeding Tephritidae achieved considerable impacts (32%)

and none extensive impacts (Table 3). Despite no significant effect of agent family or feeding guild on biocontrol success with asteraceous targets, these precedents suggest that the root-feeding flea beetle (Chrysomelidae) and stem-boring moth (Tortricidae) may be the most promising candidate agents for *S. madagascariensis* (Table 3).

Discussion

Despite the release and establishment of multiple agent species in most weed biocontrol programmes, only one or two species typically contribute to control (Denoth *et al*. 2002; McFadyen 2003). McFadyen (2003) also reported that only 55% of established agent species contributed to the control of their target weeds, while Crawley (1989) placed this figure at <50%. Although 73% of insect agent releases against invasive Asteraceae achieved establishment, only 37% of these (i.e. 27% of all releases) achieved either extensive or considerable impacts. Indeed, Asteraceae appear to be "difficult targets" for biocontrol (see Paynter *et al*. 2012); of the 38 taxa targeted worldwide, only 22 (58%) were measurably damaged by at least one agent, with six (16%) suffering extensive damage. Australia has achieved the most success which, besides the country's long and successful history in weed biocontrol (Julien *et al*. 2012), may have been influenced by the inclusion of pathogens in its programmes (A. Sheppard, pers. comm.). Australia has released six pathogens against asteraceous targets which is twice as many as the other countries, resulting in more successful impacts (Winston *et al*. 2014).

Although the probability of success in weed biocontrol often increases as more agents are released (Paynter *et al*. 2012), this is not necessarily due to the additive effects of multiple agents, but rather an increased probability of releasing a successful agent (Denoth *et al*. 2002). However, the risk of indirect non-target effects (see Pearson & Callaway 2005; Carvalheiro *et al*. 2008) also increases as more agents are released because of a higher probability of one or more species causing unpredicted environmental effects (Denoth *et al*. 2002). Releases of weed biocontrol agents should thus be limited to those most likely to offer success (e.g. Denoth *et al*. 2002; Sheppard 2003; Pearson & Callaway 2005; Goolsby *et al*. 2006). Several scoring systems and guidelines were thus devised to prioritize agent taxa, using native range criteria such as their distribution, seasonal abundance and damage to vulnerable life stages of the weed (e.g. McClay & Balciunas 2005; Goolsby *et al*. 2006; Raghu *et al*. 2006). Despite this, predictions of agent efficacy are difficult, largely because of insufficient

information to provide accurate scores for the various criteria and the risk of rejecting potentially successful agents because of poor scores for certain criteria (McClay & Balciunas 2005). Also, interactions between the agent, weed and new environment are complex and difficult to predict (Sheppard 2003). Given these shortcomings, we considered the success of equivalent agent taxa/guilds that were deployed against taxonomically related weeds to determine whether this can provide an easier predictive tool.

Although not statistically significant, root-feeding, stem-feeding and capitulum-feeding agents appeared more successful at establishing than foliage-feeding agents, possibly because of their endophagous nature. By virtue of their development within their hosts' tissues, endophagous species potentially have the advantage of being buffered from adverse environmental conditions. Similarly, while root-feeding and stem-feeding agents appeared to deliver greater impact these trends were not statistically significant. Success was also not significantly influenced by insect family, despite the apparent prominence of coleopteran (Curculionidae and Chrysomelidae) and lepidopteran (Tortricidae and Pterophoridae) agents.

Although our analysis has indicated that the prioritisation of candidate agents for *S. madagascariensis* should not be determined by preconceived expectations of success, five species are regarded as having the potential to inflict appreciable damage (Chapter 1; Chapter 2; Egli 2013; Egli and Olckers 2015). These include one capitulum feeder (Pyralidae), three stem borers (Curculionidae; Tephritidae; Tortricidae) and one root feeder (Chrysomelidae: Alticinae). In particular, the most promising species appear to be a root-feeding flea beetle, *Longitarus basotuensis* Bechyné (Chrysomelidae), and an unidentified stem-boring moth (Tortricidae). Indeed, the closely related *J. vulgaris* (= *S. jacobaea*) was controlled in several countries using a root-feeding flea beetle and two stem-boring moths (Pterophoridae and Tortricidae) (Ireson & McLaren 2012; Winston *et al*. 2014). Although Curculionidae did not demonstrate higher efficacy on asteraceous weeds, the impressive track record of weevils in weed biocontrol (Crawley 1989; Herrick & Kok 2010; Winston *et al*. 2014) suggests that the stem-boring *Gasteroclisus tricostalis* (Thunberg) should also be considered for *S. madagascariensis*. While capitulum-feeding agents have featured in the biocontrol of Asteraceae, these were largely Curculionidae that were deployed against thistles (Herrick & Kok 2010; Winston *et al*. 2014). Although capitulum-feeding Pyralidae have not yet been deployed against asteraceous weeds, the damage inflicted by the species on

S. madagascariensis (probably *Homeosoma stenotea* Hampson) suggests that it may warrant further consideration.

Our analysis of biocontrol programmes against asteraceous weeds did not support the conclusions of Crawley (1989) that, besides the cactus-feeding Dactylopiidae (Hemiptera), the insect families most likely to achieve biocontrol success were Curculionidae, Chrysomelidae and Pyralidae. In any event, besides agent impact, biocontrol success depends on several factors that include plant traits (Paynter *et al*. 2012) and the relationship between a weed's density and its negative impact (Thomas & Reid 2007). For example, "high threshold weeds" (see Thomas & Reid 2007) like *S. madagascariensis* that become more problematic at higher densities, may require lower levels of agent damage (i.e. considerable impact) to achieve success than "low threshold weeds" that require extensive impacts. While our analysis has provided some insight into which agent taxa should be prioritized for investigation in Australia and Hawaii, this will be further influenced by their fundamental host ranges, currently under consideration, and the diversity of *Senecio* species that are native to these two countries.

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CHAPTER 4

DNA barcoding provides insight into the field host range of endophagous insects associated with *Senecio madagascariensis* (fireweed) in its native range in KwaZulu-Natal, South Africa

Abstract Fireweed, *Senecio madagascariensis* Poir. (Asteraceae) is a short-lived perennial weed from southern Africa that is incurring high economic losses for livestock farmers in eastern Australia and Hawaii. It is currently the focus of a biological control programme because current control methods are proving to be ineffective. Demonstrating host specificity of biocontrol agents for fireweed in Australia is crucial because there are 87 native *Senecio* species present, several of which are co-occurring with the weed. For Hawaii, however, the demonstration of host specificity is less challenging because there are no native species in the tribe Senecioneae on these islands. Surveys of the native field host range of biocontrol agents can eliminate non-specific candidates at an early stage, thus reducing reliance on time consuming laboratory studies. Endophagous insects from families that have shown host specificity in past biocontrol programs targeting Asteraceae were targeted during this study. Thus stem-boring Curculionidae, and stem-boring and capitulum-feeding Lepidoptera and Diptera were surveyed across 18 *Senecio* species in KwaZulu-Natal, South Africa, to assess their host specificity and potential suitability as biocontrol agents for fireweed in Australia. Since a clear morphological separation of insect larvae to species level is not possible, DNA barcoding was used to differentiate between species associated with plants in the genus *Senecio* and thereby determine host specificity. The Curculionidae, Lepidoptera, and capitulum-feeding Diptera associated with fireweed all contained one or more species that displayed restriction to plants in the *S. madagascariensis* species complex. In contrast, none of the stem-boring Diptera were specific to fireweed or its species complex. This study has thus narrowed the search for potentially host specific insects that could be deployed for the biocontrol of fireweed in Australia and Hawaii and which should be prioritised for further study.

Key words: agent selection, biological control, COI gene, DNA barcoding, invasive plants, phytophagous insects, *Senecio* species

Introduction

The pasture weed *Senecio madagascariensis* Poir. (Asteraceae), native to southern Africa and Madagascar, causes huge losses to livestock farmers in Australia (McFadyen &

Morin 2012), where it has been declared a "Weed of National Significance" (Sheppard *et al*. 2013). It is also highly invasive in pastures in Hawaii (Le Roux *et al*. 2006), parts of South America (Lopez *et al*. 2008; Cruz *et al*. 2010; Le Roux *et al*. 2010) and Japan (Tsutsumi 2011). Mechanical and chemical control measures, which have negative environmental impacts (Culliney 2005), are expensive while only offering only a short-term solution due to the plant's short life history and high reproductive output (Le Roux *et al*. 2006; Sindel *et al*. 2008; DEEDI 2011; Sindel *et al*. 2012; Sheppard *et al*. 2013). There has thus been interest in biological control by both Australia and Hawaii as a less damaging and long-term solution (McFadyen & Sparks 1996).

Agent host specificity is key in ensuring the safety of weed biocontrol (McFadyen 1998) and is crucial for the biological control of *S. madagascariensis* in Australia where there are 87 native *Senecio* species (Thompson 2006). The situation in Hawaii is quite different since there are no native species in the Tribe Senecioneae and despite the size of the genus worldwide (about 1250 species) there are no *Senecio* species of economic importance (Ramadan *et al*. 2011). Any phytophagous insect that is specific to the genus *Senecio* should thus be safe for release in Hawaii, while Australia would require an agent that is specific to *S. madagascariensis* (Ramadan *et al*. 2011).

Initially, Australian and Hawaiian scientists surveyed insects and pathogens on plants in Madagascar for potential biocontrol agents (McFadyen & Sparks 1996; McFadyen & Morin 2012). Hawaii imported and later released the defoliating arctiid moth, *Secusio extensa* (Butler), despite non-target feeding on other plants in the Tribe Senecioneae, as none of these are native to Hawaii (Ramadan *et al*. 2011; Osher 2013). Australia imported and tested two lepidopterans, but neither were safe for release and the programme was later discontinued (McFadyen & Morin 2012). More recently, studies comparing the ITS 1 sequence of *S. madagascariensis* from its native and invasive range have found that the centre of origin of the Australian and Hawaiian populations is the KwaZulu-Natal region of South Africa (Scott *et al*. 1998; Radford *et al*. 2000; Le Roux *et al*. 2006). Renewed efforts to find biocontrol agents for Australia are now focused in KwaZulu-Natal as this is deemed the best place to find host specific agents (Sheppard *et al*. 2013).

Some insight into the host specificity of potential insect biocontrol agents can be gained by assessing their native field host range (Van Klinken 1999; Goolsby *et al*. 2006). Insects that are monophagous in their native range are typically monophagous in the target

plant's invaded range, so an understanding of the native host range of candidate biocontrol agents provides valuable information for their prioritization (Van Klinken 1999; Goolsby *et al*. 2006). In this study, I assessed the native field host range of the stem-boring Curculionidae, and stem-boring and capitulum-feeding Lepidoptera and Diptera associated with fireweed and 17 other *Senecio* species in KwaZulu-Natal, using molecular techniques (sequencing of the COI gene). DNA barcoding can be useful in the identification of insect larvae that are otherwise impossible to differentiate morphologically into different species (Goolsby *et al*. 2006; Rauth & Hufbauer 2009; Gaskin *et al*. 2011). In particular, the technique can be used to match immature stages with adults as well as differentiate between immature stages that are recovered on different hosts and determine whether the same or different species are involved. This study aims to highlight potentially host specific agents, which can then be prioritised for importation into quarantine for laboratory based host-range testing. The taxa targeted in this study were selected because they have been used successfully in many biocontrol programmes, including those against Asteraceae, and are likely to have an impact on *S. madagascariensis* (Chapter 3; Winston *et al*. 2014; Egli & Olckers 2017).

Materials and methods

Study species and plant surveys

The genus *Senecio* L. is one of the largest plant genera with around 1250 species worldwide (Pelser *et al*. 2007 and references therein). Southern Africa has 301 *Senecio* species (Wellman 2003), and 150 of them occur in KwaZulu-Natal (Hilliard 1977). The genus is particularly diverse, containing herbs, shrubs, succulents and vines (Hilliard 1977). *Senecio madagascariensis* belongs to a species complex that includes *S. inaequidens* DC., *S. harveianus* MacOwan, and *S. skirrhodon* DC. (Hilliard 1977). *Senecio inaequidens* and *S. harveianus* occur at high altitudes (above 1400 m above sea level) in the South African Drakensburg (Hilliard 1977) and *S. inaequidens* is invasive in Europe (Hilliard 1977; Lafuma *et al*. 2003; Bossdorf *et al*. 2008). In contrast, *S. skirrhodon* occurs at the edge of sand dunes along the eastern coast of South Africa, and may just be a coastal form of *S. madagascariensis* (Hilliard 1977).

Field surveys took place between the 20th of January 2014 and the 1st of March 2015, mainly during spring and summer, when plants were flowering and insect abundances were expected to be high (Chapter 2; Egli & Olckers 2015). Eighteen *Senecio* species were surveyed

including those in the *S. madagascariensis* species complex (Table 1). Sites were selected based on the presence of large populations of the different *Senecio* species, particularly sites supporting more than one species. Unless otherwise indicated, 10 plants separated by at least 5m were collected for each species at each of three sites (Table 1). Plants were uprooted, placed in brown paper bags and taken back to the laboratory where they were frozen for later processing. Plant material was then separated into capitula and stems. Both were dissected under a light microscope to obtain insect larvae or pupae of Curculionidae, Lepidoptera and Diptera. Insect larvae were dissected out rather than reared to adults to maximise the number of insects collected, as past experience has shown many more larvae present in the plants than are successfully reared as adults. Insect specimens were stored in 100% alcohol for genetic analysis. A voucher specimen of each *Senecio* species at each site was lodged in the Bews Herbarium (NU) at the University of KwaZulu-Natal's Pietermaritzburg campus. Plants were identified to species level using the key in "Compositae in Natal" (Hilliard 1977). These were compared with specimens in the Bews Herbarium, as well as the images of type specimens in the Kew Herbarium Catalogue (KHC 2015). Some plants could not be identified to species level but were keyed out as far as possible (Table 1).

Table 1: Date, coordinates, altitude and habitat of sites were where *Senecio* species were collected in KwaZulu-Natal.

Species	Date	Site	GPS (S;E)	Alt. (m asl)	Habitat
Senecio adnatus DC.	25/11/2014	Vernon Crookes Reserve	-30.264866° 30.595133°	479	Grassland
	09/12/2014	Correctional Services Prison Sevontein	-29.761278° 30.137408°	1460	Grassland
	22/12/2014	Mount Gilboa	-29.285622° 30.292696°	1750	Grassland
Senecio affinis DC.	20/01/2014	Bellvue	-29.635608° 30.433740°	737	Grassland
	18/02/2014	Emanzini	-29.468478° 30.370984°	733	Savannah
	08/01/2015	Ukulinga	-29.666840° 30.403181°	839	Grassland
Senecio bupleuroides DC.	08/10/2014	Hilton Road	-29.506672° 30.288927°	1070	Roadside
	12/11/2014	Ingonmankulu	-29.769228° 30.471163°	843	Grassland

Table 1: Continued

Species	Date	Site	GPS (S;E)	Alt. (m asl)	Habitat
Senecio bupleuroides	26/11/2014	Bishopstowe	-29.576262° 30.430695°	765	Roadside
Senecio glaberrimus DC.	08/10/2014	Hilton College Grassland	-29.513508° 30.300622°	1093	Grassland
	02/12/2014	Karkloof Conservation Centre	-29.346162° 30.292155°	1086	Grassland
	09/12/2014	Correctional Services Prison Sevontein [a]	-29.761278° 30.137408°	1460	Grassland
Senecio harveianus MacOwan	24/05/2014	Garden Castle	-29.74749° 29.20803°	1830	Grassland
	08/06/2014	Kamburg	-29.425860° 29.755950°	1711	Roadside
	01/03/2015	Sani Pass	-29.589648° 29.294886°	2612	Roadside
Senecio inaequidens DC.	08/06/2014	Lotheni	-29.486870° 29.701940°	1552	Roadside
	08/06/2014	Near Himeville	-29.529720° 29.622470°	1433	Roadside
	22/11/2014	Monk's Cowl	-29.049478° 29.404205°	1501	Roadside
Senecio inornatus DC.	20/01/2014	Bellvue	-29.635608° 30.433740°	737	Grassland
	20/03/2014	Hilton	-29.499149° 30.320662°	1061	Grassland
	12/02/2015	Umngeni Valley	-29.475918° 30.246727°	981	Grassland
Senecio madagascariensis Poir.	20/01/2014	Bellvue	-29.635608° 30.433740°	737	Grassland
	20/03/2014	Hilton	-29.499149° 30.320662°	1061	Grassland
	25/11/2014	Outside Vernon Crookes	-30.299717° 30.624505°	153	Roadside
Senecio oxyriifolius DC.	14/10/2014	Hilton College Grassland	-29.513508° 30.300622°	1093	Grassland
	02/12/2014	Karkloof Conservation Centre	-29.346162° 30.292155°	1086	Grassland
	22/12/2014	Mount Gilboa	-29.285622° 30.292696°	1750	Grassland
Senecio polyanthemoides Sch. Bip.	20/02/2014	Bellvue	-29.635608° 30.433740°	737	Savannah
	21/01/2014	Hillcrest	-29.758540° 30.782850°	669	Roadside

Table 1: Continued

Species	Date	Site	GPS (S;E)	Alt. (m asl)	Habitat
Senecio polyanthemoides	20/03/2014	Hilton	-29.499149° 30.320662°	1061	Forest edge
Senecio skirrhodon DC.	13/04/2014	Mtwalume	-30.499567° 30.629719°	14	Edge of sand dunes
	25/11/2014	Park Rynie	-30.326279° 30.737762°	10	Edge of sand dunes
	01/02/2015	Hibberdine	-30.574742° 30.575346°	16	Edge of sand dunes
Senecio hygrophilus Cuatrec.	04/01/2015	Garden Castle[b]	-29.755434° 29.229294°	1764	Grassland
	05/01/2015	Sani Pass Bottom	-29.646286° 29.429642°	1596	Grassland
	13/01/2014	Misty Valley Farm	-29.421546° 30.181881°	1180	Grassland
Senecio scitus Hutch. & Burtt Davy	29/11/2014	Sani Pass Hotel	-29.667503° 29.458283°	1603	Grassland
	09/12/2014	Correctional Services Prison Sevontein	-29.761278° 30.137408°	1460	Grassland
	04/01/2015	Garden Castle	-29.755434° 29.229294°	1764	Grassland
Senecio sp. nr. *adnatus* DC. 1	09/12/2014	Correctional Services Prison Sevontein	-29.761278° 30.137408°	1460	Grassland
	05/01/2015	Garden Castle	-29.755434° 29.229294°	1764	Grassland
	05/01/2015	Sani Pass	-29.646286° 29.429642°	1596	Grassland
Senecio coronatus (Thunb.) Harv.	12/11/2014	Ukulinga	-29.666840° 30.403181°	839	Grassland
	12/12/2014	Near Emanzini	-29.484783° 30.364517°	797	Savannah
Senecio retrorsus DC.	20/01/2014	Bellvue	-29.635608° 30.433740°	737	Savanna
	25/01/2014	University of KZN	-29.626191° 30.396641°	688	Grassland
Senecio sp. nr. *conrathii* N.E. Br.	25/11/2014	Vernon Crookes Reserve	-30.264866° 30.595133°	479	Savanna
Senecio sp. nr. *retrorsus* DC.	17/04/2014	Hilton	-29.499149° 30.320662°	1061	Grassland

[a] only 8 plants sampled
[b] only 9 plants sampled

Molecular work

DNA was extracted from the lepidopteran larvae using the Zymo Research Tissue & Insect DNA MiniPrep extraction kit (Zymo Research) following the manufacturer's protocol. Where possible, half the insect or 25 mg tissue was used. Modifications to the protocol were made for smaller samples to try to increase DNA extraction. For the final step, 25 µl DNA elution buffer was added to the ZymoSpin™ IIC Column, instead of 50 µl. This was heated to 70°C for 10 minutes and then centrifuged into a 1.5 ml microcentrifuge tube and pipetted back into the ZymoSpin™ IIC Column. An additional 25 µl elution buffer was added and again heated at 70°C for 10 minutes and then centrifuged into the 1.5 ml microcentrifuge tube.

For Lepidoptera specimens, Cytochome Oxidase I (COI) mitochondrial gene sequences were amplified using the forward primer LEP-F1 (5'-ATTCAACCAATCATAAAGATAT-3') and reverse primer LEP-R1 (5'-TAAACTTCTGGAT GTCCAAAAA-3'). Polymerase Chain Reactions (PCR) consisted of 25 µl reactions and contained 19.9 µl water, 2.0 µl Dream Taq buffer, 0.5 µl $MgCl_2$, 1.0 µl BSA, 0.5 µl dNTPs, 0.5 µl forward primer, 0.5 µl reverse primer, 4.0 µl sample DNA, and 0.1 µl Dream Taq DNA polymerase (Fermentas, USA). PCR cycling conditions followed those of Hebert *et al*. (2004), and were as follows: 1 min at 94°C, 6 cycles of 1 min at 94°C, 1 min 30 sec at 45°C, 1 min 15 sec at 72°C, 36 cycles of 1 min at 94°C, 1 min 30 sec at 51°C, and 1 min 15 sec at 72°C, and one cycle of 5 min at 72°C. PCR products were run through an 0.8% agarose gel for 30 minutes at 100V and visualised under a UV light. Successfully amplified products were sent to the Central Analytical Facility at Stellenbosch University (South Africa) for Sanger sequencing performed using the BigDye Chemistry, v3.1. Sequencing products were analyzed on an Applied Biosystems 3730xl Genetic Analyzer (Applied Biosystematics, Perkin Elmer). All raw sequence data were visualized and edited using BIOEdit v7.1.11 (Hall 2005).

Curculionid and dipteran larvae were submitted for DNA barcoding, using the COI sequences, to the Biodiversity Institute of Ontario at the University of Guelph, Canada. DNA extraction was performed using a commercially available kit, and amplification and sequencing were performed using standardized protocols (Hajibabaei *et al*. 2005). To prepare the sample for processing, photographs of each specimen to be barcoded were taken and uploaded onto the Barcode of Life Database (BOLD). Depending on specimen size, either the whole or half of each specimen was placed into an individual well of a 96-well plate with 100% alcohol. Larvae were subsampled and an effort was made to obtain an even spread of larvae

from all plant species and from different sites and individual plants within a species (Table 2). COI barcode data (sequences and trace files) from 37 individuals belonging to seven morphospecies were downloaded from BOLD and used in phylogenetic analyses.

The COI sequence data for the Curculionidae, Diptera and Lepidoptera data sets were aligned using CLUSTAL X 2.0.10 (Larkin *et al*. 2007). Sequences of stem-boring and capitulum-feeding Lepidoptera were analysed together as the pterophorid occurring on fireweed initially feeds in the capitula and then bores down into the stems. Sequences of stem-boring and capitulum-feeding Diptera were analysed separately. No indels or missing data were incorporated into the final alignments. Phylogenetic trees were estimated using the maximum-likelihood model-based approach. Maximum-likelihood (ML) trees were inferred using GARLI 2.0 (Zwickl 2006). The best-fit model of nucleotide substitution (Curculionidae: TVM + G; Lepidoptera: GTR+I+G; Diptera stem-boring: GTR+I+G; Diptera capitulum-feeding: TIM2+I) was selected using the Akaike Information Criterion (AIC) in jMODELTEST 2.1.7 (Darriba *et al*. 2012). Branch support was estimated using 1000 bootstrap replicates. These values were annotated onto the GARLI most likely tree using FIGTREE v1.3.1 (Rambaut 2009).

Curculionidae, Diptera and Lepidoptera specimens were assigned to Barcode Index Numbers (BINs) or barcode clusters on the trees using the clustering algorithm implemented within BOLD. These BINs represent operational taxonomic units (OTUs), which are synonymous with species (Blaxter *et al.* 2005). The use of BINs is useful when taxonomic information is lacking, as the clustering algorithm used by BOLD allows specimens to be assigned to BINs using the available sequence data (Ratnasingham & Hebert 2007).

Results

Curculionidae

Stem-boring curculionid larvae were collected on eight *Senecio* species, including all four species in the *S. madagascariensis* species complex (Table 2). A total of 78 samples were sent for analysis and 59 samples from six host plant species produced barcode compliant sequences. Analysis of the COI gene revealed eight distinct lineages of Curculionidae (Table 3; Figure 1). A total of three stem-boring lineages were present on fireweed. One lineage represented by three specimens was found to only occur on fireweed, warranting further investigation for host specificity as a potential agent. The other two lineages occurring on fireweed were represented by 15 and 23 specimens, and were also recorded on *S. skirrhodon*.

Two curculionid lineages were represented by a single specimen, one collected from *S. hygrophilus*, and one from *S.* sp. nr. *conrathii* (Table 3; Figure 1). Two lineages were represented by two specimens, one found on *S. hygrophilus*, and one on *S. inaequidens*. The remaining lineage was represented by 12 specimens, all collected on *S. harveianus*. None of the larvae collected on *S. adnatus* and *S. polyanthemoides* were successfully sequenced.

Lepidoptera

Stem-boring and capitulum-feeding Lepidoptera were found on 11 species of *Senecio* and sequenced from eight (Table 2). Of the 115 samples extracted, only 49 were successfully sequenced. Many of the capitulum-feeding lepidopteran larvae that were not successfully sequenced were very small larvae found inside the capitula and it is likely that the DNA extracted was not sufficient to allow amplification of the COI gene. The Lepidoptera formed 10 distinct lineages (Table 3; Figure 2).

Capitulum-feeding Lepidoptera were represented by five lineages, stem-boring Lepidoptera were represented by four lineages, while one lineage contained both capitulum-feeding and stem-boring Lepidoptera. While two distinct lineages were found on fireweed, these also occurred on other plants in the *S. madagascariensis* species complex. One lineage collected on fireweed was represented by 24 stem borers and was also collected from all four species in the *S. madagascariensis* species complex. The other lineage occurring on fireweed was represented by six individuals, both stem borers and capitulum feeders, and was also collected on *S. harveianus*. Three lineages of capitulum feeders were each represented by a single specimen, and were collected from *S. harvaeianus, S. hygrophilus,* and *S. oxyriifolius*. One lineage of capitulum feeders comprised two specimens, both collected on *S. oxyriifolius* and another lineage of capitulum feeders comprising four specimens was collected on *S. bupleuroides, S. glabberimus*, and *S. oxyriifolius*. One lineage of stem-borers was represented by a single specimen collected on *S. skirrhodon*. Two lineages of stem borers, one represented by four specimens and one represented by five specimens, were found only on *S. harveianus*.

Table 2: Numbers of individuals of Curculionidae, Lepidoptera, and Diptera larvae collected from each plant species, number of samples extracted (Lepidoptera) or sent for sequencing (Curculionidae and Diptera), and the number of barcode compliant sequences returned.

	Curculionidae			Lepidoptera			Diptera					
	Stem borers			Stems & Capitula			Stem borers			Capitulum feeders		
Plant species	Coll	Sent	Seq	Coll	Extr	Seq	Coll	Sent	Seq	Coll	Sent	Seq
S. adnatus	3	3	0				7	7	5	7	6	5
S. affinis										4	3	2
S. bupleuroides				10	4	1	4	3	1	283	7	7
S. coronatus				9	4	0	6	6	2	4	4	1
S. glaberrimus				3	2	2	2	2	1	11	6	6
S. harveianus	21	14	12	28	15	12	39	7	6	58	7	6
S. hygrophilus	7	4	3	2	1	1	11	9	2	88	7	7
S. inaequidens	11	4	2	7	5	4	112	8	5	86	7	7
S. inornatus							18	7	6	10	3	3
S. madagascariensis	58	28	25	44	39	20	111	10	4	165	7	4
S. oxyriifolius				9	5	4	14	7	5	6	6	5
S. polyanthemoides	10	6	0	40	30	0	16	7	4	84	6	5
S. retrorsus							1	0	0	21	6	6
S. scitus				1	1	0				10	6	5
S. skirrhodon	39	17	16	11	9	5	45	8	7			
S. sp. nr. *adnatus* 1							14	7	3	66	7	7
S. sp. nr. *conrathii*	2	2	1				2	2	1			
S. sp. nr. *retrorsus*							14	5	0	169	7	6
Total	151	78	59	155	115	49	416	95	52	1085	95	82

Coll = number collected; Sent = number sent to Biodiversity Institute of Ontario, University of Guelph for sequencing; Extr = number of samples that DNA was extracted from; Seq = number of barcode compliant sequences returned

Diptera (Stem borers)

Stem-boring dipterans were present in 16 *Senecio* species and sequenced from 14 as the single larva collected within the stems of *S. retrorsus* was not successfully sequenced; nor were any of the five larvae from *S.* sp. nr. *retrorsus* (Table 2). Of the 95 samples sent, 52 barcode compliant sequences were returned. Ten lineages of stem-boring Diptera were recorded from *Senecio* species according to the COI gene (Figure 3). Five lineages were represented by a single specimen (Table 3; Figure 3). Two of these were collected from *S. harveianus*, and the remaining three were collected from *S. bupleuroides*, *S. inornatus*, and *S.* sp. nr. *conrathii*. Two lineages were represented by two specimens, one of these lineages was collected from *S. adnatus* and the other one from *S. polyanthemoides*. One lineage was represented by seven specimens that were collected from *S. madagascariensis, S. polyanthemoides* and *S. skirrhodon*. The other two lineages were represented by 17 and 19 specimens, both of which were collected from six host plants. Two lineages were collected from fireweed; one was recorded on two other *Senecio* species while the other was recorded on an additional five *Senecio* species.

Diptera (Capitulum feeders)

Capitulum-feeding Diptera were collected and sequenced from 16 *Senecio* species. Eighty-two of the 95 samples sent for sequencing produced barcode compliant sequences (Table 2). Eight lineages of Diptera were found in the capitula of the *Senecio* species surveyed (Table 3; Figure 4). Two of these were represented by two specimens. One had both individuals collected from *S. harveianus* and the other had one specimen each from *S. bupleuroides* and *S. madagascariensis*. Two of the lineages were represented by five specimens; one of these had all specimens collected from *S. polyanthemoides* and the other had specimens collected on *S. affinis*, *S. coronatus*, and *S. inornatus*. Two lineages, one represented by seven specimens and one comprising eight specimens, had individuals collected on *S. harveianus*, *S. inaequidens*, and *S. madagascariensis*. One lineage comprising nine specimens was recovered from *S. bupleuroides, S. hygrophilus*, and *S.* sp. nr. *retrorsus*. The last lineage was represented by 44 specimens that were found on nine *Senecio* species. Two lineages of capitulum-feeding Diptera were present on fireweed and both were also found on *S. harveianus* and *S. inaequidens* (Table 3; Figure 4).

Table 3: Number of individuals of each BIN or barcode cluster (according to the COI gene) of Curculionidae, Lepidoptera (s = stem-boring, c = capitulum-feeding), stem-boring Diptera and capitulum-feeding Diptera, on each *Senecio* species. Fireweed (*S. madagascariensis*) in bold.

BIN	*adn*	*aff*	*bup*	*cor*	*gla*	*har*	*hyg*	*ina*	*ino*	***mad***	*oxy*	*pol*	*ret*	*sci*	*ski*	*nr adn*	*nr con*	*nr ret*
Curculionidae (stem borers)																		
1										**10**					13			
2										**12**					3			
3										**3**								
4								2										
5							2											
6						12												
7																	1	
8							1											
Lepidoptera (stem [=s] and capitulum [=c] borers)																		
1c						1												
2c,s						1				**5**								
3s															1			
4c							1											
5c											1							
6c											2							
7c			1		2						1							
8s						4												
9s						5												
10s						1		4		**15**					4			

Table 3: Continued

BIN	*adn*	*aff*	*bup*	*cor*	*gla*	*har*	*hyg*	*ina*	*ino*	***mad***	*oxy*	*pol*	*ret*	*sci*	*ski*	*nr adn*	*nr con*	*nr ret*
Diptera (stem borers)																		
1									1									
2						1												
3						1												
4	2																	
5										**2**		2			3			
6				2		1		5		**2**	5				4			
7			1															
8																	1	
9												2						
10	3				1	3	2		5							3		
Diptera (capitulum borers)																		
1			1				1											
2						3		2		**2**								
3						2												
4												5						
5		2		1					2									
6						1		5		**2**								
7			2				1											6
8	5		4		6		5		1		5		6	5		7		

adn = *S. adnatus*, aff = *S. affinis*; bup = *S. bupleuroides*; cor = *S. coronatus*; gla = *S. glaberrimus*; har = *S. harveianus*; hyg = *S. hygrophilus*; ina = *S. inaequidens*; ino = *S. inornatus*; mad = *S. madagascariensis*; oxy = *S. oxyriifolius*; pol = *S. polyanthemoides*; ret = *S. retrorsus*; sci = *S. scitus*; ski = *S. skirrhodon*; nr adn = *S.* sp. nr. *adnatus* 1; nr con = *S.* sp. nr. *conrathii*; nr ret = *S.* sp. nr. *retrorsus*

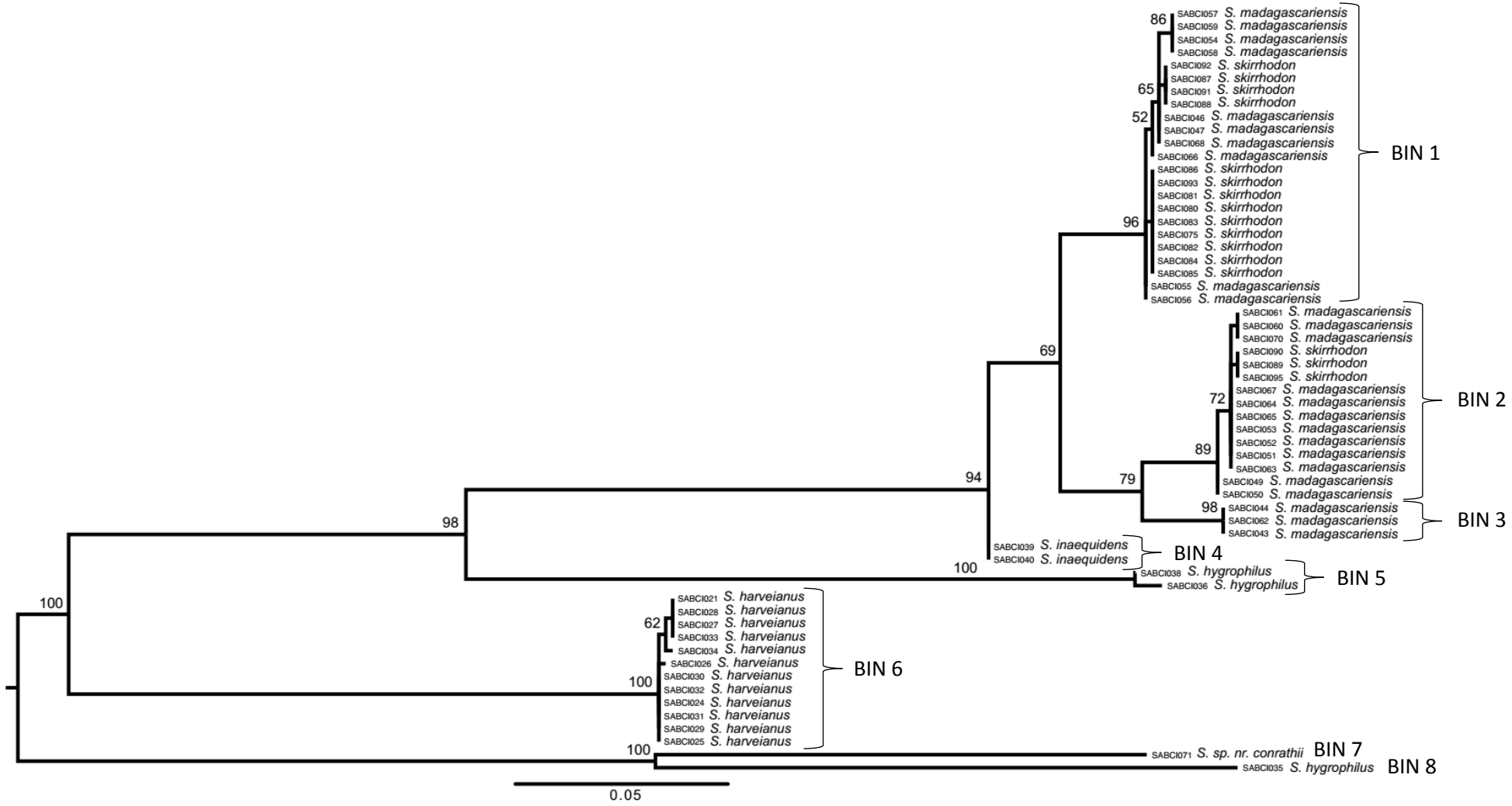

Figure 1: Mid-point rooted maximum likelihood phylogeny for stem-boring Curculionidae. Values on branches indicate bootstrap values from 1000 bootstrap replicates. Only bootstrap values larger than 50 are shown. Branch lengths are proportional to the number of substitutions. *Senecio* host plants are indicated at the end of the branches. Different BIN numbers indicate different lineages (likely species).

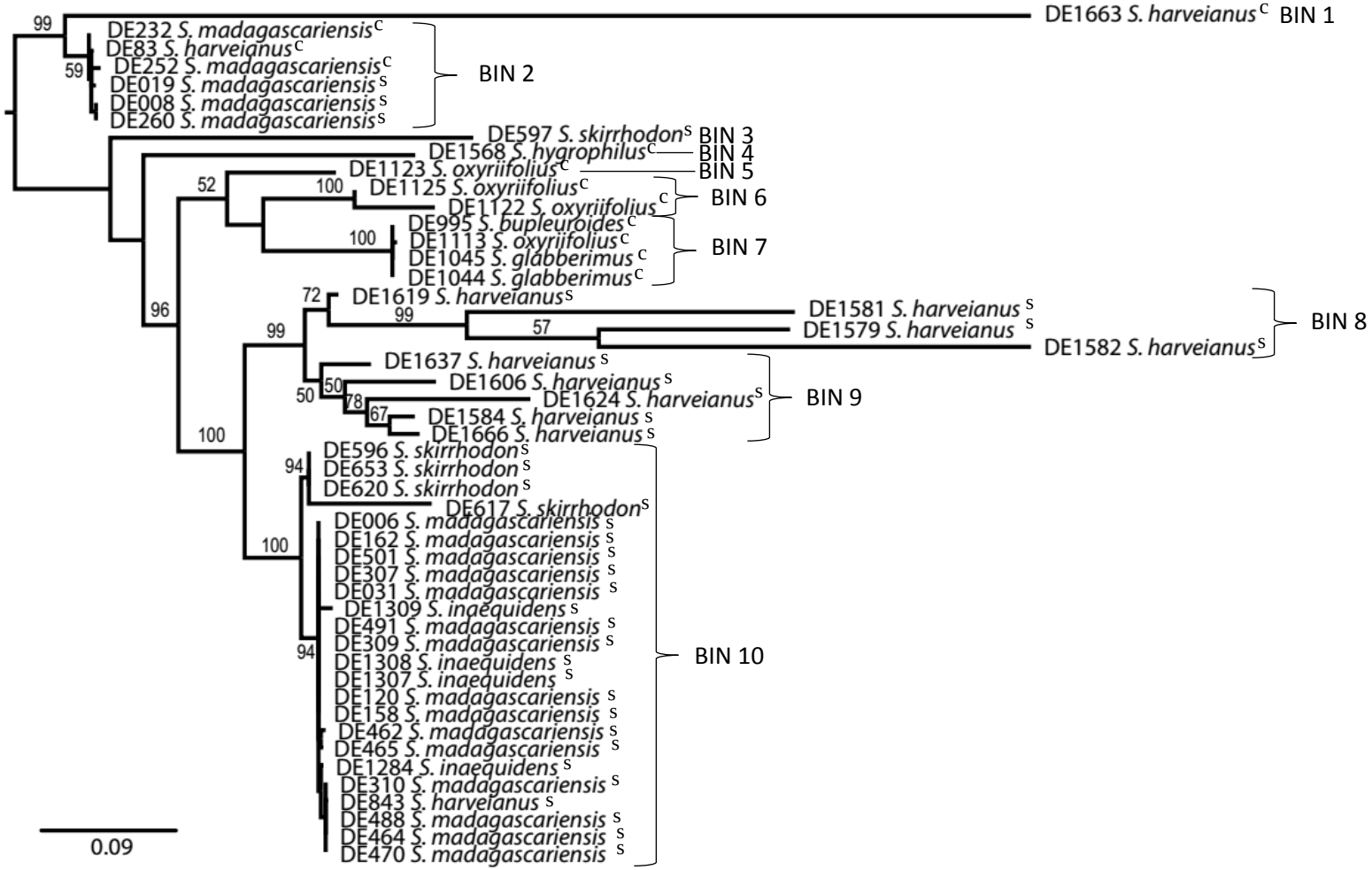

Figure 2: Mid-point rooted maximum likelihood phylogeny for stem-boring (s) and capitulum-feeding (c) Lepidoptera. Values on branches indicate bootstrap values from 1000 bootstrap replicates. Only bootstrap values larger than 50 are shown. Branch lengths are proportional to the number of substitutions. *Senecio* host plants are indicated at the end of the branches. Different BIN numbers indicate different lineages (likely species).

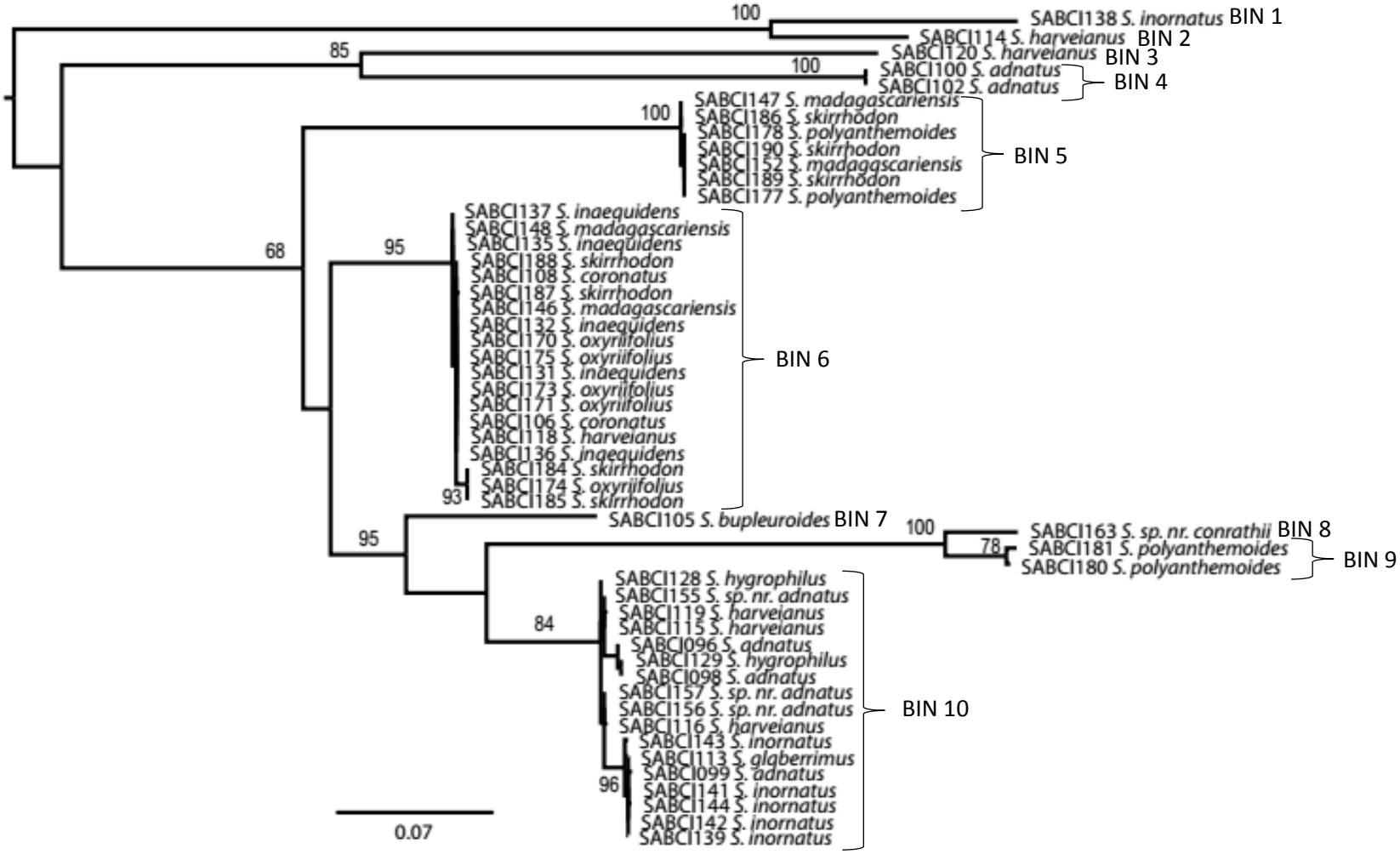

Figure 3: Mid-point rooted maximum likelihood phylogeny for stem-boring Diptera. Values on branches indicate bootstrap values from 1000 bootstrap replicates. Only bootstrap values larger than 50 are shown. Branch lengths are proportional to the number of substitutions. *Senecio* host plants are indicated at the end of the branches. Different BIN numbers indicate different lineages (likely species).

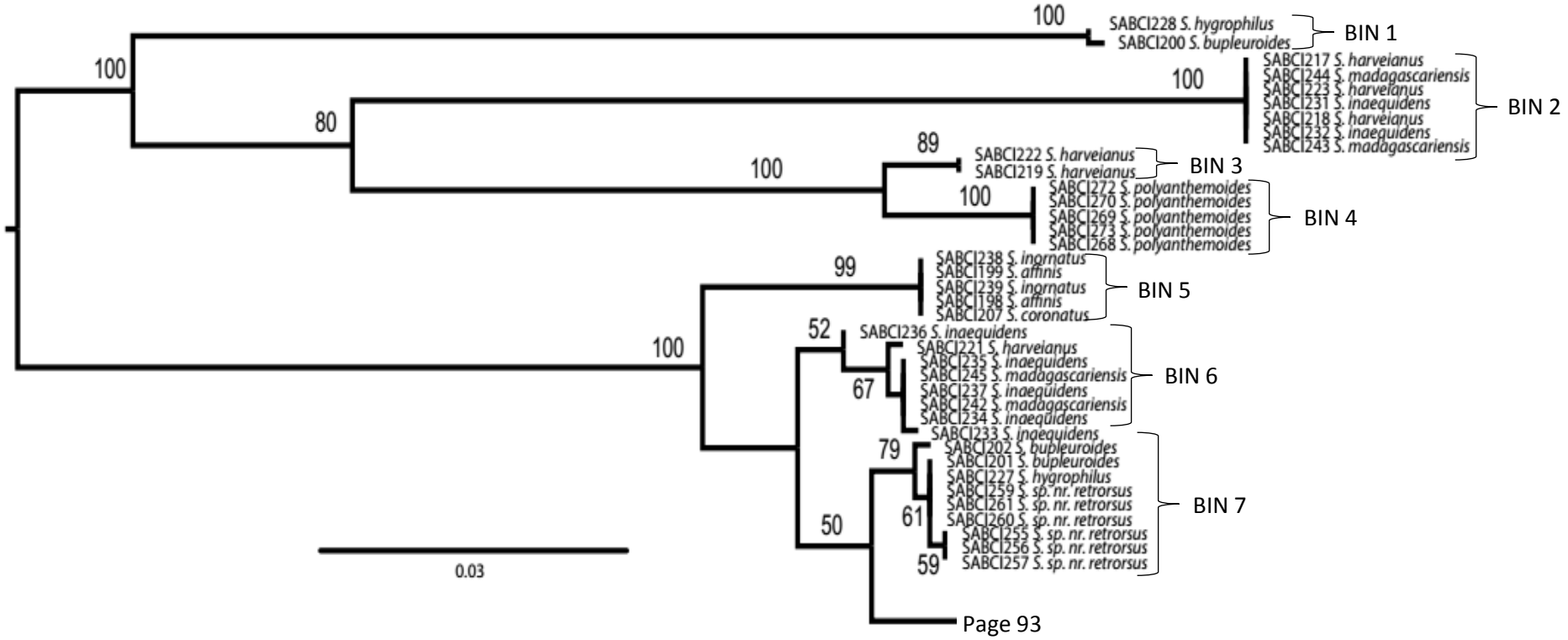

Figure 4: Mid-point rooted maximum likelihood phylogeny for capitulum-feeding Diptera. Values on branches indicate bootstrap values from 1000 bootstrap replicates. Only bootstrap values larger than 50 are shown. Branch lengths are proportional to the number of substitutions. *Senecio* host plants are indicated at the end of the branches. Different BIN numbers indicate different lineages (likely species).

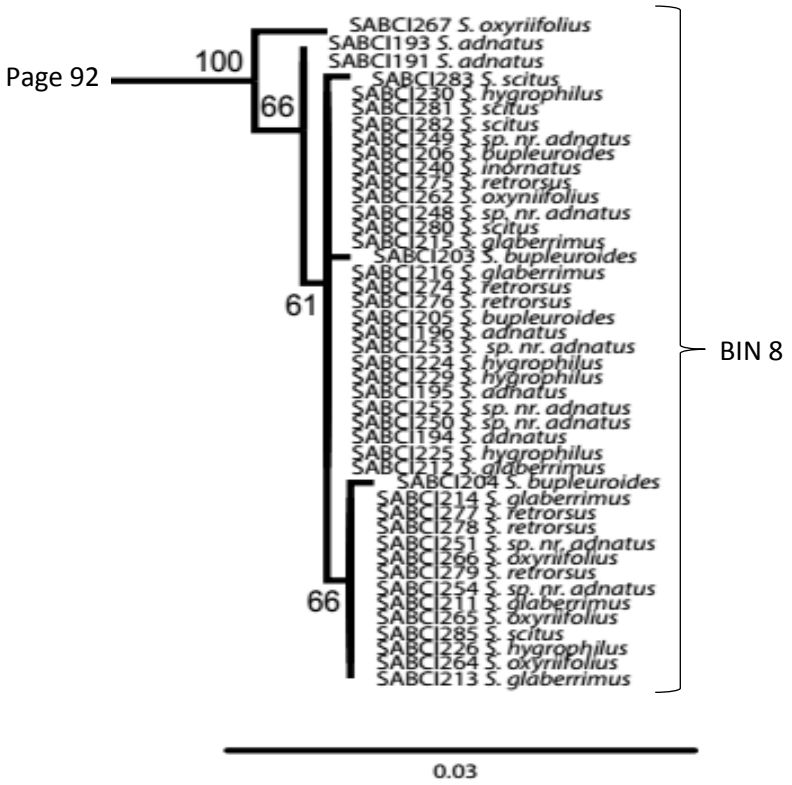

Figure 4: Continued

Discussion

Selecting insects that are most likely to be host specific, prior to their importation into quarantine for further host-range testing, is important to reduce the amount of time and money spent on unsuitable candidates for biological control. This study aimed to determine the native field host range of the endophagous insects associated with fireweed in order to prioritise those most likely to demonstrate host specificity during subsequent testing. Surveys for endophagous Curculionidae, Diptera and Lepidoptera across 18 *Senecio* species revealed specialisation within some of the taxa examined, and thus a number of insects worth further investigation as potential biocontrol agents for fireweed.

Stem-boring Curculionidae were collected on eight of the 18 *Senecio* species surveyed and successfully sequenced from six host plant species. A number of *Senecio* species that were surveyed have stems that are likely too small to support the development of larger Curculionidae such as those found in the stems of fireweed (e.g. *Gasteroclisus tricostalis* (Thunberg)). However, a few of the larger *Senecio* species also did not support any curculionid larvae. Analysis of the COI gene revealed nine lineages of Curculionidae, three of which occurred on fireweed. One of these lineages was represented by only three sequenced

specimens (recorded only on fireweed) which may not provide an accurate measure of host range, but still warrants further investigation. The two other lineages comprised 15 and 23 sequenced specimens and were collected from both fireweed and *S. skirrhodon*. The latter species is described in Hilliard (1977) as potentially comprising a coastal form of fireweed. These two plant species, if they are indeed different, are very closely related. As these two Curculionidae were not found on the other closely related species which form the *S. madagascariensis* species complex, it is likely that they are specific to fireweed and *S. skirrhodon* and thus should thus be considered as potential biocontrol agents for Australia. Adults have been sent for identification, and additional specimens are being reared for sequencing so that these larvae can be matched to the adults. Further studies focussing on the Curculionidae will help determine the host range of the third lineage.

The Curculionidae is one of the more successful taxa in the biological control of weeds as it includes many species that are often host specific and damaging (Chapter 3; Crawley 1989; Winston *et al*. 2014; Egli & Olckers 2017). The use of Curculionidae in the biological control of Asteraceae is dominated by capitulum feeders released on thistles (Chapter 3; Winston *et al*. 2014; Egli & Olckers 2017). However, a few stem borers have been released against asteraceous weeds (Appendix), and many stem-boring Curculionidae have been released in biocontrol programmes against other plant families (Winston *et al*. 2014). For example, the stem borers *Lixus cardui* Olivier and *Trichosirocalus briesei* Alonso-Zarazaga & Sánchez-Ruiz, released on a complex of *Onopordum* species, resulted in substantial control of their target weeds in Australia (Briese 2012; Winston *et al*. 2014). Crawley (1989) ranked Curculionidae as one of the most successful insect families in weed biocontrol. The degree of host restriction demonstrated indicates that it is likely that at least one of the curculionid species feeding on fireweed will be safe for release in Hawaii and possibly Australia. As no native stem-boring Curculionidae have been recovered from fireweed in Australia (Holtkamp & Hosking 1993; Harvey *et al*. 2015), there is a reduced chance of a curculionid agent recruiting parasitoids.

Lepidopteran larvae were collected from 11 of the 18 *Senecio* species. There were 10 distinct lineages of Lepidoptera and two of these included specimens collected on fireweed. Both were also collected from other species in the *S. madagascariensis* species complex, one from *S. harveianus*, and one from all three other species in the complex. This indicates some restriction to host plant, and it is possible that one or both lineages may be specific enough

for release in Australia, as plants within the above-mentioned species complex are probably more closely related to each other than fireweed is to its closest Australian relative, *S. pinnatifolius* A.Rich (Pelser *et al*. 2007). It is also very likely that these two lineages will be specific enough for release in Hawaii.

Lepidoptera have been used successfully in a number of biocontrol programs, including those against Asteraceae (Chapter 3; Winston *et al*. 2014; Egli & Olckers 2017; Appendix). The Lepidoptera feeding on fireweed belong to the families Pyralidae, Pterophoridae and Tortricidae. All of these have been deployed against invasive Asteraceae, and Tortricidae have had some success in controlling their target weeds. In the biological control of *Jacobaea vulgaris* Gaertn. in Australia, the stem-boring torticid, *Cochylis atricapitana* (Stephens), had a significant impact on plant populations (Ireson & McLaren 2012). The moth was also released in Canada, where it had a considerable impact on *J. vulgaris* populations in some parts of the country (Winston *et al*. 2014). The stem-galling tortricid, *Epiblema strenuana* (Walker), similarly had a significant impact on *Parthenium hysterophorus* (L.) in Australia (Dhileepan 2001, 2003). While Pyralidae released for the biocontrol of Asteraceae only had negligible impacts, those released on other plant families have been more successful (Chapter 3; Crawley 1989; Winston *et al.* 2014; Egli & Olckers 2017). For example, *Cactoblastis cactorum* (Berg) has had significant impacts on a number of *Opuntia* species (Cactaceae) in a number of countries where it was released (Winston *et al*. 2014) and *Arcola malloi* (Pastrana) has caused significant damage to its target plant, *Alternanthera philoxeroides* (Mart.) Griseb. (Amaranthaceae) in the USA (Winston *et al*. 2014). Pterophoridae released against Asteraceae have also caused extensive and considerable impacts on their target plants (Chapter 3; Winston *et al.* 2014; Egli & Olckers 2017; Appendix).

Of the insect taxa examined in this study, Diptera was the most abundant and most diverse, but least damaging, group associated with fireweed. Stem-boring and capitulum-feeding Diptera were present on 16 *Senecio* species. Only two of the 10 lineages of stem-boring Diptera were collected on fireweed. Both were collected from host plants outside of the *S. madagascariensis* species complex and are thus not host specific. Although these would not be safe for release in Australia, if restricted to the genus *Senecio*, or the Tribe Senecioneae, they may be suitable for release in Hawaii. Adults have been sent for identification and more specimens are being reared so that larvae can be genetically linked

to adults. Many more fly larvae were collected than were sequenced and should other stem borers not be suitable, additional sequencing of these specimens could give a better estimate of total numbers of lineages and host range. It is possible that some lineages were missed, as only four specimens from fireweed were successfully sequenced.

Two of the eight capitulum-feeding Diptera were collected on fireweed. As with the stem borers, none of these were host specific, although they were recovered only on plants belonging to the *S. madagascariensis* species complex. This indicates some degree of host plant restriction and should the capitulum-feeding lepidopterans not be suitable for release, these two lineages could be reconsidered.

Diptera have been used in weed biocontrol with varying degrees of success, and if the curculionids and lepidopterans prove unsuitable for release in Australia, then dipterans can be imported and tested. Tephritidae, in particular, have facilitated the control of a few asteraceous weeds (Winston *et al*. 2014; Appendix). *Cecidochares connexa* Macquart, a stem-galling tephritid, had a heavy impact on its target plant, *Chromolaena odorata* (L.) R. M. King & H. Rob. in the Federated states of Micronesia and the northern Mariana Islands, and a medium impact on the weed in Guam (Winston *et al*. 2014). A number of other stem- and capitulum-feeding tephritid species have had medium impacts on their target weeds (Winston *et al*. 2014). However, native stem-boring and capitulum-feeding dipterans have been recovered from fireweed in Australia (Holtkamp & Hosking 1993; Harvey *et al*. 2015), thus increasing the risk that they will recruit native parasitoids. For this reason, as well as because they are less damaging, they are a lower priority than the Lepidoptera and Curculionidae previously discussed.

Fireweed is a difficult target for biological control in Australia due to the high number of native Australian *Senecio* species, some of which are closely related to fireweed. The results of this survey indicate that there are some potentially host specific insects that are worth further investigation in the laboratory, both in South Africa and in quarantine in Australia. If host specific agents are identified for Australia, biological control may be successful as an enemy-exclusion trial found that herbivorous natural enemies play a role in in suppressing fireweed in its native range (Harvey *et al*. unpublished). This, together with the host restriction found in some endophagous insect lineages (likely species), suggests that the prospects for biological control are positive.

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CHAPTER 5

Potential efficacy of biological control of fireweed in Australia: No difference in plant biomass or native insect assemblages between native and invasive populations, despite differences in pyrrolizidine alkaloids

Abstract Fireweed (*Senecio madagascariensis* Poiret – Asteraceae), is an annual or short-lived perennial herb native to South Africa and highly invasive in Australia, where it causes reductions in the productivity of pastures and livestock losses. It was introduced to Australia prior to 1918 and has spread from the Hunter Valley area, New South Wales, north into southern Queensland and south to northern Victoria. Fireweed was declared a Weed of National Significance in Australia and is currently the target of a biological control program. Accumulating evidence suggests that fireweed may be undergoing evolutionary changes, including a shift in pyrrolizidine alkaloid concentrations and composition, along its invasion gradient. These changes may influence its interaction with herbivores including those that may be introduced for biological control. Such changes may affect the potential efficacy of a biocontrol program. This study sought to gain a thorough understanding of how well populations along the invasion gradient in Australia may respond to potential biocontrol agents. I examined insect impact and the relationships between alkaloid concentrations and insect communities on plants grown in a common garden experiment in South Africa. These plants were grown from seed sourced from populations across the invasive range in Australia and from the native range in South Africa. Five plants from each region were planted in three blocks in a randomised block design. Plants (including soil samples around the roots) were harvested after two months and plant biomass, insect herbivores recruited and alkaloid concentrations were measured. Minimal variation in insect impact on plant biomass was detected across fireweed populations. All the major insect herbivores present were prevalent on all populations with slight variation in relative abundances, despite variation in alkaloids. Virtually no relationship was detected between the variation in alkaloid concentrations and insect communities. Results from this research have positive implications for biological control. Any potential biocontrol insect agent, if host specific, is likely to be suitably damaging to Australian populations because, despite differences in alkaloid composition within and between South African and Australian populations, there were no differences in impact or insect community assemblages.

Key words: Asteraceae, biocontrol, EICA, plant-herbivore interactions, *Senecio madagascariensis*, shifting defence hypothesis

Introduction

Due to the recent increase in global travel and trade, there has been an increase in both accidental and intentional introductions of plants to areas outside of their native ranges, some of which have become invasive (Vitousek *et al*. 1997; Mack *et al*. 2000; Hulme 2009). These invasive exotic plants can have detrimental impacts on the environment by reducing biodiversity and affecting ecosystem functions (Vitousek *et al*. 1997; Mack *et al*. 2000; Culliney 2005). Exotic plants also negatively affect agriculture by reducing the productivity of pastures and incurring huge costs of control (Vitousek *et al*. 1997; Mack *et al*. 2000; Culliney 2005).

There are a number of factors that determine whether an introduced plant will become invasive in its new environment. For example, certain traits may predetermine their ability to utilise and respond to prevailing conditions, and plants may shift to faster growth strategies (Leishman *et al.* 2014) allowing them to outcompete native species (Gioria & Osborne 2014). Changes in top-down pressure from enemies such as herbivores and pathogens within the plants' introduced range can also determine whether plants become invasive (Keane & Crawley 2002) and may lead to a variety of evolutionary changes within the plants' physiology; for example, changes in the allocation of resources to defence, growth and reproduction (Muller-Scharer *et al*. 2004; Joshi & Vrieling 2005; Orians & Ward 2010; Doorduin & Vrieling 2011; Inderjit 2012).

One of the main shifts in selection pressures on plants in the introduced range is a change in herbivore communities, from one previously composed of specialists and generalists, to one dominated by generalists (Muller-Scharer *et al*. 2004). This change in herbivore community can result in a change in plant chemical defences (Muller-Scharer *et al*. 2004; Joshi & Vrieling 2005; Orians & Ward 2010; Doorduin & Vrieling 2011; Inderjit 2012). Plant defence against insect herbivores is typically in the form of secondary metabolites which can be broadly grouped into quantitative and qualitative defences. Quantitative defences are often chemicals that serve as digestibility reducers, are present in high concentrations and costly to produce, and affect specialist and generalist herbivores (e.g. lignins and tannins) (Doorduin & Vrieling 2011). Qualitative defences are chemicals such as pyrrolizidine alkaloids (PAs) and glucosinolates that are present in low concentrations, are not costly to produce, and are toxic to generalist herbivores but not specialist herbivores (Doorduin & Vrieling 2011; Inderjit 2012). Specialist herbivores are often able to sequester these toxins (Hartmann *et al*.

1997; Klitzke & Trigo 2000) and use them as host-finding cues and oviposition and feeding stimulants (van der Meijden 1996; Bernays *et al*. 2004). Higher concentrations of these chemicals will increase protection against generalist herbivores but may increase the chances of specialist herbivores locating the host plant (van der Meijden 1996). Specialist and generalist herbivores, therefore, exert opposing selection pressures on qualitative plant defences (van der Meijden 1996). This is known as the specialist/generalist dilemma and plants are predicted to produce intermediate amounts of defence chemicals to defend against generalists while reducing attack by specialists (van der Meijden 1996).

The Evolution of Increased Competitive Ability (EICA) hypothesis states that the lack of natural enemies in the introduced range results in selection favouring plants that allocate fewer resources to defence and more to growth and reproduction (Blossey & Notzold 1995). These plants are better competitors with the potential to outcompete the native plants, but are less well defended (Blossey & Notzold 1995). The Shifting Defence Hypothesis (SDH) is an extension of the EICA that takes into account the differences in selection pressure exerted by specialists and generalists (Muller-Scharer *et al*. 2004; Joshi & Vrieling 2005; Doorduin & Vrieling 2011). In the invaded range, in the absence of specialists but not generalists, plants will increase the concentration of qualitative defences that protect them against generalist herbivores and decrease the concentration of quantitative defences that protect them against specialists (Muller-Scharer *et al*. 2004; Joshi & Vrieling 2005; Doorduin & Vrieling 2011). The SDH thus predicts that in the absence of specialist herbivores in the introduced range, plants will allocate more resources to growth, reproduction and defence against generalist herbivores and less resources to defence against specialist herbivores (Muller-Scharer *et al*. 2004; Joshi & Vrieling 2005; Doorduin & Vrieling 2011). Thus, in the introduced range, invasive plants will grow larger and have higher concentrations of PA's than in their native range.

Changes in alkaloid concentrations may have an effect on insect natural enemies from the native range and thus the efficacy of potential biocontrol agents. If insects respond as predicted, specialists released for biological control are predicted to respond positively to the higher alkaloid concentrations which could improve the efficacy of biological control (Müller-Schärer *et al*. 2004). However, studies examining insect preferences between native and invasive populations have found conflicting results for both generalist and specialist natural enemies (Hinz & Schwarzlaender 2004; Inderjit 2012). Contrary to predictions, some

specialists performed better on native populations (Vrieling & Vanwijk 1994) while other studies have found no effect of differential alkaloid levels on both specialists (Castells & Berenbaum 2008; Wei *et al* 2015) and generalists (Bossdorf *et al*. 2004; Hinz & Schwarzlaender 2004; Wang *et al*. 2012).

Fireweed (*Senecio madagascariensis* Poir., Asteraceae) is an annual to short-lived perennial herb that is native to Southern Africa and Madagascar (Hilliard 1977). It has become invasive in Australia, Hawaii, parts of South America, and Japan where it invades pastures and decreases productivity (Le Roux *et al*. 2006, 2010; Tsutsumi 2011; McFadyen & Morin 2012). Fireweed contains a number of pyrrolizidine alkaloids that are toxic to livestock and can result in their death (Gardner *et al*. 2006; Cruz *et al*. 2010). In Australia, fireweed was first introduced to the Hunter Valley area, New South Wales, sometime before 1918 and has since spread north into south-east Queensland and south into Victoria creating an invasion gradient (Parsons & Cuthbertson 1992; McFadyen & Morin 2012; Harvey *et al*. 2013). At the point of introduction the plants have, therefore, coexisted with the native Australian invertebrate fauna for longer than at the invasion fronts (Harvey *et al*. 2013).

Studies on the insect herbivore fauna of fireweed in Australia have found that the plant is mostly attacked by generalist ectophages and, compared to Australian native *Senecio* species, has a higher abundance of insects but a lower species richness (Harvey *et al*. 2015). Plants at the invasion front experienced higher levels of damage and had a higher abundance of generalists and fewer specialists than plants at the core region (Harvey *et al*. 2013, 2015). It has been proposed that the plants are changing in response to herbivore and environmental pressure (Harvey *et al*. 2013) and preliminary research has demonstrated higher alkaloid concentrations at the invasion fronts than at the core point of introduction (Harvey *et al*. unpublished). These differences in alkaloid concentrations may increase the efficacy of biocontrol agents if the specialist herbivores in the native range are found to be more attracted to the invasive populations.

This study, conducted in the native range of fireweed, aimed to determine: (1) if plants from different regions in Australia and South Africa are differentially impacted (in terms of biomass) by the native herbivores and (2) the impact of changes in alkaloid concentrations and profiles in the invasive range on the abundance, richness, diversity and insect community composition of (i) all, (ii) endophagous and (iii) ectophagous insect herbivore communities in the native range. Endophagous insects such as stem borers and internal capitulum feeders

are generally regarded as specialists while ectophages such as leaf chewers and sap suckers are generalists (Cornell & Kahn 1989; Gaston *et al*. 1992). Consequently, endophagous and ectophagous insects recorded during this study represented specialists and generalists, respectively. The results of this study will give some indication of the potential efficacy of biological control by determining whether Australian fireweed plants are more, equally or less susceptible to native natural enemies than South African plants.

Materials and methods

Study species

Fireweed typically grows in disturbed or degraded areas (Hilliard 1977). The plant has a short life cycle, and can flower as early as 3 weeks after germination (Csurhes & Navie 2010). It has a high reproductive output with plants flowering throughout the year and producing on average around 300 flower heads, with each producing over 100 viable seeds capable of germinating all year round (Hilliard 1977, Csurhes & Navie 2010).

Plants used in this experiment were grown from wild seed collected from three regions in Australia across the weed's geographical range and three regions in KwaZulu-Natal, South Africa representing a similar geographical distance to populations from Australia (Table 1). Seed was collected from populations in Australia from its point of introduction (core region: Hunter Valley) where the plants have existed the longest, and from the northern and southern invasion fronts on the east coast of Australia, where the plants have existed for the least amount of time, as per herbarium records (NSW Collections database, National Herbarium of New South Wales). The three South African regions were selected to provide a similar latitudinal gradient to the collection sites in Australia and included a northern, central and southern population along the east coast. For each fireweed population, seed was collected from 10 plants.

Table 1: Details of sites where seeds for the study were collected, including three regions across Australia representing the invasion gradient and three regions in South Africa from a similar representative latitudinal gradient in KwaZulu-Natal.

Site*	Date collected	Latitude (S)	Longitude (E)	Alt. (m asl)
South Africa				
Zululand (North)	19/01/2014	-29.02547°	31.56496°	98
Hillcrest (Core)	22/12/2013	-29.75854°	30.78275°	669
Port Edward (South)	22/12/2013	-30.96413°	30.18753°	141
Australia				
Northern invasion front (North)				
A. Toorbul, QLD	24/08/2012	-27.03111°	153.09649°	11
B. Caboolture, QLD	24/08/2012	-27.07349°	152.96591°	19
C. Vetran, QLD	24/08/2012	-26.16612°	152.69917°	78
Point of introduction (Core)				
A. McClemonts Swamp Road, NSW	03/08/2011	-32.69740°	151.66213°	9
B. Koorgang Wetlands, NSW	03/08/2011	-32.83603°	151.70948°	9
C. UWS, Richmond, NSW	07/07/2011	-33.60653°	150.75440°	26
Southern invasion front (South)				
A. Damhula, NSW	16/08/2011	-36.95885°	149.86750°	30
B. Bemholia, NSW	16/08/2011	-36.65699°	149.52632°	287
C. Tilba, NSW	18/08/2011	-36.32559°	150.09376°	75

*QLD = Queensland; NSW = New South Wales

Field experiment

Seeds were planted in pots with potting soil and kept in a shade house during December 2014 and January 2015. Planting was staggered because prior experimentation determined that seeds from the northern and southern populations in Australia took longer to germinate than those from the core population and South African populations (Australia: northern invasion front - 12/12/2014; southern invasion front - 19/12/2014; core population - 01/01/2015; all South African populations: 04/01/2015). Plants were then left to grow for at least 6 weeks to ensure the formation of reasonable root stocks. Plants were cut back and flowers were removed as necessary. A random selection of healthy plants from a mix of different parent plants and populations were selected for planting in the field.

The field experiment was carried out at the University of KwaZulu-Natal Pietermaritzburg's research farm, Ukulinga (29.666228°S; 30.407561°E), from February 2015 to June 2015. The vegetation in the area is classified as Southern Tall Grassland and is dominated by grass species such as *Themeda triandra* Forssk., *Heteropogon contortus* Beauv. ex Roem. & Schult., and *Tristachya leucothrix* Nees (Acocks 1953). The study took place at a site that experiences regular disturbance in the form of ploughing.

Planting at Ukulinga took place on the 17th of February 2015. Plants were planted in three disked blocks (6 x 10 m), in 6 rows of 10 and 1 m apart. In each block, 10 plants from each region within each country were planted in a randomised block design. Thirty plants from each region in each country were planted in total. Plants were planted with around 100 g of NPK fertiliser and approximately 500 ml of water added to the soil. Each plant was watered with about 200 ml water for two days following planting. Thereafter, it rained regularly and further watering was not necessary.

Baseline measurements (height, widest canopy width, and width perpendicular to widest width) were taken once the plants had established two weeks after planting, on the 31st of March 2015, to account for any initial variation in plant size between populations.

Processing of samples

Plants were harvested on the 17th of June 2015. Plants were cut at the base of the stem and the above-ground material was bagged and taken back to the laboratory where it was stored in the freezer until further processing could take place. Fifteen plants per region (5 from each block) were selected for the insect community analysis. To account for any larvae in the immediate soil surrounding the roots, the roots and surrounding soil of a subsample of 45 of the 90 selected plants (15 per block and 2 or 3 per region within a block) were dug up using a standard spade. Soil samples were approximately 20 cm deep and 20 cm in diameter. The samples were placed in brown paper bags and taken back to the laboratory where they were placed in Berlese funnels for a week (until the 24th of June). The roots of the other 45 plants were tagged in the field and left there until the 24th of June and then removed and bagged. The number of soil samples that could be processed simultaneously was limited by the number of Berlese funnels available. The root balls including surrounding soil were similarly placed in Berlese funnels for a week to extract all soil invertebrates. Invertebrates were collected and preserved in 100% alcohol. Insect herbivores were removed from the samples to be included in the community analysis. The above-ground material was sorted into leaves, stems and flowers. Stems and flowers were dissected under a dissecting microscope and all immature insects were removed and identified to order or where possible family and then sorted into morphospecies. Externally-feeding insects were also identified accordingly.

After removing the insects, leaf and stem material were stored in a -80^{o}C freezer and then freeze dried for 24 hours. Dry plant material was ground in a sample mill before shipping

to the Hawkesbury Institute Laboratories, Western Sydney University, for chemical analysis. Roots and flowers were oven dried at 60^{o}C for 24 hours. All plant material was weighed after drying to obtain biomass.

Chemical analysis

Leaves collected from 109 plants were selected for chemical analysis (Australia: north = 16, core = 21, south = 20, total = 57; South Africa: north = 13, core = 21, south = 18, total = 52). A total of 76 stem samples were analysed (Australia: north = 13, core = 13, south = 13, total = 39; South Africa: north = 13, core = 13, south = 11, total = 37).

Alkaloids were extracted following a protocol outlined in Gardner *et al*. (2006). For each individual sample, typically 400 mg (when available) of ground leaf and stem powder was separated into two samples to test for repeatability. Each sample was extracted using 4 ml $CHCl_3$ and 4 ml 1N HCl in a sealed glass tube by mechanical rotation. The suspension was centrifuged for 3 minutes at 1,000 g and the upper aqueous acid layer was transferred with a Pasteur pipette into a second test tube. The extraction was repeated by adding 2 ml 1N HCl, mixed for 5-10 minutes, centrifuged and combined with the first. Approximately 100 mg of Zinc dust was added to the extract and mixed on the mechanical rotator for 3 hours to reduce the N-oxides. The samples were then centrifuged and decanted into a third culture tube. Ammonium hydroxide (28%) was gradually added until the samples were alkaline (pH 9-10). The PA's were then extracted using 4 ml $CHCl_3$, mixed for 5-10 minutes, centrifuged and the $CHCl_3$ layer was transferred into a fourth culture tube. The extraction was repeated with 2 ml $CHCl_3$. Anhydrous sodium sulphate was added to the combined extracts to absorb remaining water and the extracts were filtered through a SPE column. The extracts were evaporated from the vial by delivering a steady stream of nitrogen gas, and the vials were stored at −20°C until analysis.

Extracts were re-dissolved in 0.5 ml chloroform containing 10 ppm methyl stearate and transferred into a GC vial for analysis. Samples were analysed using an Agilent 7890A gas chromatograph coupled with an Agilent 5975C mass spectrometer, fitted with an HP-5ms column (30 m x 0.25 mm x 0.25 µm). Two µl of extract was injected into a multimode inlet operating in pulsed splitless mode, at a temperature of 280^{o}C. Hydrogen was used as the carrier gas at a column flow rate of 1.2 ml/min. The oven temperature profile was as follows: 100^{o}C for 1 min, ramping at 20^{o}C/min to 200^{o}C, then 10^{o}C/min to 300^{o}C with a 5 minute hold.

The MS transfer line was set at 280°C. Mass spectra were obtained at a scan range of 60-500 m/z. Data analysis was performed using Agilent MSD Chemstation E.02.02.1431.

To determine the repeatability of the two replicate samples for each plant individual, the coefficient of variation (standard deviation/mean; CV) of the summed total concentration of each chemical was calculated and then expressed as a percentage. The tolerance level for poor repeatability was set at 20% and samples with a CV higher than this were excluded from the analysis.

Statistical analysis

Univariate and multivariate analyses of data were carried out using the same three factor model where country (Australia, South Africa) and region (North, Core, South) were considered as fixed factors and block (1, 2, 3) was considered as a random factor. Univariate datasets were analysed using DataDesk 6.1® and multivariate datasets were analysed using PERMANOVA+ Version 1.0.2 add-on statistical package (Anderson 2001; McArdle & Anderson 2001) within PRIMER 6 Version 6.1.12 (Clarke 1993; Clarke & Gorley 2006).

Univariate analyses: To test for differences in final plant biomass between countries and among regions, an analysis of covariance (ANCOVA) was carried out using the model described above. To account for effects of different plant sizes at initial planting on biomass, initial volume (dm^3) was fitted as a covariate. Similarly, an ANCOVA was used to test for differences between countries and among regions in the abundance, morphospecies richness and Shannon Diversity (H' = -SUM (Pi*Log(Pi)), where Pi is the proportional abundance of the *i*th species) of the total, endophagous and ectophagous herbivores. Plant biomass (g) was fitted as a covariate to account for differences caused by plant size. The effects of country and region on the concentrations of PAs extracted from the leaves and the stems of fireweed were tested with an analysis of variance (ANOVA). Where the interaction between country and region was significant, Scheffe's post-hoc test was used to determine where the differences were. Prior to analysis, all univariate data were $\log_{10}(x+1)$ transformed to meet the assumptions of normality and homogeneity of variances.

Multivariate analyses: To determine if there were differences in the total, endophagous and ectophagous herbivore assemblages found on plants from populations across the three regions in Australia and South Africa, analyses of the invertebrate herbivore assemblages were performed with a three factor permutational analysis of variance

(PERMANOVA) using the model design described above. Biomass (g) ($log_{10}(x+1)$) was included as a covariate. The analysis used S17 Bray-Curtis similarity and Type I Sum of Squares. The number of permutations was set at 9999. The species contributions to dissimilarity between countries was determined using similarity percentages (SIMPER) analyses. The cut-off for low contributions was set at 90%.

To test for differences in the composition of PAs extracted from 1) the leaves and 2) the stems of fireweed plants, a PERMANOVA was run. The analysis was based on S17 Bray-Curtis similarity. Type III Sum of Squares was used and the number of permutations was set at 9999. Data were $log_{10}(x+1)$ transformed prior to analysis. Where the interaction between country and region was significant, pairwise tests were run to determine where the differences lie. A SIMPER analysis indicated the contribution of each alkaloid to dissimilarity between countries and between pairs of regions that differed significantly. The cut-off for low contributions was set at 90%.

The relationship between the composition of alkaloids in the leaves and stems of fireweed and the total, endophagous and ectophagous insect herbivore assemblages was investigated in PRIMER. Alkaloid data were $log_{10}(x+1)$ transformed and normalised prior to analysis. Resemblance matrices were calculated using Euclidean distances for alkaloid data (for the purposes of these analyses it was entered as environmental data) and S17 Bray-Curtis similarity for abundance data. To determine how closely the alkaloid composition data were related to the insect assemblage data, the RELATE function was used. The Spearman's rank correlation method was used and the number of permutations was set at 9999. Where a significant relationship was found between alkaloid composition and insect community, a BEST analysis (using Spearman's rank correlation) was run to determine which alkaloids provided the highest correlation between the alkaloid data and the herbivore community data. The BIOENV method was selected and a permutation test was run with 999 permutations. Thereafter, a distance-based linear model (DistLM) was used to determine the relative contribution of each of the alkaloids that gave the highest correlation between alkaloid composition and insect community according to the BEST analysis. The step-wise selection procedure and AICc selection criteria were used and the number of permutations was set at 9999. Marginal tests were run to determine the significance of the effect of the alkaloids included.

Results

Biomass

Total plant biomass ranged from 3.64 g to 93.05 g. Total plant biomass did not vary between South Africa and Australia (F = 1.9444, df = 1, P = 0.1670; Figure 1). Despite a trend towards higher plant biomass in the core region of South Africa compared to all other regions, there were no significant differences in plant biomass among regions (F = 1.1999, df = 2, P = 0.3065; Table 2; Figure 1).

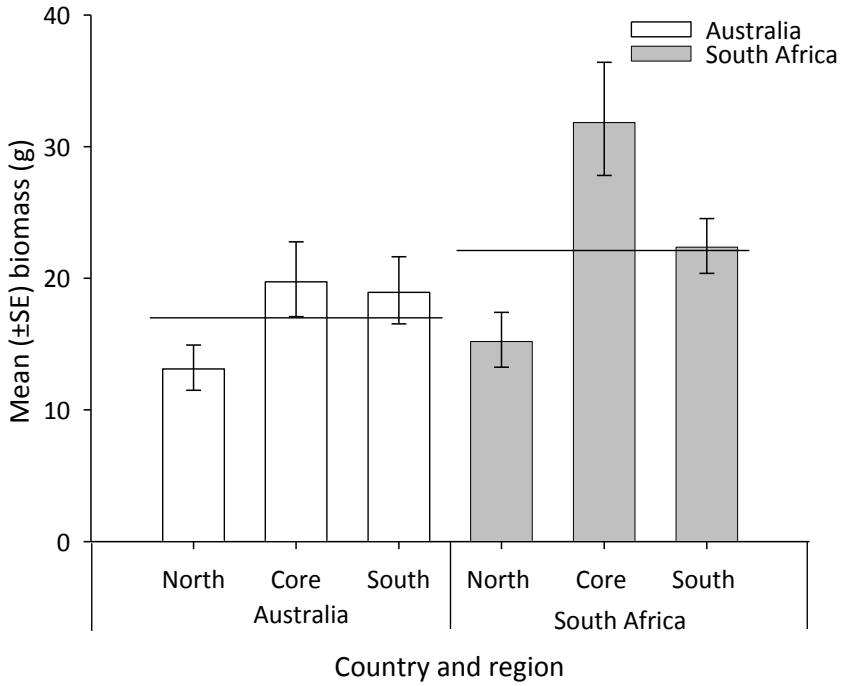

Figure 1: Mean (±SE) biomass (g) of *Senecio madagascariensis* plants grown from seed collected from three regions in Australia (white bars) and South Africa (grey bars). Lines across bars represent country means.

Insect herbivores

A total of 4705 insects belonging to 153 morphospecies were collected. Herbivores made up most of the individuals (89%) and 59% of the morphospecies, while most of the rest were parasitoids of endophagous immature stages (Figure 2). Ectophagous insects were responsible for nearly two thirds of the individuals and one third of the morphospecies richness of all insects (Figure 2). Stem borers were about 6 times as abundant and twice as species rich as capitulum feeders (Figure 2). Insects belonged to several orders including, in order of decreasing abundance, Hemiptera (just over half of all individuals), Diptera (about a

quarter of insects collected), Hymenoptera, Coleoptera, Thysanoptera, Lepidoptera and unidentified orders (Figure 2). Hymenoptera was the most species rich order, making up 42% of morphospecies (Figure 2). This was followed by, in order of decreasing richness, Hemiptera, Diptera, Coleoptera and Thysanoptera, Lepidoptera and unidentified orders (Figure 2).

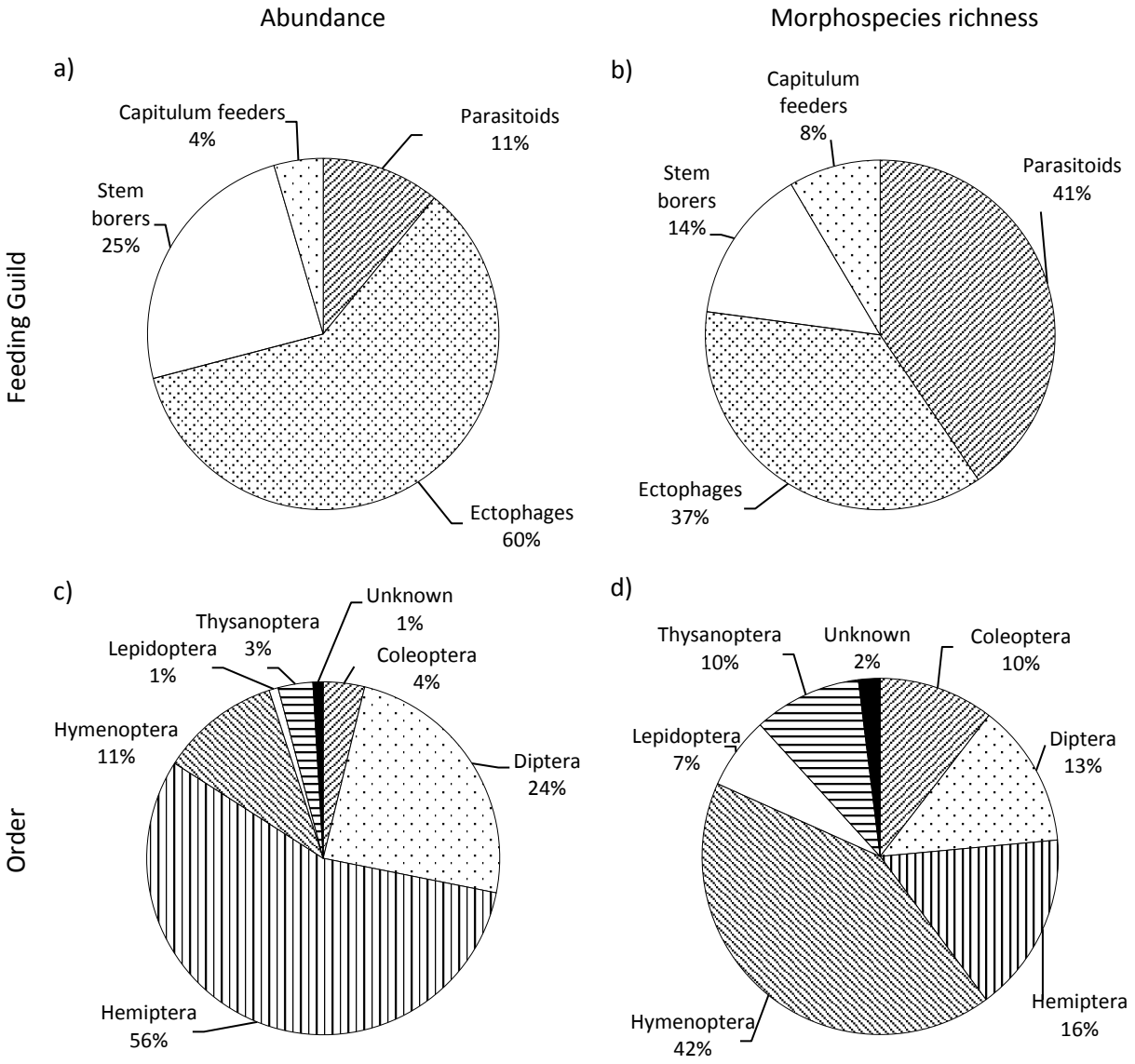

Figure 2: Percentage contribution to a) abundance and b) morphospecies richness of each feeding guild, and percentage contribution to c) abundance and d) morphospecies richness of each insect order representing the insect communities collected on all fireweed plants.

Table 2: Results of three factor ANCOVA for the effect of country (Australia, South Africa), region, their interaction, and block on total plant biomass and stem and leaf alkaloid concentrations, and the abundance, morphospecies richness and Shannon Diversity (H'=-SUM(Pi*Log(Pi))) of total insect herbivores and endophagous and ectophagous herbivores. All data were $\log_{10}(\times+1)$ transformed. Statistically significant results are highlighted in bold.

		Biomass[a]	Alkaloid concentration		Abundance[b]			Richness[b]			Diversity[b]		
			Leaves	Stems	All	Endo	Ecto	All	Endo	Ecto	All	Endo	Ecto
Covariate	F	5.256	-	-	10.051	84.414	0.5418	67	52.137	23.747	38.426	31.3	25.885
	df	1, 81	-	-	1, 81	1, 81	1, 81	1, 81	1, 81	1, 81	1, 81	1, 81	1, 81
	P	**0.0245**	-	-	**0.0021**	**0.0001**	0.4638	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**
Country	F	1.9444	4.7291	10.151	5.903	0.6175	3.3172	0.5145	0.0213	1.1574	6.2327	0.1012	0.1659
	df	1, 81	1, 101	1, 68	1, 81	1, 81	1, 81	1, 81	1, 81	1, 81	1, 81	1, 81	1, 81
	P	0.167	**0.032**	**0.0022**	**0.0173**	0.4343	0.0723	0.4753	0.8844	0.2852	**0.0146**	0.7512	0.6848
Region	F	10.862	3.9124	1.0471	0.4611	0.1632	0.9758	0.138	0.7527	0.7857	0.0112	0.8747	0.8788
	df	2, 81	2, 101	2, 68	2, 81	2, 81	2, 81	2, 81	2, 81	2, 81	2, 81	2, 81	2, 81
	P	**0.0001**	**0.0231**	0.3566	0.6322	0.8497	0.3813	0.8713	0.4744	0.4593	0.9889	0.4209	0.4192
Country × Region	F	1.1999	3.9019	1.8293	1.6201	0.9949	0.5519	0.7129	0.4549	0.3944	1.4025	0.5146	0.8184
	df	2, 81	2, 101	2, 68	2, 81	2, 81	2, 81	2, 81	2, 81	2, 81	2, 81	2, 81	2, 81
	P	0.3065	**0.0233**	0.1683	0.2042	0.3742	0.578	0.4933	0.6362	0.6754	0.2519	0.5997	0.4447
Block	F	3.577	0.9235	0.1806	4.7009	1.0547	5.1494	1.6809	0.5548	3.7437	5.4627	0.6642	1.5525
	df	2, 81	2, 101	2, 68	2, 81	2, 81	2, 81	2, 81	2, 81	2, 81	2, 81	2, 81	2, 81
	P	**0.0325**	0.4005	0.8352	**0.0117**	0.353	**0.0078**	0.1926	0.5763	**0.0279**	**0.0059**	0.5174	0.2179

[a] Initial volume (dm^3) was fitted as a covariate to account for any variation in initial size at time of planting (df = 1)

[b] Biomass (g) was fitted as a covariate to account for any variation due to plant size (df = 1)

Populations of fireweed from Australian-sourced seed had a significantly higher total herbivore abundance than those from South African-sourced seed (Table 2; Figure 3a) but a significantly lower Shannon Diversity (Table 2; Figure 3c). There was no difference in morphospecies richness of all herbivores, endophagous herbivores and ectophagous herbivores between the two countries, and no regional differences were significant ($P > 0.05$; Table 2; Figure 3b, 3e, 3h). Abundance and Shannon Diversity of endophagous and ectophagous herbivores did not differ significantly between countries or among the Australian and South African regions (Table 2; Figure 3d, 3f, 3g, 3i).

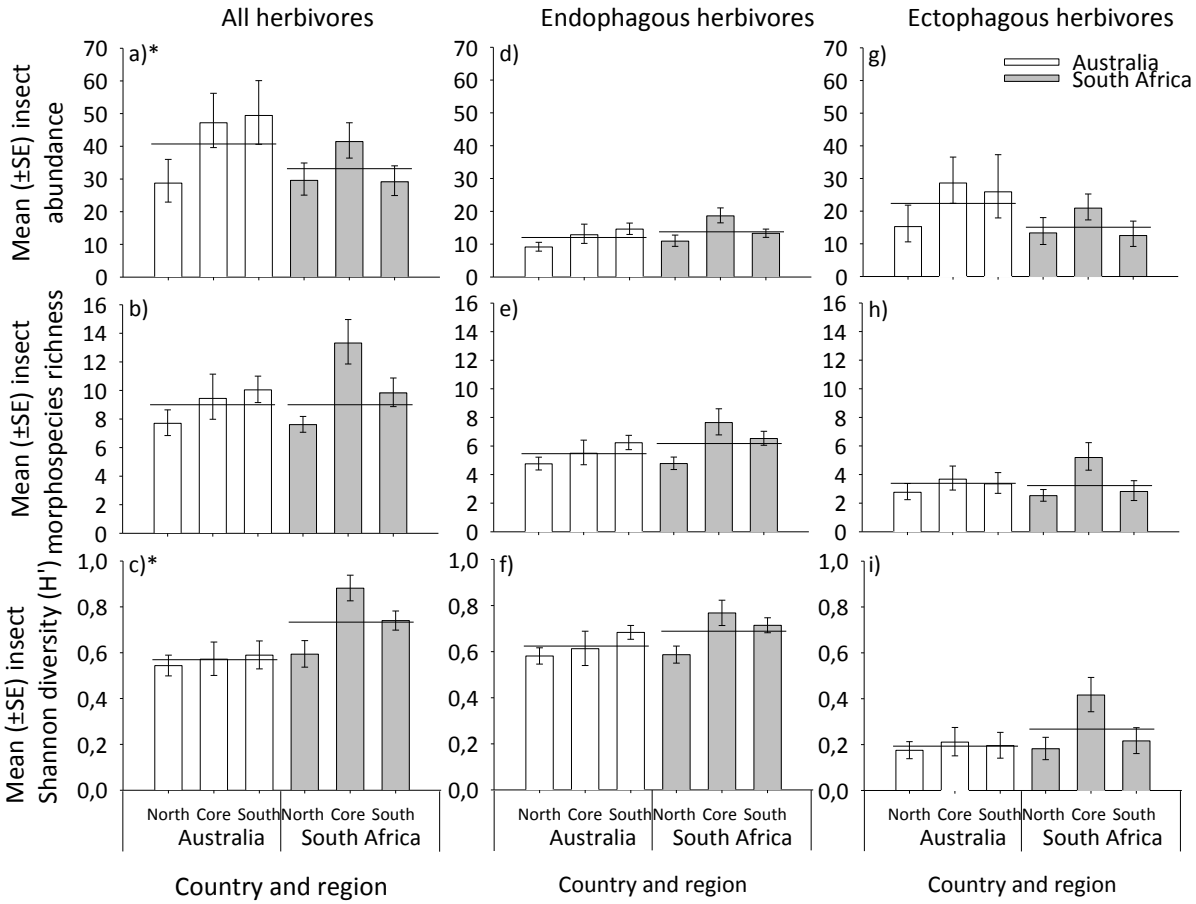

Figure 3: Mean (±SE) insect herbivore abundance, morphospecies richness and Shannon diversity (H') of all herbivores combined (a-c), endophagous herbivores (d-f) and, ectophagous herbivores (g-i) associated with fireweed plants grown from seed collected across three regions in Australia (white bars) and South Africa (grey bars). Lines across bars represent country means; * indicates significant differences between country means.

Table 3: Results of a three factor PERMANOVA for the effects of country (Australia, South Africa), region, their interaction, and block on total, endophagous and ectophagous insect community composition; and leaf and stem alkaloid composition. Statistically significant results are highlighted in bold.

		Insect communities			Alkaloid composition	
		All	Endo	Ecto	Leaves	Stems
Covariate[a]	Pseudo-F	5.6584	10.898	2.1624	-	-
	df	1, 64.94	1, 78.83	1, 73.15	-	-
	P	**0.0001**	**0.0001**	0.066	-	-
Country	Pseudo-F	3.4575	2.0531	3.6362	13.152	6.6479
	df	1, 81.83	1, 81.54	1, 80.05	1, 103	1, 68
	P	**0.0058**	**0.0298**	**0.009**	**0.0001**	**0.0004**
Region	Pseudo-F	0.9228	0.8275	0.9744	2.2743	1.9125
	df	2, 82.53	2, 82.04	2, 80.68	1, 103	1, 68
	P	0.5065	0.6729	0.433	**0.0109**	0.0547
Country x Region	Pseudo-F	0.7805	0.7827	0.7998	2.2209	1.3645
	df	2, 81	2, 81	2, 79	1, 103	1, 68
	P	0.6733	0.728	0.6092	[b]**0.0105**	0.199
Block	Pseudo-F	2.7295	1.5428	2.1954	1.6675	1.5391
	df	2, 81	2, 81	2, 79	1, 103	1, 68
	P	**0.0052**	0.0649	**0.0305**	0.0713	0.1257

[a] Biomass (g) was fitted as a covariate to account for variation due to differences in plant size (df = 1)
[b] Results of pairwise tests are presented in Table 6

Table 4: Results of one-factor SIMPER for the total, endophagous and ectophagous insect assemblages collected on fireweed populations, grown from seed sourced from Australia and South Africa. The average Bray-Curtis dissimilarities are given for total, endophagous and ectophagous herbivorous insects. For each population, the mean abundance is recorded along with the percentage contribution and cumulative contribution to the overall group dissimilarity; with morphospecies and guild therein ordered in decreasing contribution to the overall group similarity.

Morphospecies	Mean abundance		Contrib%	Cum%	Guild
	Australia	S.A.			
All insects	Average dissimilarity = 60.48				
Hemiptera: *Hilda*	35.53	18.42	48.08	48.08	Sap sucker
Diptera: Dip 10	5.02	5.11	8.02	56.10	Stem borer
Diptera: Dip 09	2.44	2.51	5.30	61.40	Stem borer
Diptera: Dip 11	0.87	1.71	3.34	64.74	Stem borer
Coleoptera: Curc 01	0.98	1.11	3.04	67.78	Stem borer
Diptera: Dip 18	0.53	1.02	2.70	70.48	Stem borer
Diptera: Dip 05	0.89	0.78	2.52	73.00	Capitulum/seed feeder
Thysanoptera: Thrips 01	0.62	0.71	1.86	74.87	Flower feeder
Coleoptera: Nit 01	0.71	0.20	1.38	76.25	Capitulum/seed feeder
Diptera: Dip 13	0.62	0.29	1.35	77.60	Stem borer
Diptera: Dip 04	0.38	0.27	1.25	78.84	Capitulum/seed feeder
Hemiptera: Hem 03	0.44	0.31	1.09	79.94	Sap sucker
Unknown: Unkn 01	0.18	0.36	1.07	81.01	Stem borer
Diptera: Dip 03	0.31	0.36	1.05	82.06	Capitulum/seed feeder
Diptera: Dip 15	0.13	0.42	1.01	83.07	Stem borer
Hemiptera: Hem 02	0.40	0.31	0.95	84.02	Sap sucker
Diptera: Dip 07	0.31	0.22	0.90	84.92	Capitulum/seed feeder
Hemiptera: *Nysius* 01	0.18	0.42	0.88	85.81	Sap sucker
Lepidoptera: Lep 05	0.22	0.27	0.85	86.66	Stem borer
Thysanoptera: Thrips 11	0.24	0.24	0.80	87.46	Flower feeder
Diptera: Dip 16	0.24	0.20	0.80	88.26	Stem borer
Unknown: Unkn 04	0.09	0.31	0.76	89.02	Stem borer
Hemiptera: Hem 07	0.18	0.24	0.64	89.66	Sap sucker
Diptera: Dip 17	0.13	0.16	0.61	90.27	Stem borer

Table 4: Continued

Morphospecies	Mean abundance		Contrib%	Cum%	Guild
	Australia	S.A.			
Endophages	Average dissimilarity = 59.77				
Diptera: Dip 10	5.02	5.11	21.23	21.23	Stem borer
Diptera: Dip 09	2.44	2.51	13.85	35.08	Stem borer
Diptera: Dip 11	0.87	1.71	9.04	44.12	Stem borer
Coleoptera: Curc 01	0.98	1.11	7.76	51.88	Stem borer
Diptera: Dip 05	0.89	0.78	7.18	59.05	Capitulum/seed feeder
Diptera: Dip 18	0.53	1.02	6.69	65.74	Stem borer
Diptera: Dip 13	0.62	0.29	3.97	69.71	Stem borer
Coleoptera: Nit 01	0.71	0.20	3.80	73.52	Capitulum/seed feeder
Diptera: Dip 04	0.38	0.27	3.11	76.63	Capitulum/seed feeder
Diptera: Dip 03	0.31	0.36	2.75	79.37	Capitulum/seed feeder
Diptera: Dip 15	0.13	0.42	2.54	81.92	Stem borer
Diptera: Dip 07	0.31	0.22	2.52	84.44	Capitulum/seed feeder
Unknown: Unkn 01	0.18	0.36	2.49	86.93	Stem borer
Lepidoptera: Lep 05	0.22	0.27	2.24	89.16	Stem borer
Diptera: Dip 16	0.24	0.20	2.19	91.35	Stem borer
Ectophages	Average dissimilarity = 59.95				
Hemiptera: *Hilda*	37.19	18.42	74.58	74.58	Sap sucker
Thysanoptera: Thrips 01	0.65	0.71	3.47	78.05	Flower feeder
Hemiptera: Hem 03	0.47	0.31	1.84	79.89	Sap sucker
Hemiptera: Hem 02	0.42	0.31	1.69	81.58	Sap sucker
Thysanoptera: Thrips 11	0.26	0.24	1.67	83.25	Flower feeder
Hemiptera: *Nysius* 01	0.19	0.42	1.58	84.83	Sap sucker
Hemiptera: Hem 07	0.19	0.24	1.09	85.92	Sap sucker
Coleoptera: Col 01	0.12	0.20	0.97	86.90	Flower feeder
Coleoptera: FleaB	0.05	0.20	0.93	87.82	Root/leaf feeder
Hemiptera: Aphid 01	0.14	0.13	0.89	88.71	Sap sucker
Hemiptera: *Nysius* 02	0.09	0.27	0.86	89.57	Sap sucker
Thysanoptera: Thrips 05	0.09	0.09	0.73	90.30	Flower feeder

There were significant differences in total, endophagous and ectophagous insect herbivore community composition found on Australian and South African fireweed populations (Table 3). Despite strong dissimilarity in insect herbivores of around 60% for all assemblages compared between Australian and South African populations (Table 4), differences were largely driven by variation in abundance and not the presence or absence of important morphospecies, as all morphospecies contributing to dissimilarity were present on both country's populations. Twenty eight percent of species were only recorded once, with nearly equal numbers on Australian and South African populations.

Ninety percent of the dissimilarity between Australian and South African plants was caused by 24 insect morphospecies. Half of the insects responsible for the dissimilarity were stem borers; sap suckers and capitulum/seed feeders each made up one fifth of the insects and flower feeders represented less than 10% of the insects. The tettigometrid *Hilda patruelis* Stal contributed 48.1% to the dissimilarity in total herbivore community found between Australian and South African plants (Table 4). Four stem-boring dipterans and a stem-boring weevil were the next most important species responsible for community differences between populations and together with *H. patruelis* contributed 70.4% of the differences between countries in the overall herbivore community.

Fifteen of the total morphospecies were responsible for 90% of the dissimilarity in endophagous insect communities found on South African and Australian plants; two thirds of these were stem borers, while the remaining one third were seed feeders. Dipterans made up three quarters of the insects contributing the most to dissimilarity. The stem-boring dipterans, Dip10, Dip09 and Dip11 (Tephritidae or Agromyzidae) contributed the most to the dissimilarity between the two countries, contributing 21.2%, 13.8%, and 9.0% respectively (Table 4). This was followed by the stem-boring weevil, Curc01, which contributed 7.7%.

Twelve morphospecies were responsible for 90% of the dissimilarity in ectophagous insect communities between Australian and South African plants; just over half were flower feeders, one third were sap suckers and the rest were root or leaf feeders. *Hilda patruelis*, which was twice as abundant on Australian populations, contributed the most (74.6%) to the difference between Australian and South African plants followed by Thrips01 (3.5%) and then two sap-sucking hemipterans, Hem03 and Hem02, contributing 1.8% and 1.7%, respectively (Table 4). The flea beetle (*Longitarsus basutoensis* Bechnyé), with root-feeding larvae and leaf-feeding adults, was responsible for 0.9% of the differences between countries (Table 4).

Alkaloids

A total of 10 pyrrolizidine alkaloids were extracted from the leaves and stems of fireweed plants: senecivernine, senecionine, integerrimine, mucronatinine, retrosine, usaramine, otosenine, desacetyl doronine, florosenine, and doronine. The total PA concentrations ranged from 26 µg/g to 29 670 µg/g dry weight in leaves, and 188 µg/g to 13 849 µg/g dry weight in stems.

Total alkaloid concentration within the leaves was significantly higher in Australian plants than South African plants (F = 4.7291, df = 1, 101, P = 0.032; Table 2; Figure 4a). Significant differences were also found among regions but these showed no distinct patterns (F = 3.9019, df = 2, 101, P = 0.0233). For example, the southern South African population had a significantly higher total leaf concentration than the core South African population but was similar to all other populations regardless of country. The core population from South Africa was significantly different to the core and south Australian populations in leaf alkaloid concentrations. In contrast, stem samples displayed a significant difference in alkaloid concentrations between countries (F = 10.151, df = 1, 681, P = 0.0022) with concentrations in Australian populations being nearly double that of South African populations, but showed no significant differences among regions (P < 0.05; Table 2; Figure 4b).

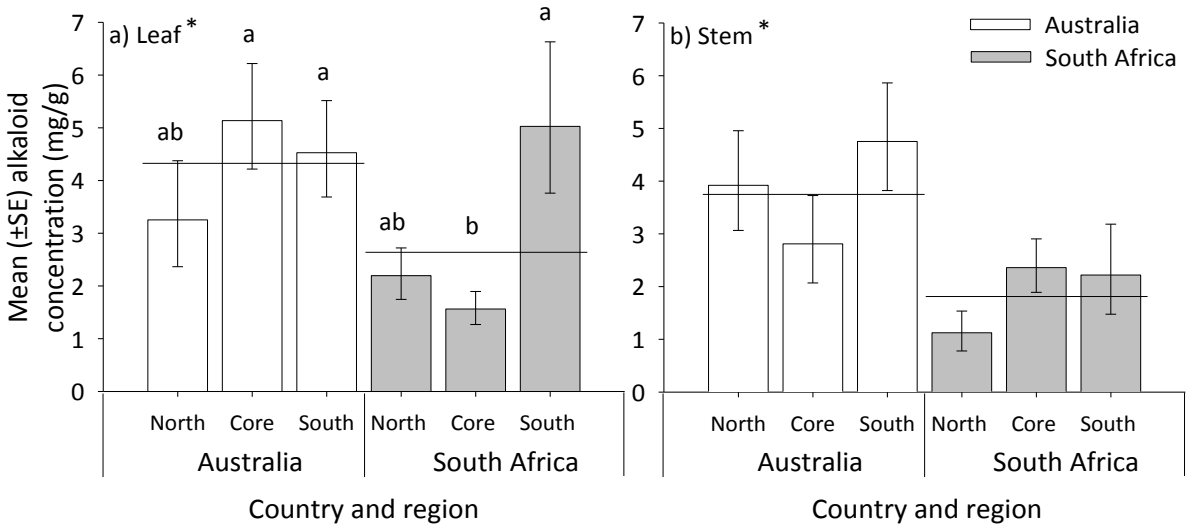

Figure 4: Mean (±SE) total alkaloid concentrations in the a) leaves and b) stems of fireweed from three regions in Australia (white bars) and three regions in South Africa (grey bars). Lines across bars represent country means, * indicates significant differences between country means. Bars with different letters are significantly different at P < 0.05 according to Scheffe's post-hoc pairwise comparison tests.

Differences in the leaf alkaloid concentrations were mostly driven by concentrations of doronine and florosenine which were 2.8 and 4.5 times higher, respectively, in Australian populations than in South African populations. These two alkaloids were also responsible for the significant variation in alkaloid composition between countries and regions (Table 3). Together, they contributed just over 40% of the differences between countries which had a

dissimilarity of 74% (Table 5). Doronine and florosenine also caused most of the dissimilarity in PA composition between pairs of Australian and South African populations, with the exception of the Australian north population versus the South African south population and the Australian north population versus the South African north population (Table 6). The differences between these two pairs were largely driven by senecivernine and retrosine, which were also responsible for the differences among South African populations. There was no significant variation among Australian populations, or between the South African north and south populations.

Although differences in total stem concentrations were largely driven by much higher concentrations of desacetyl doronine and doronine in Australian populations, they were not responsible for the significant variation in composition between countries. Nearly half the dissimilarity (72%) was due to differences in retrosine and doronine. There was no regional variation in the composition of PAs extracted from the stems.

Table 5: Results of a one factor SIMPER for the alkaloids extracted from fireweed populations grown from seed sourced from Australia and South Africa. The average Bray-Curtis dissimilarities are given for alkaloids extracted from leaves and alkaloids extracted from stems. For each population, the mean concentration (mg/g) is recorded along with the percentage contribution and cumulative contribution to the overall group dissimilarity; with alkaloids ordered in decreasing contribution to the overall group dissimilarity.

Alkaloid	Mean concentration		Contrib%	Cum%
	Australia	S. Africa		
Leaf alkaloids	Mean diss. = 73.60			
Doronine	1.66	0.34	21.64	21.64
Florosenine	1.51	0.52	20.28	41.92
Senecivernine	0.64	0.98	13.70	55.62
Retrosine	0.60	1.12	13.40	69.03
Mucronatinine	0.49	0.87	10.51	79.54
Desacetyl doronine	0.63	0.06	6.99	86.53
Otosenine	0.47	0.05	5.62	92.14
Stem Alkaloids	Mean diss. = 72.37			
Doronine	1.56	0.43	28.20	28.20
Retrosine	0.67	0.95	21.40	49.61
Desacetyl doronine	1.05	0.12	14.16	63.77
Florosenine	0.65	0.40	13.66	77.43
Otosenine	0.42	0.14	6.39	83.81
Senecionine	0.23	0.13	4.91	88.72
Senecivernine	0.13	0.22	3.99	92.72

Table 6: Results of a one factor SIMPER for the alkaloids extracted from leaves of fireweed populations grown from seed sourced from across three regions in Australia (Aus) and South Africa (SA). The average Bray-Curtis dissimilarities are given for pairs of regions (C = Core; N = North; S = South) that were significantly different from each other according to the multiple comparisons from the PERMANOVA. For each population, the mean concentration (mg/g) is recorded along with the percentage contribution and cumulative contribution to the overall group dissimilarity; with alkaloids ordered in decreasing contribution to the overall group dissimilarity. P values from pairwise comparisons are given.

Alkaloid	Mean concentration		Contrib%	Cum%
Mean diss. = 70.92	Aus C	SA C	P = 0.0001	
Florosenine	1.98	0.24	25.41	25.41
Doronine	1.46	0.10	21.44	46.85
Senecivernine	0.92	0.51	14.15	61.01
Mucronatinine	0.81	0.49	10.68	71.69
Retrosine	0.63	0.43	8.36	80.05
Otosenine	0.51	0.03	6.68	86.73
Senecionine	0.21	0.07	4.78	91.51
Mean diss. = 74.63	Aus N	SA C	P = 0.0019	
Florosenine	0.67	0.24	17.10	17.10
Doronine	0.88	0.10	16.50	33.60
Senecivernine	0.74	0.51	13.38	46.98
Retrosine	0.48	0.43	11.08	58.06
Desacetyl doronine	1.10	0.03	10.93	68.99
Mucronatinine	0.27	0.49	10.87	79.86
Otosenine	0.73	0.03	10.11	89.97
Senecionine	0.22	0.07	3.91	93.88
Mean diss. = 74.08	Aus S	SA C	P = 0.001	
Doronine	2.44	0.10	32.23	32.23
Florosenine	1.68	0.24	23.60	55.83
Retrosine	0.65	0.43	9.98	65.80
Desacetyl doronine	0.62	0.03	8.66	74.46
Senecivernine	0.30	0.51	8.41	82.87
Mucronatinine	0.33	0.49	7.92	90.78
Mean diss. = 70.56	Aus C	SA S	P = 0.0296	
Florosenine	1.98	1.24	21.51	21.51
Doronine	1.46	0.88	17.30	38.81
Senecivernine	0.92	1.77	17.09	55.90
Retrosine	0.63	2.02	15.03	70.92
Mucronatinine	0.81	1.51	12.33	83.26
Otosenine	0.51	0.12	5.01	88.26
Senecionine	0.21	0.32	4.05	92.32

Table 6: Continued

Alkaloid	Mean concentration		Contrib%	Cum%
Mean diss. = 76.63	Aus N	SA S	P = 0.0473	
Senecivernine	0.74	1.77	18.36	18.36
Retrosine	0.48	2.02	16.61	34.96
Florosenine	0.67	1.24	14.54	49.51
Doronine	0.88	0.88	13.96	63.47
Mucronatinine	0.27	1.51	11.75	75.21
Desacetyl doronine	1.10	0.13	8.47	83.68
Otosenine	0.73	0.12	7.09	90.77
Mean diss. = 74.67	Aus S	SA S	P = 0.0002	
Doronine	2.44	0.88	23.88	23.88
Florosenine	1.68	1.24	19.57	43.45
Retrosine	0.65	2.02	15.58	59.03
Senecivernine	0.30	1.77	15.02	74.05
Mucronatinine	0.33	1.51	10.36	84.41
Desacetyl doronine	0.62	0.13	6.21	90.62
Mean diss. = 70.75	Aus C	SA N	P = 0.0001	
Florosenine	1.98	0.01	22.50	22.50
Doronine	1.46	0.01	19.85	42.35
Retrosine	0.63	1.03	14.73	57.08
Senecivernine	0.92	0.66	13.80	70.88
Mucronatinine	0.81	0.62	11.29	82.17
Otosenine	0.51	0.00	6.08	88.25
Senecionine	0.21	0.11	4.20	92.45
Mean diss. = 75.84	Aus N	SA N	P = 0.0009	
Retrosine	0.48	1.03	19.24	19.24
Senecivernine	0.74	0.66	14.99	34.22
Doronine	0.88	0.01	13.96	48.18
Florosenine	0.67	0.01	12.36	60.54
Mucronatinine	0.27	0.62	11.96	72.50
Desacetyl doronine	1.10	0.03	10.01	82.51
Otosenine	0.73	0.00	8.79	91.30
Mean diss. = 72.65	Aus S	SA N	P = 0.0001	
Doronine	2.44	0.01	29.10	29.10
Florosenine	1.68	0.01	20.18	49.29
Retrosine	0.65	1.03	15.84	65.12
Senecivernine	0.30	0.66	10.02	75.14
Mucronatinine	0.33	0.62	8.79	83.94
Desacetyl doronine	0.62	0.03	7.52	91.46

Table 6: Continued

Alkaloid	Mean concentration		Contrib%	Cum%
Mean diss. = 69.70	SA C	SA S	P = 0.0017	
Senecivernine	0.51	1.77	23.54	23.54
Retrosine	0.43	2.02	20.99	44.53
Mucronatinine	0.49	1.51	16.50	61.03
Florosenine	0.24	1.24	13.41	74.44
Doronine	0.10	0.88	9.96	84.40
Senecionine	0.07	0.32	4.05	88.45
Usaramine	0.10	0.24	3.79	92.24
Mean diss. = 60.06	SA C	SA N	P = 0.0451	
Retrosine	0.43	1.03	28.82	28.82
Senecivernine	0.51	0.66	22.85	51.67
Mucronatinine	0.49	0.62	19.90	71.57
Florosenine	0.24	0.01	8.32	79.88
Senecionine	0.07	0.11	4.83	84.72
Usaramine	0.10	0.11	4.04	88.76
Doronine	0.10	0.01	3.91	92.67

There was a significant relationship between the composition of leaf alkaloids and the endophagous insect communities (r_s = 0.251, P = 0.00019), but not the total herbivore (r_s = 0.121, P = 0.0501) or the ectophagous herbivore communities (r_s = 0.012, P = 0.4138). No significant relationship was found between the composition of stem alkaloids and any of the insect communities (P > 0.05). The endophagous insect community was significantly correlated (r_s = 0.299, P = 0.005) with the concentrations of five leaf alkaloids, namely senecionine, integerrimine, usaramine, otosenine, and doronine. These five alkaloids, included in the DistLM model for the endophagous community, explained 14.55% of the variation in that community. Senecionine explained the most variation in the endophagous community (6.41%) while doronine explained the least (0.37%).

Discussion

When introduced to new environments, plant populations can undergo evolutionary and adaptive changes that may facilitate invasion. For example, plants may reallocate resources from defence to growth and reproduction, or change their chemical defences (Blossey & Notzold 1995; Muller-Scharer *et al*. 2004; Joshi & Vrieling 2005; Doorduin & Vrieling 2011). Australian populations of fireweed were found to have higher concentrations of PAs than native South African populations (Harvey *et al*. unpublished). This increase in PA

concentration could be a response to a change in insect community from one containing about 33% specialists to one with less than 10% specialists (Harvey *et al*. 2015). These changes may influence interactions with native herbivores. This study sought to determine (1) if fireweed plants from the native and invasive range would be differentially affected by natural enemies in the native range and (2) if invasive populations would attract the same suite of natural enemies as native populations, as this could affect the potential efficacy of biocontrol. There was no difference in plant biomass between the native and invasive fireweed populations that were exposed to native herbivores under the same field conditions. Insect communities were similar across populations with only slight differences in relative abundances, despite significant variation across populations in alkaloid concentrations and profiles. No obvious relationship between the insect community and alkaloid composition was detected. This indicates that the Australian fireweed populations are equally susceptible to insect damage from the native herbivores and that biological control is a viable management strategy for the control of fireweed in Australia.

The lack of any differences in biomass between native and invasive fireweed populations is contrary to one of the predictions of the EICA and SDH hypotheses that plants from the invaded range will display increased growth in the introduced range but reduced growth in the native range (Blossey & Notzold 1995; Muller-Scharer *et al*. 2004; Joshi & Vrieling 2005; Doorduin & Vrieling 2011). The results of this study support those of Muller & Martens (2005), who found no difference in biomass between native and invasive populations of *Lepidium draba* L. (Brassicaceae), and Meyer *et al*. (2005) who found no differences in plant height between native and invasive populations of *Solidago gigantea* Ait. (Asteraceae). A review by Hinz & Schwarzlaender (2004) found that increased vigour in invasive populations was only recorded in half of the studies where they were grown together with native populations in a common garden experiment.

The increase in size experienced by some plants in their invaded range can be due to reallocation of resources from defence to growth and reproduction (Blossey & Notzold 1995; Muller-Scharer *et al*. 2004; Joshi & Vrieling 2005; Doorduin & Vrieling 2011). While Australian fireweed plants appeared to allocate more to defence in the form of PAs, they did not have a lower biomass than native plants. This is likely because alkaloids are relatively cheap to produce and there is often no trade-off between either growth or reproductive output and PA concentrations (Vrieling & Vanwijk 1994; Stastny *et al*. 2005). The trade-off between

growth and defence is more common with more costly quantitative chemicals that are usually deployed as a defence against specialists (Poorter & de Jong 1999; Glawe *et al*. 2003). It is unlikely that specialist defences have decreased in Australian plants as specialist herbivores were not more abundant on invasive populations and Australian plants did not display greater biomass. Fireweed in Australia does not seem to be allocating more resources to increase biomass.

Australian fireweed plants had a significantly higher total abundance of herbivores than South African plants. Australian plants may thus have evolved to grow bigger (Blossey & Notzold 1995; Muller-Scharer *et al*. 2004; Joshi & Vrieling 2005; Doorduin & Vrieling 2011) or to be more tolerant to herbivory (Muller-Scharer *et al*. 2004; Zou *et al*. 2008; Wang *et al*. 2011) but due to higher insect loads, only grew to the same size as South African plants. However, this is unlikely as insect abundances were low and the difference was small (mean of 41 and 33 individuals per plant on Australian and South African populations, respectively). The difference in insect abundance was mostly due to differences in the abundance of the sap-sucking tettigometrid, *H. patruelis*, which was nearly twice as abundant on Australian plants. This insect was also ranked as low priority for biological control (Chapter 1). At such low densities, this insect may not have a significant impact on the plant. While it is unclear if Australian plants have higher growth/tolerance or if the tettigometrid is not very damaging, Australian plants, when exposed to native natural enemies, did not grow bigger and were equally susceptible to insect herbivores.

There were no differences in abundance, richness or diversity of either endophagous or ectophagous insects between native and invasive fireweed populations, despite differences in alkaloid profiles and concentrations. The SDH predicts that endophagous insects should prefer the Australian populations as they have a higher concentration of PAs (Muller-Scharer *et al*. 2004; Joshi & Vrieling 2005; Doorduin & Vrieling 2011) which often act as feeding or oviposition cues for specialists (van der Meijden 1996; Bernays *et al*. 2004). The higher concentration of PAs in the Australian populations did not have a significant effect on the overall specialist herbivore community. Experiments on specialists' reactions to changes in PA concentration have had mixed results, with some studies finding that specialists prefer invasive populations (Stastny *et al*. 2005; Wang *et al*. 2011, 2012), while other studies found no preferences (Van dam *et al*. 1995; Vrieling & de Boer 1999; Castells & Berenbaum 2008; Wei *et al*. 2015). Conversely, Vrieling and Vanwijk (1994) found performance of a specialist

flea beetle to be lower at higher concentrations of PAs. Although specialists may react to other chemicals (Castells & Berenbaum 2008), no difference was found in endophagous herbivore abundance, richness and diversity, which suggests that any other changes the plants may have undergone (but that were not measured here) have not affected the specialist insects. Overall, the specialists in this study did not show preference for either Australian or native fireweed populations.

Similarly, there was no difference in ectophagous herbivore abundance, richness or diversity. This is also contrary to predictions of the SDH which states that PAs are typically a defence against generalist herbivores and that increased concentrations will result in increased defence (van der Meijden 1996; Muller-Scharer *et al*. 2004; Joshi & Vrieling 2005; Doorduin & Vrieling 2011). Native plants which are less well defended are thus expected to host higher numbers of generalists (Cano *et al*. 2009). My results support a number of studies that found no difference in the preferences of generalists between native and invasive populations, despite differences in alkaloid concentrations (Hinz & Schwarzlaender 2004; Wang *et al*. 2012). However, a number of studies have found that generalists react negatively to PAs (Van dam *et al*. 1995; Wei *et al*. 2015). Any adaptive changes that fireweed has undergone since its introduction to Australia do not appear to have much of an impact on either the native endophagous or ectophagous insect communities.

It is possible that the designation of endophages as specialists and ectophages as generalists was too broad/inaccurate to show a significant trend. When comparing the community assemblages, there were slight differences between the native and invasive populations. These differences were due to mostly small differences in the relative abundance of different insects, and were not related to differences in alkaloid profiles. Furthermore, no insects were unique to either population. Had specialists preferred invasive populations and generalists native populations, it would be expected that there would be more separation than was recorded in the PERMANOVA results. The only insect that showed a big difference between the two plant populations was the sap sucker *H. patruelis.* Although a generalist, *H. patruelis* might have been attracted to one of the alkaloids that is present in higher concentrations in Australian plants (Bernays *et al*. 2004; Wei *et al*. 2015), or may be reacting to something else in the plant that was not measured. Generally, the insects did not show a preference for either fireweed population.

The results of this experiment indicate that biocontrol is a viable option as the Australian plants, despite differences in alkaloid profiles and higher alkaloid concentrations, are not differentially affected by insects in terms of biomass, and are equally attractive to native insect herbivores. Importantly, there was not much difference in the abundance of certain insects that were prioritised for biological control (e.g. stem-boring curculionid and lepidopteran). The root-feeding flea beetle, *L. basutoensis*, which has a high priority, was more abundant on South African fireweed populations although numbers were extremely low. However, since numbers of each species were relatively low, it is not possible to draw meaningful conclusions about the preferences of individual species. It would, therefore, be useful to test the preferences of prioritised insects on plants from native and invasive populations and determine the response of plants from both provenances to insect damage. Additionally, while I found no difference in alkaloids from plants along the invasion gradient in Australia in this study (due to small sample size and differing conditions), there were significant differences recorded by Harvey *et al*. (unpublished data). It would thus be useful to include these different Australian populations in any subsequent tests of insect agent preferences.

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GENERAL DISCUSSION AND CONCLUSIONS

Invasive plants have extensive negative impacts on the environment and agriculture. They reduce biodiversity and productivity, and incur high control costs (Pimentel *et al*. 2000, 2001). Since chemical and mechanical control methods are costly, damaging to the environment and often provide only short-term solutions, biological control is increasingly being viewed as a more cost effective, environmentally friendly and self-sustaining control method (McFadyen 1998; Zimmermann *et al*. 2004; Culliney 2005). *Senecio madagascariensis* (fireweed) has invaded pastures in Australia and Hawaii causing huge losses to livestock production (Le Roux *et al*. 2006, 2010; McFadyen & Morin 2012). There has thus been interest in biological control as a long-term and safe control strategy (Ramadan *et al*. 2011; McFadyen & Morin 2012; Sheppard *et al*. 2013). Genetic studies have confirmed that KwaZulu-Natal, South Africa, is the centre of origin of invasive Australian and Hawaiian populations (Scott *et al*. 1998; Radford *et al*. 2000; Le Roux *et al*. 2006). Efforts to source insect agents for Australia are thus being focussed in this region.

Fireweed is a difficult target for biological control in Australia because of the large number of native *Senecio* species there (Thompson 2006). Some of these are closely related to fireweed (Prentis *et al*. 2007) and finding a host specific agent may therefore be difficult. Fireweed has also recruited several Australian *Senecio* herbivores, including specialists together with their parasitoids (Harvey *et al*. 2015) from native Australian *Senecio* species. There is thus the risk that a biocontrol agent may similarly move onto native Australian *Senecio* species, as well as recruit parasitoids from native Australian *Senecio* insects (Paynter *et al*. 2010). The situation in Hawaii is vastly different as Hawaii has no native *Senecio* species, nor any species within the tribe Senecioneae, ensuring that host-specificity requirements are less strict (Ramadan *et al*. 2011). The research reported in this PhD thesis aimed to prioritise insects as potential biocontrol agents and confirm the susceptibility or resistance of Australian fireweed populations to these insects as a result of changes in their pyrrolizidine alkaloid profiles and concentrations.

Plant populations were surveyed to identify the major herbivorous taxa associated with fireweed in KwaZulu-Natal (Chapter 1). The incidence and abundance of the herbivore fauna was assessed and the native insect taxa and feeding guilds were compared to those

that are associated with fireweed in Australia (i.e. recruited from native Australian *Senecio* species). Abundance can affect the efficacy of biocontrol as insects that are able to build up in high numbers are more likely to inflict sufficient damage on the target plants (Goolsby *et al*. 2006). Incidence is also important as insects that occur over a wider geographic range are more likely to tolerate a wider range of climatic conditions (Goolsby *et al*. 2006). Additionally, the recruitment of similar insect taxa (in the same feeding guilds) by the plants in the invasive range may result in competition between the agents and native insects and increase the likelihood that introduced agents will recruit native parasitoids, thereby reducing their efficacy (Paynter *et al*. 2010).

In some biocontrol programmes insects that attacked the target weed for only part of the year did not have sufficient impact on survivorship or reproductive output to ensure control (Woodburn & Cullen 1995; Cullen & Sheppard 2012). It was thus necessary to release a suite of agents that attack the target weed at different times during the year to ensure sustained damage (Woodburn & Cullen 1995; Cullen & Sheppard 2012). The abundance of the major herbivorous taxa associated with fireweed was surveyed once per season, over the period of a year, in order to determine the insects' potential to inflict sustained damage on the plants (Chapter 2).

Agents were also prioritised in relation to the successes and failures of similar insect taxa and feeding guilds used in previous biocontrol programs against Asteraceae (Chapter 3). Analysis of the literature revealed that certain taxa are used frequently and are effective. A number of the insect families found on fireweed have been used before in the biocontrol of Asteraceae and have resulted in control of the target weed. Many of the programs against Asteraceae targeted thistles (tribe Cardueae) which are distantly related to, and morphologically very different from, fireweed (Funk *et al*. 2009). The only plant in the tribe Senecioneae that had been targeted over a measureable time frame is *Jacobaea vulgaris* (= *Senecio jacobaea*), which is closely related and similar in morphology to fireweed (Funk *et al*. 2009; Winston *et al*. 2014). Similar insect assemblages occur on both plants and biological control of *J. vulgaris* has largely been a success in both Australia and the USA, with no non-target feeding recorded (McFadyen & Morin 2012; Winston *et al*. 2014).

The native field host range of stem-boring Curculionidae, and of stem-boring and capitulum-feeding Lepidoptera and Diptera was assessed (Chapter 4). As the endophagous insects collected during surveys of other native *Senecio* species in KwaZulu-Natal comprised

larvae, which are virtually impossible to separate to species level based on morphology, the COI gene was sequenced to determine insect identity. The phylogenies based on these sequences revealed that certain taxa were restricted to plants in the *S. madagascariensis* species complex. Generally, insects that display signs of host specificity in the field in their native range have displayed host specificity in laboratory-based host-range tests. Consequently, field host range data such as these can be a good indicator of whether the prioritized agents are likely to be host specific in the invaded range (Van Klinken 1999; Goolsby *et al*. 2006).

Increases in the concentrations of defensive chemicals such as pyrrolizidine alkaloids (PAs), which are abundant in fireweed, in the invasive populations may affect the efficacy of biological control agents (Muller-Scharer *et al*. 2004). Pyrrolizidine alkaloids are considered to provide defence against generalist enemies, but also host-finding or oviposition cues for specialist enemies which respond positively to increases in PA concentrations (Muller-Scharer *et al*. 2004). However, studies have found varying responses of specialist enemies to changes in alkaloid concentrations (Macel *et al*. 2002; Joshi & Vrieling 2005). Thus, the relationship between PA concentrations and native insect assemblages was investigated in an open-field experiment (Chapter 5).

Based on these aspects, several insects were prioritised for further investigation in quarantine in Australia or in the laboratory/field in South Africa. The main taxa considered and their potential efficacy as biocontrol agents are discussed.

Coleopteran candidates

Initial field surveys revealed 10 coleopteran taxa feeding within the stems and capitula, as well as externally on the foliage and roots. Of these, the stem-boring weevil and the root-feeding flea beetle were considered to be the most promising biocontrol candidates for both Australia and Hawaii.

Stem-boring weevil (Figure 1a-b)

The curculionid, tentatively identified as *Gasteroclisus tricostalis* (Thunberg), appeared to be the most damaging of the stem borers associated with fireweed. Though only present at a third of sites surveyed, it was the most common and abundant of the coleopteran stem borers. Curculionidae were usually present in low numbers, typically one to two larvae

per stem with a mean (± SE) of 2.94 (± 0.29), although one plant contained 17 curculionid larvae. The high numbers of curculionid larvae occasionally found in single plants and the lack of stem-boring Curculionidae feeding on fireweed in Australia (Holtkamp & Hosking 1993; Harvey *et al*. 2015) suggest that Curculionidae may experience lower levels of parasitism in Australia and that release from their natural enemies could result in much higher numbers per plant in the invaded range. Impact assessments will be able to determine whether stem boring significantly affects the plant's growth and reproductive output and how many larvae per plant are required to achieve this.

Seasonal surveys revealed that Curculionidae were present throughout most of the year but were absent in August (winter). Abundance was also significantly lower in May and November. If the curculionids are not able to cause enough damage to fireweed plants during the months when larvae are present in the stems, more effective biocontrol may be achieved by using a combination of agents from the same or other guilds.

Curculionidae have been fairly successful in weed biological control. Some 21 species were released on 80 occasions against Asteraceae in the world's most active (in biological control) countries. Sixty nine of these releases achieved establishment (86%), of which six resulted in extensive impacts and 21 in considerable impacts (Chapter 3; Egli & Olckers 2017). However, many of these curculionids are capitulum feeders that were released against thistles and no capitulum-feeding curculionids were recorded in fireweed capitula. Stem-boring weevils have also been effective. Eight species have been deployed in 10 releases, with seven releases establishing and three causing considerable impact. Stem borers used against Asteraceae have high establishment success and were the second most effective feeding guild, causing extensive impacts in 9% of releases (Chapter 3; Egli & Olckers 2017). Similarly, an analysis by Crawley (1989) found that the most successful insect families in weed biological control were, in decreasing order, Dactylopiidae, Curculionidae, Chrysomelidae and Pyralidae.

All three lineages of Curculionidae associated with fireweed showed restriction to fireweed or to plants within the *S. madagascariensis* species complex. Adults have been sent for identification and will be genetically matched to larvae, thereby clarifying which species are likely to be host specific. The Curculionidae have thus been prioritised for further study.

Root-feeding flea beetle (Figure 1c-d)

The flea beetle, now confirmed as *Longitarsus basutoensis* Bechyné (Chrysomelidae: Alticinae), was seldom collected due to the initial sampling method. The larvae feed externally on the roots and remain in the soil when plants are uprooted, while the highly mobile adults also escape when plants are collected. This is likely the reason that their overall incidence and abundance was low and flea beetles were only collected in February and May during the seasonal survey (although some larvae were collected during the Ukulinga field trials in June). These results are, therefore, not an accurate indication of the flea beetle's incidence, abundance and phenology and more targeted sampling is required to determine this. Soil samples and sweep netting would be more effective collection methods for the larvae and adults, respectively.

Chrysomelidae have been used often in biological control of Asteraceae and are one of four families that have inflicted extensive damage (Chapter 3; Egli & Olckers 2017). A total of 18 species have been released on 27 occasions. Eleven of these releases established, of which two resulted in extensive damage and four in considerable damage. Only two of these were root feeders; both were *Longitarsus* species released for the biological control of *J. vulgaris* (Winston *et al*. 2014). Of the five releases undertaken against *J. vulgaris*, all established, two caused considerable damage, and two caused extensive damage. Although the establishment success of Chrysomelidae is low (41%), that of root feeders is high (79%). Twelve percent of root feeders have inflicted extensive impacts on their target weeds and 44% considerable impacts, higher than for any other feeding guild used against Asteraceae (Chapter 3; Egli & Olckers 2017).

Longitarsus species with root-feeding larvae have been very successful in the biocontrol program against *Jacobaea vulgaris* (= *Senecio jacobaea*) (Ireson & McLaren 2012). Because of this and the fact that no flea beetles have been recorded on fireweed in Australia (Holtkamp & Hosking 1993; Harvey *et al*. 2015), the flea beetle has been prioritised for further study.

Lepidopteran candidates

A few species of stem-boring and capitulum-feeding Lepidoptera are associated with fireweed. The results of these studies deemed the most important of these to be the stem-boring Tortricidae and capitulum-feeding Pyralidae.

Stem-boring moths (Figure 1e)

The stem-boring lepidopterans (Tortricidae), which appeared to comprise two species, were present in lower numbers than the curculionid, but were more widely distributed and may be able to tolerate a wider range of climatic conditions. As with the curculionid, numbers per plant were typically low although one plant had 11 lepidopteran larvae in the stems and another contained 14. Again, this indicates that despite generally low numbers, many lepidopteran larvae can occur within a single plant and it is possible that in the invaded range, higher numbers will be found per plant. Unfortunately, the presence of stem-boring Lepidoptera in the stems of fireweed plants in Australia (Holtkamp & Hosking 1993; Harvey *et al*. 2015) may aid in the recruitment of native parasitoids and thus reduce the lepidopteran's ability to build up high numbers.

Lepidoptera were not present in the stems throughout the year and were only recovered in February and May. They would, therefore, only be able to damage the plant during part of the year and if not enough damage is inflicted during this time, they may not be effective agents. If not effective alone, using the tortricid in combination with another stem-boring insect agent may provide a solution. The peak abundance of the Tortricidae overlaps with that of the curculionid, but not with that of the stem-boring Diptera (see below), and it may be best to combine the tortricid with the dipteran if necessary.

Tortricidae have been used more than any other family of Lepidoptera in the biological control of Asteraceae (Chapter 3; Egli & Olckers 2017). Seven species, of which three are stem borers, have been released. Six of the seven releases of stem borers resulted in establishment, of which three had extensive impacts and one considerable impact on the target weed. Tortricidae are one of four families released against Asteraceae that inflicted extensive impact on the target plant. The family Tortricidae, therefore, has a good track record in the biocontrol of Asteraceae (Winston *et al*. 2014), and the tortricid released for the biocontrol of *J. vulgaris* resulted in extensive and considerable damage in Australia and Canada, respectively (Ireson & McLaren 2012). One stem-boring lepidopteran lineage (presumably a tortricid) and one stem-boring and capitulum-feeding lepidopteran lineage (presumably a pterophorid) were both collected only on plants in the *S. madagascariensis* species complex. These insects are therefore worthy of further consideration.

Capitulum-feeding moth (Figure 1f)

The capitulum-feeding moth, tentatively identified as *Homeosoma stenotea* Hampson (Pyralidae), was not that widespread or abundant, occurring at fewer sites and in lower numbers than other capitulum feeders. The moth was, however, the most damaging of the capitulum feeders and a single larvae could destroy all achenes within a flower head. The pyralid has thus been prioritised as the most effective capitulum feeder on fireweed. If numbers are high enough in the invaded range, these insects have the potential to reduce reproductive output. However, the capitulum-feeding Lepidoptera found on fireweed in Australia (Holtkamp & Hosking 1993; Harvey *et al*. 2015) could result in competition and aid in recruiting parasitoids, thereby reducing the ability of the pyralid to reach high numbers and thus its effectiveness.

Pyralidae were only present in November and in very low numbers. While the lack of larvae throughout the rest of the year could be due to the insect's life cycle, it may also be that larvae were missed during sampling because the moth generally occurs in very low numbers. A later study, where plants were collected in June, revealed Lepidopteran larvae within the capitula, although in much lower numbers (mean of 0.067 per plant) than in any other surveys, suggesting that they are not common at that time of year. Its peak abundance is in contrast with the tephritid larvae, considered the second most damaging capitulum feeders. A combination of these two agents could help ensure that the plant's floral material is attacked consistently throughout the year.

Though no capitulum-feeding Lepidoptera in the family Pyralidae have been used in the biological control of Asteraceae (Chapter 3; Egli & Olckers 2017), stem-boring Pyralidae and flower-feeding Lepidoptera have been used. Although capitulum feeders have high establishment success (77%), they have been the least effective of the endophagous insects with only 6% causing extensive damage, 34% considerable damage, and 60% negligible damage. Pyralidae have not fared well in the biological control of Asteraceae. The only pyralid to have been deployed had no effect on the target weed in any of the three countries where it was released. Despite this, the capitulum-feeding pyralid should not be ruled out without further investigation as an analysis by Crawley (1989) ranked Pyralidae as the 4th most effective family used in weed biocontrol.

Dipteran candidates

Diptera was the most diverse and abundant of the endophagous insect orders collected on fireweed, but was also the least damaging. However, a few taxa may have potential as biocontrol agents.

Stem-boring flies

Stem-boring Agromyzidae and Tephritidae both showed a high incidence and low abundance on fireweed. As with the other stem borers, though abundances were typically low, occasionally over 20 dipteran larvae were recovered from a single plant, indicating that they do have the ability to occur in high numbers. The fact that they were recovered from many sites indicates that they may be adapted to a wider range of climatic conditions than species only present at a few sites. The tephritid in particular was widespread, occurring at 91% of sites sampled. However, dipteran larvae were less damaging than those of the stem-boring weevil and moth and are thus deemed as lower priority. Additionally, stem-boring Agromyzidae were recovered from fireweed in Australia (Holtkamp & Hosking 1993; Harvey *et al*. 2015), and the dipterans are thus likely to recruit native Australian parasitoids.

The stem-boring Tephritidae and Agromyzidae were present throughout the year, though agromyzid abundances peaked during May and August and that of the tephritid during May. This is when the stem-boring Curculionidae were least abundant. Though the stem-boring flies are less damaging, their mean abundance was higher than that of the curculionid and tortricid stem borers. Should the curculionid or tortricid be ineffective due to low numbers for part of the year, adding one of the dipterans as an additional biocontrol agent might ensure sustained damage on the plant as their peak abundance occurs at different times. No stem-boring Agromyzidae have been used in the biocontrol of Asteraceae, but three species of stem-boring Tephritidae have been released on 10 occasions (Chapter 3; Egli & Olckers 2017). All of these releases established and four caused considerable damage.

Neither of the two stem-boring dipteran lineages collected on fireweed were restricted to fireweed, suggesting that they are unsuitable for Australia. However, if they are specific to *Senecio* species they may be safe for release in Hawaii. As very few stem-boring dipterans associated with fireweed were successfully sequenced, additional sequencing of stem-boring dipterans may reveal more lineages, some of which may be host specific.

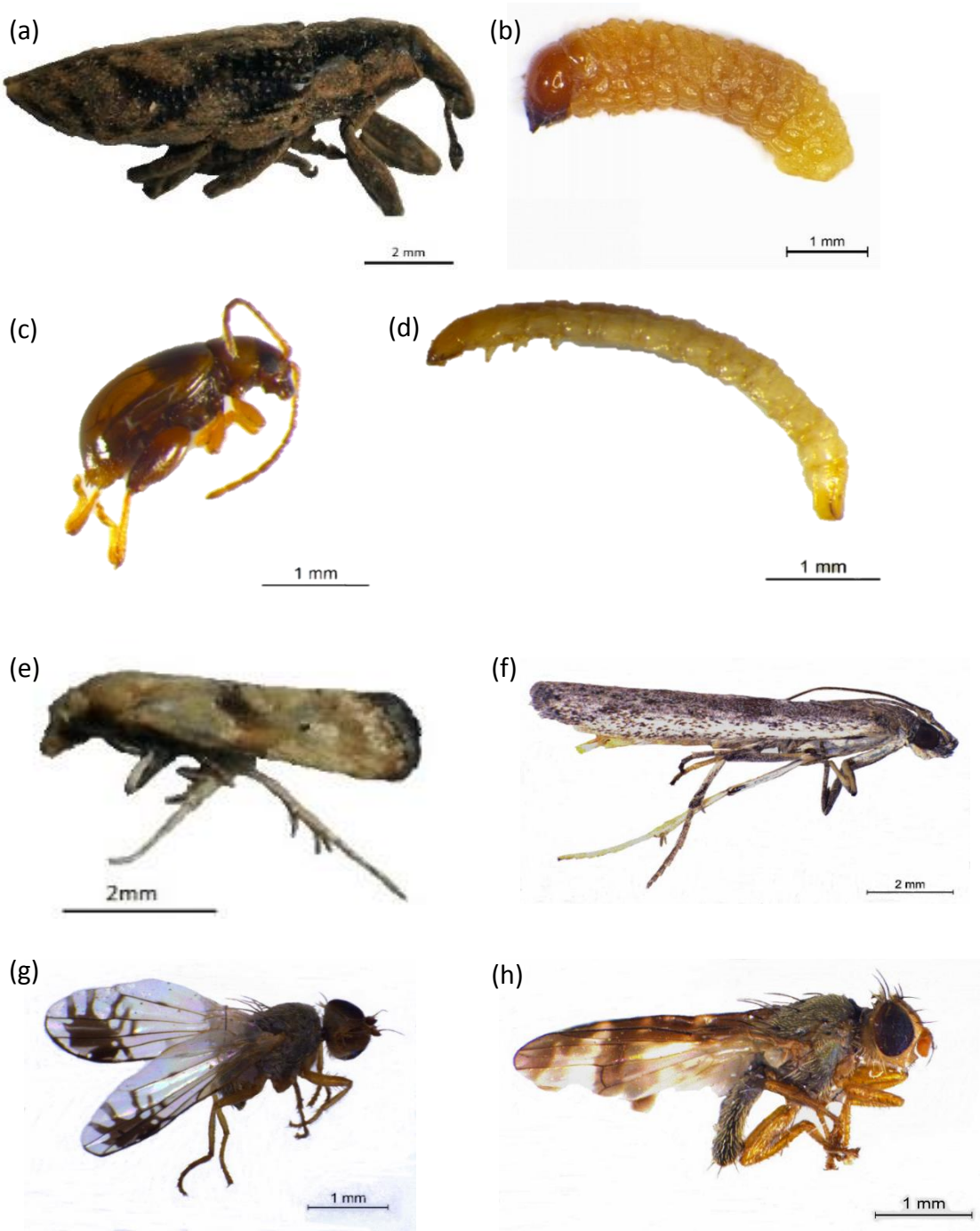

Figure 1: Candidate agents for the biological control of fireweed: (a) adult and (b) larva of the stem-boring weevil; (c) adult and (d) larva of the root-feeding flea beetle; (e) stem-boring tortricid; (f) capitulum-feeding pyralid; (g) and (h) capitulum-feeding tephritids.

Capitulum-feeding flies (Figure 1g-h)

The capitulum-feeding Tephritidae, which comprise more than one species, were present at half of the sites sampled and in higher numbers than the pyralid. They were less damaging than the pyralid, but more damaging than the other capitulum-feeding insects (e.g.

Agromyzidae). Where Tephritidae were present in the capitula, only a single larva was present which destroyed most of the seeds within the capitulum. If, at high numbers, this is enough to significantly reduce reproductive output, tephritids may have value as biocontrol agents. However, native Tephritidae were recorded in the capitula of fireweed in Australia (Holtkamp & Hosking 1993; Harvey *et al*. 2015) and if one is released for biological control, it seems likely to recruit Australian tephritid parasitoids.

Capitulum-feeding tephritid numbers were highest in May when capitulum numbers were highest and were absent in February when lepidopterans were present. If either insect is unable to control the plant because of its absence for part of the year, using these two insects in combination could result in higher levels of floral damage. The family Tephritidae has been fairly successful in the biocontrol of Asteraceae (Chapter 3; Egli & Olckers 2017). Establishment success is high (73% of releases) and eight releases resulted in considerable damage. Both lineages found on fireweed were restricted to the *S. madagascariensis* species complex. While not a priority taxon, the tephritids may have some biocontrol value as they are the second most damaging capitulum feeders.

Potential efficacy of biological control of fireweed

Fireweed contains a number of pyrrolizidine alkaloids. These types of chemicals typically defend against generalist insects which are present in the plant's invasive range, but are used as host-finding and oviposition cues by specialist enemies that are largely absent from the invasive range (Muller-Scharer *et al*. 2004; Joshi & Vrieling 2005; Doorduin & Vrieling 2011). Generalists and specialists, therefore, inflict opposing selection pressures on PA concentrations (van der Meijden 1996). The Shifting Defence Hypothesis (SDH) predicts that plants in the invasive range will have higher concentrations of PA's, as the result of a change in selection pressure due to a shift in insect assemblages from one dominated by specialists in the native range to one dominated by generalists in the invasive range (Muller-Scharer *et al*. 2004; Inderjit 2012). A greenhouse trial compared the concentrations of PAs from across the native range with those at the point of introduction and the invasion fronts in the invasive range in Australia (Harvey *et al*. unpublished). As predicted by the SDH, plants from invasive populations had higher concentrations of PAs than those from native populations (Harvey *et al*. unpublished). Within the invaded range, fireweed populations at the point of introduction (where it has existed for longer and recruited more endophagous (specialist) insects)

displayed lower alkaloid concentrations than at the invasion fronts, where the plant has existed for a shorter period of time and has recruited fewer endophagous insects (Harvey *et al*. 2013, unpublished).

Changes in PA concentration can have implications for biological control as the SDH suggests that specialist biocontrol agents will be more attracted to plants with higher alkaloid concentrations (Muller-Scharer *et al*. 2004). While this has been found for some specialist species (Joshi & Vrieling 2005) it has not proved true for all (Macel *et al*. 2002). An open-field trial conducted during this study found that while Australian plants have a higher concentration of alkaloids, there was no difference in the insect communities between native and invasive fireweed populations, no significant relationship between insect communities and alkaloid concentration, and no difference in natural enemy impact on plant biomass between native and invasive populations. Comparing the mean abundance of the most important insects (i.e. that were prioritised for biological control) between the South African and Australian plants revealed little to no difference and indicates that while none of the potential agents are more attracted to invasive (Australian) fireweed, they are also not negatively affected by increases in defensive chemicals. The increased concentrations of PA's in Australian fireweed should, therefore, not reduce the potential efficacy of biological control.

The potential efficacy of biological control was also evaluated through an open-field enemy-exclusion trial carried out in KwaZulu-Natal (Harvey *et al*. unpublished). The impacts of insect herbivores, fungal pathogens and intra-specific plant competition on the biomass and reproductive output of fireweed was tested using pesticide treatments that excluded natural enemies at different plant densities (Harvey *et al*. unpublished). In plots where insects were excluded, plants displayed a higher mean biomass and produced more flowers than in the control plots, at both low and high plant densities (Harvey *et al*. unpublished). Fireweed populations are, therefore, to some extent, negatively affected by natural enemies in the native range. A study comparing the effects of competition by South African grasses and Australian grasses on fireweed found no negative effects of grass competition (from either group) on fireweed biomass, although simulated defoliation had an effect (Fynn 2016). Natural enemy impact (albeit simulated) thus seemed to have more of an effect on fireweed biomass than grass competition. The results of these experiments suggest that biological control should have an impact on fireweed populations in Australia.

Conclusions and further research

The results of this study have highlighted a number of insects that could potentially be used for the biocontrol of fireweed in Australia, but also Hawaii. Genetic sequencing of the immature (endophagous) stages has strongly suggested that several of the insects associated with fireweed are host specific, but more intensive host-specificity testing is required for confirmation. Together with other native range studies conducted as part of this collaboration between the CSIRO and UKZN, this research has also indicated that biological control may be effective.

Currently, the focus of future research is on the stem-boring weevil (probably *Gasteroclisus tricostalis*), the stem-boring moth(s) (unknown species), and the root-feeding flea beetle (*Longitarsus basutoensis*). Adults of all taxa have been sent for identification, with confirmation of identity for some still pending. Identified adult weevil and moth specimens (but also adults of other stem-boring and capitulum-feeding taxa) can then be sequenced and matched to the larvae collected in the host-range surveys. This will clarify exactly which weevils and moths are host specific in the field. As no host-range data for the flea beetle were recorded during this study, future surveys that involve the sweep-netting of *Senecio* species across KwaZulu-Natal, as well as the collection of soil samples around the roots to extract the larvae, will give an indication of whether it is host specific. Open-field trials or laboratory-based host-range tests that include fireweed's closest Australian native relative, *Senecio pinnatifolius*, can be carried out in the native range (if permits are granted) to screen which insects are most likely to be safe for release in Australia. These can then be imported into quarantine in Australia for further host-range testing.

Though the seasonal surveys have given some indication of when the prioritized taxa and their immature stages are present, thorough studies of their life cycles are required. The development of culturing techniques (notably in the case of *L. basutoensis*) will be important to establish laboratory cultures that can be used for host-range testing, both in South Africa and in Australia. Studies on the impact of these insects on the survival, growth, and reproductive output of fireweed will also indicate whether they are likely to cause significant impacts in the invaded range. Although the open-field experiment found no effect of PA concentration on insect associations, further studies examining the preference and performance of the prioritized insect species on native and invasive fireweed populations would be useful to confirm equivalent susceptibility. Climate matching using programmes

such as Climex or MaxEnt may also provide some insight into whether the insects are likely to establish in Australia. For this, data on the insects' thermal limits, determined by laboratory trials or presence/absence data from around KwaZulu-Natal, would need to be collected.

Future research steps in this biological control programme are to develop culturing techniques and carry out host-range experiments in the laboratory and field for the stem-boring weevil, stem-boring moths and root-feeding flea beetle, as these have been assigned the highest priority. Should none of these be considered suitable for release in Australia, similar research to that described above can be carried out on the remaining candidates, namely the stem-boring agromyzid and tephritid, and the capitulum-feeding pyralid and tephritid(s). It is likely that at least one of these potential agents will be suitable for release in Australia, and certainly a number of them will be suitable for release in Hawaii, which has less stringent host-specificity requirements in this particular situation. The prospects for the biocontrol of fireweed in its invaded countries are thus promising, more so in countries where there are no native *Senecio* species or the genus is poorly represented.

General recommendations

Based on the results of this study, the following recommendations are made in the context of "best practices" for weed biocontrol programmes:

(1) Studies in the countries (or centre) of origin of invasive plants should be pursued as much as possible. Although extended studies, as opposed to short collection trips, may be costly, the benefits are considerable. Activities such as seasonal studies on the agents' population dynamics, surveys on related plant species to determine the agents' native host range, open-field trials to determine the susceptibility/resistance of plants from the invaded range to the agents, and natural-enemy exclusion trials can add substantial value to biocontrol programmes.

(2) Genetic sequencing of insect immature stages associated with a range of potential host plants in the weed's native range can provide an early indication of the host range of candidate agents, particularly since the presence of immature stages on a plant indictes host utilization. This approach could save time that would otherwise be spent on host-range tests and ensure that unsuitable agents (i.e. those recovered on several host plants) are rejected at an early stage of the

programme. Besides matching different insect species with their host plants, sequencing techniques can also reveal the presence of additional (or cryptic) species that were not detected during earlier surveys.

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APPENDIX: SUPPLEMENTARY MATERIAL FOR CHAPTER 3

Insects that were released as biological control agents against invasive species of Asteraceae in Australia, Canada, Hawaii (USA), New Zealand, South Africa and the mainland USA (excluding Hawaii) and, and the outcomes thereof. Agent impacts are explained in the Materials and methods, Chapter 3. Data were retrieved from Winston *et al*. (2014).

Plant species Country of origin				
Insect agent species (Order: Family)	Feeding guild	Country	Establishment	Impact of agent
		Tribe Anthemideae		
***Tripleurospermum inodorum* (L.) Sch. Bip.** Eurasia				
Microplontus edentulus (Schultze) Coleoptera: Curculionidae	Stem borer	Canada	Yes	None
Omphalapion hookerorum (Kirby) Coleoptera: Brentidae	Flower & seed feeder	Canada	Yes	Considerable
Rhopalomyia tripleurospermi Shukravá & Hinz Diptera: Cecidomyiidae	Flower galler	Canada	Yes	Considerable
		Tribe Astereae		
***Baccharis halimifolia* L.** North America				
Anacassis fuscata (Klug) Coleoptera: Chrysomelidae	Foliage feeder	Australia	No	N/A
Anacassis phaeopoda Buzzi Coleoptera: Chrysomelidae	Foliage feeder	Australia	No	N/A
Aristotelia ivae Busck Lepidoptera: Gelechiidae	Foliage feeder	Australia	Yes	Negligible

Bucculatrix ivella Busck Lepidoptera: Bucculatricidae	Leaf miner	Australia	Yes	Negligible
Heilipodus intricatus (Boheman) Coleoptera: Curculionidae	Stem borer	Australia	No	N/A
Hellinsia balanotes (Meyrick) Lepidoptera: Pterophoridae	Stem borer	Australia	Yes	Negligible
Lioplacis elliptica Stål Coleoptera: Chrysomelidae	Foliage feeder	Australia	No	N/A
Lorita baccharivora Pogue Lepidoptera: Tortricidae	Foliage feeder	Australia	No	N/A
Megacyllene mellyi (Chevrolat) Coleoptera: Cerambycidae	Stem borer	Australia	Yes	Considerable
Metallactus nigrofasciatus Suffrian Coleoptera: Chrysomelidae	Foliage feeder	Australia	No	N/A
Metallactus patagonicus Suffrian Coleoptera: Chrysomelidae	Foliage feeder	Australia	No	N/A
Rhopalomyia californica Felt Diptera: Cecidomyiidae	Stem tip galler	Australia	Yes	Considerable
Trirhabda bacharidis (Weber) Coleoptera: Chrysomelidae	Foliage feeder	Australia	Yes	Negligible
Tribe Calenduleae				
Chrysanthemoides monilifera* (L.) Norl. subsp. *monilifera Southern Africa				
Chrysolina fasciata (De Geer) Coleoptera: Chrysomelidae	Foliage feeder	Australia	No	N/A
Chrysolina scotti Daccordi Coleoptera: Chrysomelidae	Foliage feeder	Australia	No	N/A
Chrysolina sp. B Coleoptera: Chrysomelidae	Foliage feeder	Australia	No	N/A

Comostolopsis germana Prout Lepidoptera: Geometridae	Leaf roller	Australia	No	N/A
Mesoclanis magnipalpis Bezzi Diptera: Tephritidae	Flower head feeder	Australia	No	N/A
Mesoclanis polana Munro Diptera: Tephritidae	Flower head feeder	Australia	No	N/A
Tortrix sp. Lepidoptera: Tortricidae	Leaf roller	Australia New Zealand	No Yes	N/A Negligible
***Chrysanthemoides monilifera* (L.) Norl. subsp. *rotundata* (DC.) Norl.** Southern Africa				
Cassida sp. 3 Coleoptera: Chrysomelidae	Foliage feeder	Australia	Yes	Negligible
Chrysolina scotti Daccordi Coleoptera: Chrysomelidae	Foliage feeder	Australia	No	N/A
Chrysolina sp. B Coleoptera: Chrysomelidae	Foliage feeder	Australia	No	N/A
Comostolopsis germana Prout Lepidoptera: Geometridae	Leaf roller	Australia	Yes	Considerable
Mesoclanis magnipalpis Bezzi Diptera: Tephritidae	Flower head feeder	Australia	No	N/A
Mesoclanis polana Munro Diptera: Tephritidae	Flower head feeder	Australia	Yes	Considerable
Tortrix sp. Lepidoptera: Tortricidae	Leaf roller	Australia	Yes	Negligible
Tribe Cardueae				
***Carduus acanthoides* L.** Eurasia, N. Africa				
Rhinocyllus conicus (Frölich) Coleoptera: Curculionidae	Flower & seed feeder	Canada New Zealand	Yes AH*, Yes	Negligible Unknown

		USA	Yes	Negligible
Trichosirocalus horridus (Panzer)	Foliage feeder	Canada	Yes	Negligible
Coleoptera: Curculionidae		USA	Yes	Considerable
Urophora solstitialis (L.)	Flower galler	Canada	Yes	Negligible
Diptera: Tephritidae		USA	No	N/A
***Carduus nutans* L. (includes different subspecies)**				
Eurasia, N. Africa				
Cheilosia grossa (Fallén)	Stem borer	USA	No	N/A
Diptera: Syrphidae				
Psylliodes chalcomera (Illiger)	Foliage feeder	USA	No	N/A
Coleoptera: Chrysomelidae				
Rhinocyllus conicus (Frölich)	Flower & seed feeder	Australia	Yes	Considerable
Coleoptera: Curculionidae		Canada	Yes	Extensive
		New Zealand	Yes	Considerable
		USA	Yes	Considerable
Trichosirocalus horridus (Panzer)	Foliage feeder	Canada	Yes	Negligible
Coleoptera: Curculionidae		New Zealand	Yes	Considerable
		USA	Yes	Considerable
Trichosirocalus mortadelo Alonso-Zarazaga & Sánchez-Ruiz	Foliage feeder	Australia	Yes	Extensive
Coleoptera: Curculionidae				
Urophora solstitialis (L.)	Flower galler	Australia	Yes	Considerable
Diptera: Tephritidae		Canada	Yes	Unknown
		New Zealand	Yes	Negligible
		USA	No	N/A
***Carduus pycnocephalus* L.**				
Eurasia, N. Africa				
Cheilosia grossa (Fallén)	Stem borer	USA	Yes	Considerable
Diptera: Syrphidae				

Rhinocyllus conicus (Frölich)	Flower & seed feeder	New Zealand	AH*, Yes	Negligible
Coleoptera: Curculionidae		USA	Yes	Considerable
Trichosirocalus horridus (Panzer)	Foliage feeder	New Zealand	Yes	Unknown
Coleoptera: Curculionidae		USA	Yes	Considerable
***Carduus tenuiflorus* Curt.**				
Europe, N. Africa				
Cheilosia grossa (Fallén)	Stem borer	USA	Yes	Negligible
Diptera: Syrphidae				
Rhinocyllus conicus (Frölich)	Flower & seed feeder	New Zealand	AH*, Yes	Negligible
Coleoptera: Curculionidae		USA	AH*, Yes	Considerable
Trichosirocalus horridus (Panzer)	Foliage feeder	New Zealand	AH*, Yes	Unknown
Coleoptera: Curculionidae		USA	AH*, Yes	Considerable
***Centaurea cyanus* L.**				
Eurasia				
Chaetorellia australis Héring	Seed feeder	USA	AH*, Yes	Negligible
Diptera: Tephritidae				
***Centaurea diffusa* Lam.**				
Eurasia				
Agapeta zoegana (L.)	Root feeder	Canada	Yes	Negligible
Lepidoptera: Tortricidae		USA	AH*, Yes	Negligible
Bangasternus fausti (Reitter)	Seed feeder	USA	Yes	Negligible
Coleoptera: Curculionidae				
Chaetorellia acrolophi White & Marquardt	Flower head feeder	USA	Yes	Negligible
Diptera: Tephritidae				
Cyphocleonus achates (Fåhraeus)	Root feeder	Canada	AH*, Yes	Negligible
Coleoptera: Curculionidae		USA	AH*, Yes	Negligible
Larinus minutus Gyllenhal	Flower head feeder	Canada	Yes	Extensive
Coleoptera: Curculionidae		USA	Yes	Extensive

Larinus obtusus Gyllenhal	Seed feeder	USA	AH*, Yes	Negligible
Coleoptera: Curculionidae				
Metzneria paucipunctella Zeller	Flower head feeder	Canada	Yes	None
Lepidoptera: Gelechiidae		USA	AH*, Yes	Negligible
Pelochrista medullana (Staudinger)	Root borer	Canada	No	N/A
Lepidoptera: Tortricidae		USA	Yes	Negligible
Pterolonche inspersa Staudinger	Root feeder	Canada	Yes	Unknown
Lepidoptera: Pterolonchidae		USA	Yes	None
Sphenoptera jugoslavica Obenberger	Root feeder	Canada	Yes	Considerable
Coleoptera: Buprestidae		USA	Yes	Considerable
Terellia virens (Loew)	Seed feeder	USA	Yes	Unknown
Diptera: Tephritidae				
Urophora affinis (Frauenfeld)	Flower head galler	Canada	Yes	Considerable
Diptera: Tephritidae		USA	Yes	Considerable
Urophora quadrifasciata (Meigen)	Flower head feeder	Canada	Yes	Negligible
Diptera: Tephritidae				
***Centaurea jacea* L. nothosubsp. *pratensis* (W.D.J. Koch) Čelak**				
Europe				
Bangasternus fausti (Reitter)	Seed feeder	USA	AH*, No	N/A
Coleoptera: Curculionidae				
Cyphocleonus achates (Fåhraeus)	Root feeder	USA	AH*, No	N/A
Coleoptera: Curculionidae				
Larinus minutus Gyllenhal	Flower head feeder	USA	AH*, Yes	Negligible
Coleoptera: Curculionidae				
Larinus obtusus Gyllenhal	Seed feeder	USA	AH*, Yes	Negligible
Coleoptera: Curculionidae				
Sphenoptera jugoslavica Obenberger	Root feeder	USA	AH*, No	N/A
Coleoptera: Buprestidae				
Urophora quadrifasciata (Meigen)	Flower head feeder	Canada	Yes	Negligible
Diptera: Tephritidae				

Centaurea jacea* L. subsp. *jacea				
Europe				
Larinus obtusus Gyllenhal	Seed feeder	USA	AH*, Yes	Considerable
Coleoptera: Curculionidae				
***Centaurea jacea* L. subsp. *nigra* (L.) Bonnier & Layens**				
Europe				
Larinus obtusus Gyllenhal	Seed feeder	USA	AH*, Yes	Considerable
Coleoptera: Curculionidae				
***Centaurea solstitialis* L.**				
Eurasia, N. Africa				
Bangasternus orientalis (Capiomont)	Flower head feeder	USA	Yes	Negligible
Coleoptera: Curculionidae				
Chaetorellia australis Héring	Seed feeder	USA	Yes	Negligible
Diptera: Tephritidae				
Eustenopus villosus (Boheman)	Seed feeder	USA	Yes	Considerable
Coleoptera: Curculionidae				
Larinus curtus Hochhut	Flower head feeder	USA	Yes	Negligible
Coleoptera: Curculionidae				
Urophora jaculata Rondani	Flower head feeder	USA	No	N/A
Diptera: Tephritidae				
Urophora sirunaseva (Héring)	Flower galler	USA	Yes	Negligible
Diptera: Tephritidae				
***Centaurea stoebe* L. sens. lat. (= *Centaurea maculosa* Lam.)**				
Eurasia				
Agapeta zoegana (L.)	Root feeder	Canada	Yes	Considerable
Lepidoptera: Tortricidae		USA	Yes	Negligible
Bangasternus fausti (Reitter)	Seed feeder	USA	AH*, Yes	Negligible
Coleoptera: Curculionidae				

Chaetorellia acrolophi White & Marquardt	Flower head feeder	Canada	Unknown	N/A
Diptera: Tephritidae		USA	Yes	Negligible
Cyphocleonus achates (Fåhraeus)	Root feeder	Canada	Yes	Considerable
Coleoptera: Curculionidae		USA	Yes	Considerable
Larinus minutus Gyllenhal	Flower head feeder	Canada	Yes	Negligible
Coleoptera: Curculionidae		USA	AH*, Yes	Considerable
Larinus obtusus Gyllenhal	Seed feeder	Canada	Yes	Considerable
Coleoptera: Curculionidae		USA	Yes	Considerable
Metzneria paucipunctella Zeller	Flower head feeder	Canada	Yes	Negligible
Lepidoptera: Gelechiidae		USA	Yes	Negligible
Pelochrista medullana (Staudinger)	Root borer	Canada	No	N/A
Lepidoptera: Tortricidae		USA	Yes	Negligible
Pterolonche inspersa Staudinger	Root feeder	Canada	No	N/A
Lepidoptera: Pterolonchidae		USA	AH*, No	N/A
Sphenoptera jugoslavica Obenberger	Root feeder	Canada	AH*, Yes	Negligible
Coleoptera: Buprestidae		USA	AH*, Yes	Negligible
Terellia virens (Loew)	Seed feeder	Canada	No	N/A
Diptera: Tephritidae		USA	Yes	Negligible
Urophora affinis (Frauenfeld)	Flower head feeder	Canada	Yes	Negligible
Diptera: Tephritidae		USA	Yes	Considerable
Urophora quadrifasciata (Meigen)	Flower head feeder	Canada	Yes	Negligible
Diptera: Tephritidae				
***Centaurea virgata* Lam. subsp. *squarrosa* (Boiss.) Gugler**				
Eurasia, Asia M.				
Agapeta zoegana (L.)	Root feeder	USA	AH*, unconfirmed	N/A
Lepidoptera: Tortricidae				
Bangasternus fausti (Reitter)	Seed feeder	USA	AH*, Yes	Extensive
Coleoptera: Curculionidae				
Cyphocleonus achates (Fåhraeus)	Root feeder	USA	AH*, Yes	Unknown
Coleoptera: Curculionidae				

Larinus minutus Gyllenhal Coleoptera: Curculionidae	Flower head feeder	USA	AH*, No	N/A
Pterolonche inspersa Staudinger Lepidoptera: Pterolonchidae	Root feeder	USA	AH*, No	N/A
Sphenoptera jugoslavica Obenberger Coleoptera: Buprestidae	Root feeder	USA	AH*, Yes	Considerable
Terellia virens (Loew) Diptera: Tephritidae	Seed feeder	USA	AH*, No	N/A
Urophora affinis (Frauenfeld) Diptera: Tephritidae	Flower head feeder	USA	AH*, Yes	Negligible
***Cirsium arvense* (L.) Scop.** Eurasia				
Altica carduorum Guérin-Méneville Coleoptera: Chrysomelidae	Foliage feeder	Canada	No	N/A
		New Zealand	No	N/A
		USA	No	N/A
Cassida rubiginosa O.F. Müller Coleoptera: Chrysomelidae	Foliage feeder	New Zealand	Yes	Unknown
Ceratapion onopordi Kirby Coleoptera: Apionidae	Stem borer	New Zealand	Unconfirmed	N/A
Hadroplontus litura (F.) Coleoptera: Curculionidae	Stem borer	Canada	Yes	Negligible
		New Zealand	No	N/A
		USA	Yes	Negligible
Larinus carlinae (Olivier) Coleoptera: Curculionidae	Flower head feeder	Canada	Yes	Negligible
		USA	Yes	None
Lema cyanella (L.) Coleoptera: Chrysomelidae	Foliage feeder	Canada	No	N/A
		New Zealand	Yes	None
Rhinocyllus conicus (Frölich) Coleoptera: Curculionidae	Flower & seed feeder	Canada	AH*, Yes	Negligible
		New Zealand	AH*, Yes	Negligible
		USA	AH*, Yes	Negligible

Urophora cardui (L.)	Stem galler	Canada	Yes	Negligible
Diptera: Tephritidae		New Zealand	Yes	Unknown
		USA	Yes	Negligible
***Cirsium palustre* (L.) Scop.**				
Eurasia				
Rhinocyllus conicus (Frölich)	Flower & seed feeder	Canada	AH*, Yes	None
Coleoptera: Curculionidae		New Zealand	AH*, Yes	Negligible
Trichosirocalus horridus (Panzer)	Foliage feeder	Canada	AH*, Yes	Unknown
Coleoptera: Curculionidae		New Zealand	Yes	Unknown
***Cirsium vulgare* (Savi) Ten.**				
Eurasia, N. Africa				
Cheilosia grossa (Fallén)	Stem borer	USA	Yes	Negligible
Diptera: Syrphidae				
Rhinocyllus conicus (Frölich)	Flower & seed feeder	Australia	Yes	Unknown
Coleoptera: Curculionidae		Canada	AH*, Yes	Unknown
		South Africa	Yes	Negligible
		USA	AH*, Yes	Negligible
Trichosirocalus horridus (Panzer)	Foliage feeder	Australia	AH*, Yes	Unknown
Coleoptera: Curculionidae		Canada	AH*, Yes	Unknown
		New Zealand	Yes	Unknown
		USA	AH*, Yes	Negligible
Urophora stylata (Fabricius)	Seed galler	Australia	Yes	Considerable
Diptera: Tephritidae		Canada	Yes	Considerable
		New Zealand	Yes	Unknown
		South Africa	No	N/A
		USA	Yes	Considerable

***Onopordum acanthium* L.**				
Eurasia, N. Africa				
Rhinocyllus conicus (Frölich)	Flower & seed feeder	Canada	AH*, Yes	Unknown
Coleoptera: Curculionidae		USA	AH*, No	N/A
Trichosirocalus horridus (Panzer)	Foliage feeder	Canada	Yes	Unknown
Coleoptera: Curculionidae		USA	AH*, unconfirmed	N/A
***Onopordum acaulon* L.**				
Eurasia, N. Africa				
Eublemma amoena (Hübner)	Leaf miner	Australia	No	N/A
Lepidoptera: Erebidae				
Larinus latus Herbst	Flower head feeder	Australia	Unknown	N/A
Coleoptera: Curculionidae				
***Onopordum* spp. (complex of parent & hybrid forms of *O. acanthium* L. and *O. illyricum* L. in Australia**				
Eurasia, N. Africa				
Botanophila spinosa Rondani	Foliage feeder	Australia	No	N/A
Diptera: Anthomyiidae				
Eublemma amoena (Hübner)	Leaf miner	Australia	Yes	Considerable
Lepidoptera: Erebidae				
Larinus latus Herbst	Flower head feeder	Australia	Yes	Considerable
Coleoptera: Curculionidae				
Lixus cardui Olivier	Stem borer	Australia	Yes	Considerable
Coleoptera: Curculionidae				
Tephritis postica Loew	Flower head feeder	Australia	No	N/A
Diptera: Tephritidae				
Trichosirocalus briesei Alonso-Zarazaga & Sánchez-Ruiz	Crown borer	Australia	Yes	Considerable
Coleoptera: Curculionidae				

Urophora terebrans (Loew) Diptera: Tephritidae	Flower head feeder	Australia	No	N/A
***Rhaponticum repens* (L.) Hidalgo** Central Asia				
Aulacidea acroptilonica Tyurebaev Hymenoptera: Cynipidae	Shoot galler	Canada USA	Yes Yes	Unknown Unknown
Jaapiella ivannikovi Fedotova Diptera: Cecidomyiidae	Shoot galler	Canada USA	Yes Yes	Unknown Unknown
***Silybum marianum* (L.) Gaertn.** Europe, N. Africa				
Rhinocyllus conicus (Frölich) Coleoptera: Curculionidae	Flower & seed feeder	Australia South Africa USA	Yes AH*, No Yes	Negligible N/A None
Tribe Cichorieae				
***Chondrilla juncea* L.** Eurasia				
Bradyrrhoa gilveolella (Treitschke) Lepidoptera: Pyralidae	Root feeder	Australia Canada USA	No Yes Yes	N/A Unknown Unknown
Cystiphora schmidti (Rübsaamen) Diptera: Cecidomyiidae	Leaf & stem galler	Australia USA	Yes Yes	Considerable Negligible
***Pilosella aurantiaca* (L.) F.W. Schultz & Sch. Bip.** Europe				
Aulacidea subterminalis Niblett Hymenoptera: Cynipidae	Leaf & stolon galler	Canada USA	Unknown Unknown	Unknown Unknown
***Pilosella flagellaris* (Willd.) Arv.-Touv.** Europe				
Aulacidea subterminalis Niblett Hymenoptera: Cynipidae	Leaf & stolon galler	Canada	Unknown	Unknown

***Pilosella officinarum* Vaill.**				
Eurasia				
Aulacidea subterminalis Niblett	Leaf & stolon galler	New Zealand	Yes	Negligible
Hymenoptera: Cynipidae				
Cheilosia psilophthalma (Becker)	Foliage feeder	New Zealand	No	N/A
Diptera: Syrphidae				
Cheilosia urbana (Meigen)	Root feeder	New Zealand	No	N/A
Diptera: Syrphidae				
Macrolabis pilosellae (Binnie)	Leaf & stolon galler	New Zealand	Yes	Negligible
Diptera: Cecidomyiidae				
Oxyptilus pilosellae Zeller	Foliage feeder	New Zealand	No	N/A
Lepidoptera: Pterophoridae				
***Sonchus arvensis* L.**				
Eurasia				
Cystiphora sonchi (Bremi)	Leaf galler	Canada	Yes	Negligible
Diptera: Cecidomyiidae				
Liriomyza sonchi Hendel	Leaf miner	Canada	No	N/A
Diptera: Agromyzidae				
Tephritis dilacerata (Loew)	Flower head feeder	Canada	No	N/A
Diptera: Tephritidae				
Tribe Eupatorieae				
***Ageratina adenophora* (Spreng.) R.M. King & H. Rob.**				
Mexico				
Procecidochares utilis Stone	Stem galler	Australia	Yes	Negligible
Diptera: Tephritidae		Hawaii	Yes	Considerable
		New Zealand	Yes	Negligible
		South Africa	Yes	Considerable
Xanthaciura connexionis Benjamin	Flower head feeder	Hawaii	No	N/A
Diptera: Tephritidae				

***Ageratina riparia* (Regel) R.M. King & H. Rob.**				
Mexico				
Oidaematophorus beneficus Yano & Heppner	Foliage feeder	Hawaii	Yes	Considerable
Lepidoptera: Pterophoridae				
Procecidochares alani Steyskal	Stem galler	Australia	Yes	Negligible
Diptera: Tephritidae		Hawaii	Yes	Considerable
		New Zealand	Yes	Considerable
Xanthaciura connexionis Benjamin	Flower head feeder	Hawaii	No	N/A
Diptera: Tephritidae				
***Chromolaena odorata* (L.) R.M. King & H. Rob.**				
N., C. & S. America				
Calycomyza eupatorivora Spencer	Leaf miner	South Africa	Yes	Negligible
Diptera: Agromyzidae				
Lixus aemulus Petri	Stem borer	South Africa	Unknown	N/A
Coleoptera: Curculionidae				
Pareuchaetes aurata aurata (Butler)	Foliage feeder	South Africa	No	N/A
Lepidoptera: Erebidae				
Pareuchaetes insulata (Walker)	Foliage feeder	South Africa	Yes	Considerable
Lepidoptera: Erebidae				
Pareuchaetes pseudoinsulata Rego Barros	Foliage feeder	South Africa	No	N/A
Lepidoptera: Erebidae				
Tribe Heliantheae				
***Ambrosia artemisiifolia* L.**				
North America				
Epiblema strenuana (Walker)	Stem galler	Australia	AH*, Yes	Extensive
Lepidoptera: Tortricidae				
Stobaera concinna (Stäl)	Sap sucker	Australia	AH*, Yes	Negligible
Hemiptera: Delphacidae				

Zygogramma bicolorata Pallister Coleoptera: Chrysomelidae	Foliage feeder	Australia	AH*, Yes	Considerable
Zygogramma suturalis (F.) Coleoptera: Chrysomelidae	Foliage feeder	Australia	No	N/A
***Parthenium hysterophorus* (L.)** N., C. & S. America				
Bucculatrix parthenica Bradley Lepidoptera: Bucculatricidae	Leaf miner	Australia	Yes	Negligible
Carmenta sp. nr. *ithacae* (Beutenmüller) Lepidoptera: Sesiidae	Root feeder	Australia	Yes	Negligible
Conotrachelus albocinereus Fiedler Coleoptera: Curculionidae	Stem galler	Australia	Yes	Negligible
Epiblema strenuana (Walker) Lepidoptera: Tortricidae	Stem galler	Australia	Yes	Extensive
Listronotus setosipennis (Hustache) Coleoptera: Curculionidae	Stem borer	Australia	Yes	Considerable
Platphalonidia mystica (Razowski & Becker) Lepidoptera: Tortricidae	Stem borer	Australia	Yes	Negligible
Smicronyx lutulentus Dietz Coleoptera: Curculionidae	Seed feeder	Australia	Yes	Considerable
Stobaera concinna (Stäl) Hemiptera: Delphacidae	Sap sucker	Australia	Yes	Unknown
Zygogramma bicolorata Pallister Coleoptera: Chrysomelidae	Foliage feeder	Australia	Yes	Considerable
***Xanthium strumarium* L. sens. lat. (includes several species)** N., C. & S. America				
Epiblema strenuana (Walker) Lepidoptera: Tortricidae	Stem galler	Australia	AH*, Yes	Negligible

Euaresta aequalis Loew	Seed feeder	Australia	Yes	None
Diptera: Tephritidae				
Mecas cana saturnina (LeConte)	Stem borer	Australia	Unknown	N/A
Coleoptera: Cerambycidae				
Nupserha vexator (Pascoe)	Stem borer	Australia	Yes	Negligible
Coleoptera: Cerambycidae				
Tribe Inuleae				
***Pluchea carolinensis* (Jacq.) G. Don**				
Tropical America				
Acinia picturata (Snow)	Flower head feeder	Hawaii	Yes	Negligible
Diptera: Tephritidae				
Dicomeris aenigmatica (Clarke)	Foliage feeder	Hawaii	Yes	None
Lepidoptera: Gelechiidae				
Tribe Senecioneae				
***Jacobaea vulgaris* Gaertn.**				
Eurasia, N. Africa				
Botanophila jacobaeae (Hardy)	Seed feeder	Australia	No	N/A
Diptera: Anthomyiidae		New Zealand	Yes	Negligible
Botanophila seneciella (Meade)	Seed feeder	Canada	Yes	Negligible
Diptera: Anthomyiidae		New Zealand	No	N/A
		USA	Yes	Negligible
Cochylis atricapitana (Stephens)	Stem & crown borer	Australia	Yes	Extensive
Lepidoptera: Tortricidae		Canada	Yes	Considerable
		New Zealand	Unknown	N/A
Longitarsus flavicornis (Stephens)	Root feeder	Australia	Yes	Considerable
Coleoptera: Chrysomelidae				

Longitarsus jacobaeae (Waterhouse)	Root feeder	Australia	Yes	Negligible
Coleoptera: Chrysomelidae		Canada	Yes	Considerable
		New Zealand	Yes	Extensive
		USA	Yes	Extensive
Platyptilia isodactyla (Zeller)	Stem & crown borer	Australia	Yes	Extensive
Lepidoptera: Pterophoridae		New Zealand	Yes	Considerable
Tyria jacobaeae (L.)	Foliage feeder	Australia	Yes	None
Lepidoptera: Arctiidae		Canada	Yes	Negligible
		New Zealand	Yes	Considerable
		USA	Yes	Considerable
Tribe Vernonieae				
***Elephantopus mollis* Kunth**				
C. America, West Indies				
Tetraeuaresta obscuriventris (Loew)	Flower head feeder	Hawaii	Yes	Negligible
Diptera: Tephritidae				

*AH = Alternative (i.e. not the primary) host