Biology, seasonal abundance and host range of capitulum-feeding insects associated with the invasive weed *Senecio madagascariensis* (Asteraceae) in its native range in KwaZulu-Natal, South Africa

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PREFACE

The research described in this dissertation was carried out in the School of Life Sciences (Pietermaritzburg campus), College of Agriculture, Engineering and Science, University of KwaZulu-Natal, from April 2017 to December 2020 under the supervision of Prof T. Olckers, Dr D. Egli and Dr S. Willows-Munro.

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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COLLEGE OF AGRICULTURE, ENGINEERING AND SCIENCE DECLARATION OF PLAGIARISM

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ABSTRACT

Native to KwaZulu-Natal, South Africa, *Senecio madagascariensis* (fireweed) is a herbaceous plant that has become highly invasive in many countries where it was accidentally introduced in contaminated fodder. Rapid growth rates, high fecundity and toxic secondary compounds that poison livestock have caused severe economic impacts in infested pastures and rangelands. Biological control, using imported natural enemies from South Africa, is being pursued as a long-term control option for invaded countries, particularly Australia where invasions are most severe. This study forms part of a collaboration with the CSIRO in Australia to source and assess potential insect biocontrol agents that could be imported into Australia. The aims of this study were to: (i) investigate and identify species of capitulum-feeding insects on fireweed populations; (ii) determine the seasonal abundance of capitulum-feeding insects on fireweed populations in the field; (iii) differentiate between the different lepidopteran and dipteran species associated with fireweed by means of DNA barcoding; and (iv) verify the host range of these insects by surveying related *Senecio* species in the field and comparing the associated insects using DNA barcoding. Insects with capitulum-feeding larvae included Coleoptera, Lepidoptera and Diptera and were most abundant during late summer and autumn. The most important potential biocontrol agents were the lepidopterans *Homoeosoma stenotea* (Pyralidae) and an unidentified species of *Platyptilia* (Pterophoridae), while the flies, which included two unidentified species of *Trupanea* (Tephritidae), were less abundant. DNA barcoding of the COI gene revealed distinct genetic lineages (possible species) of lepidopterans that were recorded on eight of the 36 surveyed *Senecio* species, with most specimens conforming to *H. stenotea* and *Platyptilia* sp. *Homoeosoma stenotea* was recorded on three, and *Platyptilia* sp. on one, non-target *Senecio* species, respectively. The species of

Trupanea were restricted to *S. madagascariensis*, but since they were collected only during seasonal surveys, studies of their host specificity were not concluded. Since the two lepidopteran species do not appear to be strictly host specific, they may not be suitable biocontrol agents for countries like Australia that have a diverse native *Senecio* flora. However, countries that lack native or economically important *Senecio* species may choose to further consider these potential agents.

Keywords: Biocontrol agent ecology; DNA barcoding; fireweed; flower-feeding agents; native range studies; weed biocontrol

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Chapter 1: Introduction

1.1. Plant invasions

The global human population has displayed an exponential increase, facilitating the introduction of plant species beyond their geographic range limits. The prevalence and advancement of transportation systems and international trade has promoted alien plant introductions to more distant regions and foreign countries (Meyerson & Mooney, 2007; Hulme, 2009). The consequence is that some of the alien plant species, purposely or accidentally introduced, become invasive in their new environments; a few of these introduced species were also considered problematic in their native countries. An invasive alien plant species is one whose distribution covers a considerable area beyond the area of introduction as a result of extensive reproduction and has overcome the barriers imposed by the biotic and abiotic factors of the new environment (Richardson *et al.*, 2000).

Several studies have investigated the mechanisms that underlie the ability of alien plants to subdue non-native habitats, with various hypotheses proposed to explain this. These hypotheses include release from natural enemies (Keane & Crawley, 2002), and habitat adaptation (niche modification) due to phenotypic plasticity (Davidson *et al.*, 2011). The enemy release hypothesis (Keane & Crawley, 2002) states that invasive plants introduced to exotic countries proliferate because of the absence of regulation imposed by their co-evolved natural enemies, particularly specialists from their native distribution.

A suite of shared plant traits has been recognised among plant invaders (Pyšek & Richardson, 2008; van Kleunen *et al.*, 2015). These traits range from rapid growth to a reproductive capacity that enables naturalization. Regional studies concluded on the significance of reproductive traits between invasive and non-invasive species, although congeneric studies

limited the general application of most characteristic patterns (Pyšek & Richardson, 2008). For example, the importance of monoecy and hermaphroditism is recognised through mate availability, and self-compatibility is important when considering pollinator limitation (Williamson & Fitter, 1996; Sutherland, 2004). Other studies indicated the role of pollen vector attraction, with invasive plants being more insect pollinated (Crawley *et al*., 1996; Williamson & Fitter, 1996). Moreover, flowering phenology is considered significant in invasive plants, with early flowering and extended flowering periods promoting soil seed bank and seed dispersal (Crawley *et al*., 1996; Pyšek *et al*., 2003). The role of smaller seed size for successful invasion, particularly in invasive herbs, is recognised through increased seed output and efficient dispersal when compared to larger seeds (Thompson *et al*., 1993; Hamilton et al., 2005).

Physiological traits also enable invasive plants to withstand biotic and abiotic environmental factors that may hinder proliferation (van Kleunen *et al.*, 2010). These include competitive advantages due to allelopathy. Clonal growth, leaf area, relative growth rate of seedlings also have been reported to be positively correlated with successful invasion in other studies (Pyšek, 1997; Hamilton *et al*., 2005). The role of some of these traits that enable naturalization is negated by the presence of natural enemies in the native range of the alien species, thus preventing invasion in the native range (Keane & Crawley, 2002). In the introduced range, naturalization in the absence of natural enemies (i.e., enemy release) serves as the foundation for classical biological control on the basis that natural enemies, which supress the reproductive and vegetative vigour of an alien plant species within its native range, could be introduced into the new invaded range to exert control (McFadyen, 1998).

The native fauna that comprise natural enemies of most invasive plants are largely insects, but also plant pathogens. Significant herbivory by larger fauna (such as herbivorous mammals) is usually unattainable since most invasive plants are highly unpalatable due to their secondary compounds that are physiologically toxic to animals (Gardner *et al.*, 2006; Cruz *et al.*, 2010). It is unlikely that native insects, which are adapted to cope with these plant defences, will be accidentally introduced along with their invasive host plant. Thus, the introduction of natural enemies (biological control) should be purposeful and substantiated by research to negate the possibility of them affecting non-target native plants, as well as to confirm their potential to inflict damage on the target weed species.

The perspective of climate matching between the invader's native region and its invasive range has been understood to be a key factor in the efficacy of these natural enemy introductions (e.g., Singh & Olckers, 2017). Yet, there is evidence of the ability of exotic plants to shift ecological niches and inhabit areas that are climatically different from those in their native range (Gallagher *et al.*, 2010). This lack of niche conservatism in exotic plant species could aggravate the threat already posed by them to native biodiversity and ecological processes that vary amongst ecosystems, and lessen the impact of biological control (Gallagher *et al*., 2010).

1.2. Pathways for the introduction of exotic plants

Exotic species introductions are often facilitated by the practice of forestry, agroforestry, and commercial botany. Commercial forestry plays a huge role in introducing invasive woody plants; for example, the cultivation of *Pinus*, *Eucalyptus*, and *Acacia* species for timber (Hoffmann *et al.*, 2011). Horticultural practices further promote invasiveness due to the selection of genetically inheritable traits that are often affiliated with this ability (Richardson

& Rejmánek, 2011). For example, horticultural trees for forestry are modified for vigorous growth, reduced generation time, and bestowed with an ability to withstand extremely disturbed, eroded habitats (Grotkopp *et al.*, 2010; Reichard, 2011). Artificial selection for prolific fruiting further attracts fruit/seed dispersers to exotic plants that were introduced as ornaments (van Kleunen *et al.*, 2018).

Although most botanical gardens are now established for conservation purposes, commercial botany activities continue to introduce more non-native species available for being sold (van Kleunen *et al*., 2018). In fact, a comprehensive, international study by Hulme (2015) on living plant collections from 3 000 botanical gardens reported that 99% of the major global weeds occurred in at least one living collection, with only 23% comprising threatened species. The potential for plant invasions from just one living collection is negligible, but the occurrence of more than one botanical garden within a region would increase the likelihood of escape (Hulme, 2015).

Accidental introductions in animal fodder and crop seeds are prevalent for agricultural weeds in pastural and crop lands. International trade of crop seeds provides a pathway for unintentional introductions through contamination; however, seed cleaning and quarantine measures have been adopted to regulate accidental introductions (Mack & Lonsdale, 2001). There are several other pathways for the introduction of exotic plants such as transferring plants as biofuels and through international trade. Thus, to halt exacerbating the threat of plant invasions should begin with regulatory measures to prevent the introduction of species exhibiting invasive attributes.

1.3. Effects of invasive plants

Different types of invasive plants (i.e., herbs, succulents, trees, and shrubs) pose various threats in their new ranges. Trees and shrubs are now recognised as the most significant contributors to the major global invaders (Richardson & Rejmánek, 2011). The aggressive spread of invasive species has shown them to be the second most important threat to global biodiversity, after habitat loss (Wilcove *et al.*, 1998). Alien plant invasions are often linked to habitat destruction (Crawley, 1987), as invasive species exhibit an outstanding ability to thrive in disturbed environments. Moreover, their negative impacts are not only on ecosystem processes, but are also economic and social (Van Wilgen *et al.*, 2001; Pejchar & Mooney, 2009). Ecological effects are reflected on different levels of ecological organization, and the magnitudes of these effects are dependent on the complexity of each level of ecological organization (e.g., impacts on ecological community (such as interruption of a food chain) vs. impact on a species population (such as patch occurrence) (Pejchar & Mooney, 2009).

1.3.1. Ecological impacts

Plant diversity in most ecosystem types has been presumed to be highly threatened by invasive alien plants. This is because the extinction of native species, as well as a reduction in their species richness and abundance, is more prevalent in invaded localities and habitats (Bellard *et al.*, 2016). Alien invasive plants successfully outcompete native species within the same habitat, this is because invasive plants are often more successful colonisers of severely disturbed habitats compared to natives (Powell *et al.*, 2013). The outcome of invasive colonisations further determines the invasibility (the degree of susceptibility to invasion) of the already infested area (Davis *et al.*, 2000). The fluctuating resource availability theory predicts that an increase in available resources (i.e., soil nutrients, water, etc.) lessens the competitive vigour of cohabiting species, although allowing prolific exotic species to increase

on unexploited resource-rich patches within a given locality (Davis *et al*., 2000; Davis & Pelsor, 2001). The competitive displacement by invasive plants is most significant on common species in a given locality, yet rare species are also at risk (Powell *et al*., 2013).

Hybridisation between native and invasive plants is a major threat to native biodiversity. This is because even if the hybrids are sterile, the population of the native species is at risk from discounted reproductive efforts (Mack *et al.*, 2000). Moreover, there is the possibility of the resulting offspring becoming a new and aggressive invasive species. For example, hybridisation between the exotic North American cordgrass (*Spartina alterniflora* Loisel.; Poaceae) and a native cordgrass (*Spartina maritima* (Curtis) Fernald) in England constantly produced sterile offspring, until an occurrence of chromosome doubling, which generated fertile and invasive offspring, identified as *S. anglica* Hubb. (Mack *et al*., 2000). There are thus other significant impacts of invasive plants apart from the direct threats to native plant biodiversity (i.e., competition and hybridisation with native relatives) (Gurevitch & Padilla, 2004).

Grasslands and savannahs are under threat from pasture weeds such as mesquite (*Prosopis* spp.; Fabaceae), famine weed (*Parthenium hysterophorus* L.; Asteraceae), and invasive grasses (e.g., tussock paspalum (*Paspalum quadrifarium* Lam.)). Such invaders increase fuel biomass, and thus increase the frequency and intensity of fires (Mack *et al*., 2000). After burns, the exotic species re-sprout, while the native perennials that cannot functionally withstand frequent fires are displaced. Native perennial grasses that are more palatable to herbivorous wild animals are typically displaced by unpalatable or less palatable exotics (Mack *et al*., 2000).

Water resources have been significantly threatened by exotic plant invasions, and negative impacts include disruptions to stream flow, destabilization of riverbanks, and degraded freshwater ecosystems (Richardson & Van Wilgen, 2004). Major aquatic weeds like the water hyacinth (*Eichhornia crassipes* (Mart) Solms; Pontederiaceae) increase evapotranspiration, displace native aquatic plants through blocking light penetration, and reduce populations of native invertebrates and fish through the reduction of available oxygen (Richardson & Van Wilgen, 2004). Several other ecological impacts of invasive plants include indirect effects such as disrupted food chains and changes in host associations.

1.3.2. Economic and social impacts

The economic costs of invasive plants are manifested in various ways, including the costs of managing invasions through chemical, mechanical, and biological control; and the costs incurred from the direct impacts of invasions such as reduced grazing pastures for commercial livestock (Van Wilgen *et al*., 2001). Extensive infestations from invasive weeds also reduce property values, thus halting the selling and buying of homes and affecting the livelihoods of property owners (Pimentel, 2014). Conservation economies improve local communities, but extensive invasions may depreciate biodiverse lands, and discourage tourism (Perrings, 2005).

The economic impact of weeds in commercial agriculture is severe. The decline in the value of crop production due to agricultural weeds was estimated to be US$ 1.5 billion annually (Pimentel *et al*., 2001), and this value has presumably increased over time. Pastoral weeds like fireweed (*Senecio madagascariensis* Poir.; Asteraceae) reduce grazing capacities for commercial livestock, incurring reduced profits (Richardson & Van Wilgen, 2004; Sheppard *et al.*, 2013). Commercial agriculture plays a major role in the economy of developing countries. In developing countries, where most of the population relies on subsistence farming, major

weeds like *Chromolaena odorata* (L.) King & Rob. (Asteraceae) decrease both arable land and grazing land (Perrings, 2005). This causes an increase in households living in abject poverty, which further burdens the developing economy.

Aggressive aquatic weeds disrupt irrigation systems and form an ideal habitat for mosquitos, which are vectors of several human diseases (Perrings, 2005). The formation of dense cover in rivers and lakes affects human livelihoods in recreational activities such as boating and reduces services like fishing and water supply (Richardson & Van Wilgen, 2004). Moreover, the spread of invasive plants could impede plans for public infrastructure development where land is heavily infested (Perrings, 2005). Altogether, these challenges halt sustainable community development and consequently deflate the capital investment that could potentially advance the local economy.

International trade is a major pathway for the introduction of invasive plants, and thus could lead to the establishment of restrictive policies on trading with other countries to avoid accidental introductions of exotic plants (Dalmazzone, 2000; Hulme, 2009). Less restrictive countries are more susceptible to alien plant invasions (Dalmazzone, 2000). This could severely impede the economies of developing countries, whose reliance on economic growth is through trading with developed countries, which are usually the ones with more restrictive trade policies (Perrings, 2005).

The management of plant invasions is costly to governments and private property owners. This comes from clearing equipment and the size of the work force required to facilitate manual clearing in small infested areas (Holt, 2004). Chemical control is the most common control method in agriculture but is constrained in natural ecosystems due to risks to non-target plant diversity (Holt, 2004). The purchase of herbicides is costly, particularly in cases

where highly specific products (effective on fewer, specific plants) are required (Holt, 2004). Techniques involved in prevention, such as quarantine facilities, and early detection activities, such as surveillance equipment, might not be affordable for some countries (Holt, 2004). Biological control thus seems to be the most cost-effective, long-term, and environmentally friendly control method.

1.4. Biological control

1.4.1. History and advancement of the discipline

Classical weed biological control became a professional discipline during the 1900s on the premise of the natural enemy release hypothesis. The initial applications were in India and Hawaii (USA) followed by Australia for the major weeds lantana (*Lantana camara* L.; Verbenaceae) and prickly pear cactus (*Opuntia stricta* Haw.; Cactaceae), and the discipline was later extended to other countries, notably Canada, New Zealand, and South Africa (Briese, 2000; Timmons, 2005; Kumar, 2015). The techniques involved have been advanced over time to prevent direct and indirect impacts on non-target plant species, or the importation and release of ineffective agents (Sheppard *et al.*, 2005).

Advancement of biocontrol included the realisation that different life stages of biological control agents could exhibit different host ranges, and that host specificity following release is determined by gene expression in the new environment (Sheppard *et al*., 2005). Several tests are conducted over an extended time frame to validate the suitability of an agent in terms of host-specificity and potential damage (McFadyen, 1998). Moreover, the collaboration of researchers in the invaded country and the weed's native country promotes the selection of suitable agents, even prior to quarantine introductions into the invaded country (Pedrosa-Macedo *et al.*, 2003; Ferrar *et al.*, 2004).

Advances in molecular analysis have allowed the inclusion of non-target test plants that are more likely to be directly impacted due to their phylogenetic relatedness to the target weed (Briese, 2005). Thorough test plant lists provide precision for the host range prediction of potential agents. Molecular studies on the target plant have prevented the misidentification of native plants as the target and the weed being misidentified as a native species (Radford *et al.*, 2000). Additionally, hybridization between the weed and either closely related native taxa, or another closely related invasive species, can be identified (Gaskin *et al.*, 2011). Molecular techniques can also reveal the exact origin of the weed, which could improve the chances of sourcing suitable biocontrol agents (Scott *et al.*, 1998; Radford *et al.*, 2000).

The identification of biological control agents could also be validated by molecular analysis where distinguishable morphological traits are not clear. This could minimise the misinterpretation of host specificity, as the presence of a misidentified close relative of the candidate agent could lead to false conclusions of polyphagy (Gaskin *et al*., 2011). Moreover, intraspecific variation amongst agent populations, which may influence the expression of their field host range, can be identified in their native range (Sheppard *et al.*, 2005; Klein & Seitz, 2008).

1.4.2. Success of biological control

The success of biological control programmes has been widely reported in many countries (Fowler *et al.*, 2000; McFadyen, 2000), such as the successful control of *Sesbania punicea* (Cav.) Benth. (Fabaceae) and other weeds in South Africa (Hoffmann & Moran, 1999). The declaration of success for biological control projects has often varied according to different definitions of success. However, success should be based on the regulation of the weed's population, rather than on individual agent performance (McFadyen, 2000). This is because

more than one biocontrol agent may be released against a target weed, but the ineffectiveness of the majority of the released agents does not imply programme failure. Six biological control agents were released against jointed cactus, *Opuntia aurantiaca* Lindl. (Cactaceae) in South Africa, yet only the cochineal insect *Dactylopius austrinus* De Lotto (Dactylopiidae) was able to reduce the weed's population (Moran & Zimmermann, 1991; McFadyen, 2000). Additionally, the lepidopteran *Cactoblastis cactorum* Berg (Pyralidae) released against *Opuntia stricta* (Haw.) Haw. was considerably more effective in reducing population densities than the eight other agents released (McFadyen, 2000).

The establishment and proliferation of biological control agents in their new range is often variable across different regions and climatic conditions. For example, the weevil *Anthonomus santacruzi* Hustache (Curculionidae), which was released against *Solanum mauritianum* Scopoli (Solanaceae) in South Africa, thrives in low altitude coastal areas but is restricted in higher altitude inland regions (Singh & Olckers, 2017; Mkhize & Olckers, 2019). Such variations in agent impact highlight the fact that biological control programmes should be given several years before the declaration of success or failure in terms of controlling weed populations (McFadyen, 2000; Moran *et al.*, 2013). Additionally, comprehensive records of weed population dynamics prior to the onset of a biological control project are important for the evaluation of each control programme after an adequate time frame (i.e., post release evaluations) (McFadyen, 2000).

Despite the limitations of inadequate financial resources and inefficient political processes in the release of new agents, which impede success (McFadyen, 2000; Moran *et al*., 2013), biological control remains the most sustainable and environmentally friendly method for controlling alien invasive plants. Often, the most prominent challenge in introducing a new

agent into a new environment is to determine the risk of non-target attacks. This is investigated by importing the potential agent into quarantine in the invaded country. Hence, the potential host range of the biocontrol agent is determined by exposing it to plants that are closely related to the target weed and its ecological proxies, in tests that include no-choice and choice scenarios. The occurrence of feeding and oviposition on the non-target test plants in relation to that on the target plant is used to assess the probability of non-target attacks.

1.5. *Senecio madagascariensis* Poir.

1.5.1. Ecology

Senecio madagascariensis Poir. (Asteraceae) is an annual or perennial herb that is native to South Africa and Madagascar (Hilliard, 1977) and is commonly known as fireweed in invaded countries. The plant has small, yellow inflorescences (capitula) that are typical of the Asteraceae (Figure 1.1). Flowering occurs throughout the year but can be prolonged during summer (McFadyen & Morin, 2012). The plant typically occurs in disturbed areas such as grazing pastures, roadsides, urban vegetation, and woodlands. The species is invasive in several countries that include Argentina, Australia, Brazil, Japan, Kenya, and the USA (Hawaii) (Sindel *et al.*, 1998). Sindel *et al.* (2008) confirmed that fireweed had not yet reached its potential distribution in Australia as predicted in 1992 (Sindel & Michael, 1992). In Japan, continued spread was predicted to be primarily hindered by temperature, with greater invasive potential only in regions where *S. madagascariensis* already occurs in patches (Tsutsumi, 2011).

The colonization of *S. madagascariensis* is promoted by the frequency and intensity of disturbance (Sheppard & Olckers, 2012), with pastures and managed grasslands being more prone to its invasion. Competition from perennial grasses is vital for the suppression of

fireweed populations during the wet summer season in Australia, where infestations are particularly severe (Sheppard & Olckers, 2012). However, in the dry winter season, fire management regimes aimed at promoting grass recruitment also aid fireweed invasion, as fire is favourable for its recruitment (Sindel *et al.*, 1998; Sheppard & Olckers, 2012).



Figure 1.1. Individual plants of fireweed (*Senecio madagascariensis*) with yellow capitula and white pappuses, in a small population growing in urban vegetation. Images by N. Mkhize.

Fynn *et al*. (2019) investigated the suppression of fireweed by the interaction of simulated herbivory (for biocontrol agents) and interspecific competition from six grass species (South Africa natives and introduced grasses). The reproductive capacity of fireweed was not affected by either competition or simulated herbivory. In the absence of simulated herbivory, the competitive ability of fireweed was reduced by grass competition, resulting in reduced plant biomass (Fynn *et al*., 2019). However, when fireweed plants were defoliated the suppression of plant biomass by grass competition was lessened (Fynn *et al*., 2019). It was

proposed that this was probably due to allelopathic responses (facilitated by pyrrolizidine alkaloids) that were induced by defoliation, therefore reducing the competitive ability of grasses on *S. madagascariensis* (Fynn *et al*., 2019).

Despite being self-incompatible (Sindel *et al*., 1998), the efficiency of fireweed in attracting honeybee and hoverfly pollinators promotes extensive seed production (Sindel *et al*., 1998; Sindel, 2009). Efficient seed dispersal is attained by the seed's pappus (modified calyx) which attaches to animal bodies and is easily transported by wind (McFadyen & Morin, 2012).

1.5.2. Biological control of fireweed

Fireweed has been considered for biological control in Australia since 1991 (Sheppard & Olckers, 2012). The weed causes significant problems such as reduced pasture productivity (Sheppard *et al*., 2013; see Figure 1.2) and livestock poisoning from the pyrrolizidine alkaloids that are produced by fireweed plants (Cruz *et al.*, 2010). Due to avoidance by animal grazers as a result of its unpalatability, fireweed outcompetes pastoral species that are selectively grazed (McFadyen & Morin, 2012). Conventional control measures, including herbicide applications, mechanical removal, and pasture management are costly due to the weed's prolonged flowering and seed production and are difficult to implement (McFadyen & Morin, 2012; Sheppard *et al*., 2013).

Initial efforts towards the biological control of *S. madagascariensis* involved surveys of its populations in Madagascar (Marohasy, 1989). This was prior to the later findings that Australian fireweed was more closely matched with populations in KwaZulu-Natal Province, South Africa (Scott *et al.*, 1998; Radford *et al.*, 2000). *Senecio madagascariensis* resembles species in the *Senecio lautus* Willd. complex in Australia. Hence, it was initially not regarded as an exotic, as it was perceived to be a biotype of the Australian native *Senecio pinnatifolius*

A. Rich. (Sindel *et al*., 2008; McFadyen & Morin, 2012). Additionally, another Australian native, *Senecio brigalowensis* Thomps., has been misidentified as fireweed due to its weedy nature in parts of the country (McFadyen & Morin, 2012).

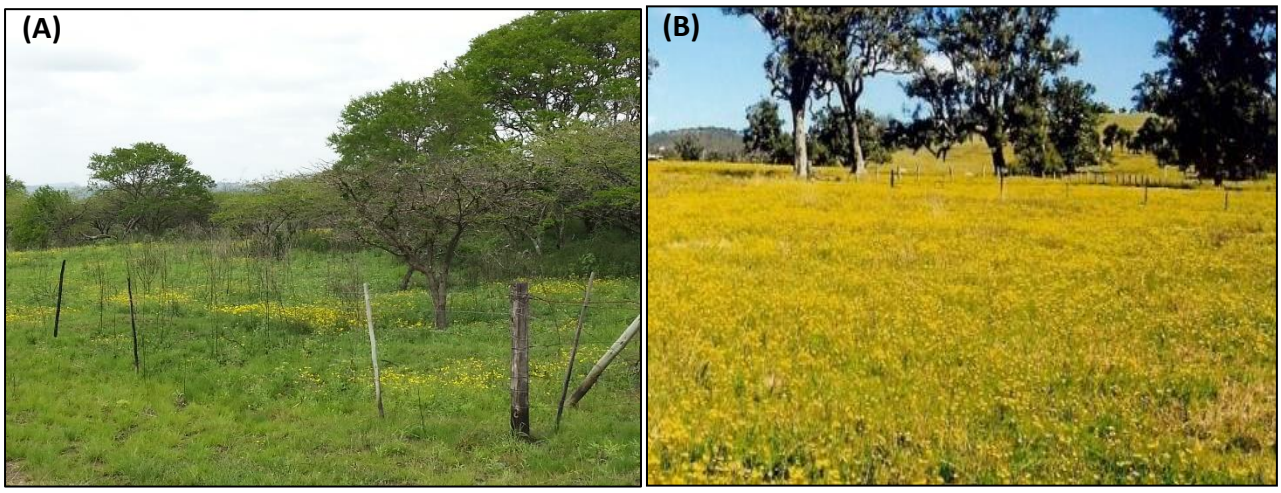


Figure 1.2. Native populations of fireweed in KwaZulu-Natal, South Africa (A) and invasive populations in Australia (B). Images by T. Olckers (A) and A. Sheppard (B).

The flower-feeding moth *Phycitodes* sp. nov. (Pyralidae) and stem-boring moth *Lobesia* sp. nov. (Tortricidae) were quarantined in Australia as candidate agents, after surveys in Madagascar (McFadyen & Sparks, 1996). Host-range testing in quarantine deemed that both moths were unsuitable for release, as oviposition and larval development was recorded on a diverse range of native Australian *Senecio* species (McFadyen & Sparks, 1996). No agent importations were made from early surveys in South Africa, due to restricted funding and lack of collaboration (McFadyen & Morin, 2012). The prevailing spread of fireweed and its later designation as a "Weed of National Importance" in Australia rejuvenated biocontrol efforts in South Africa. Comprehensive natural-enemy surveys in KwaZulu-Natal Province potentially offered more promising agents, once the uncertainty on the origin of Australian fireweed was resolved (Scott *et al*., 1998; Radford *et al*., 2000).

Hence, funding was made available in 2009 to resume biological control efforts in South Africa in collaboration with the University of KwaZulu-Natal, for native-range assessments of fireweed's natural enemies (Sheppard & Olckers, 2012). The project aimed to highlight insect herbivores associated with fireweed that are highly host-specific, given the diversity of *Senecio* species in Australia. Earlier surveys in South Africa in 2002, on rust fungi associated with fireweed, discovered pure and hybrid strains of *Puccinia lagenophorae* Cooke (Pucciniaceae: Pucciniomycetes) (McFadyen & Morin, 2012). However, the rust isolates were not host-specific and demonstrated less pathogenicity compared to *P. lagenophorae* strains already present on fireweed and other *Senecio* species in Australia.

Fifteen insect taxa were recorded as potential agents for fireweed following recent quantitative surveys in KwaZulu-Natal (Egli & Olckers, 2015; 2020). The seasonal abundance varied amongst these taxa, with only four present across all seasons (Egli & Olckers, 2015). Seasonal abundance in the native range is an important indicator of the potential of a candidate agent to supress weed populations in the invaded range, particularly since fireweed flowers almost throughout the entire year.

Concerning the biological control of other invasive Asteraceae, *Parthenium hysterophorus* L. agents targeting structural parts of the plant (roots and stems) were highly effective in Australia, not only on individual plants but also at the population level (Dhileepan, 2007). A flower-feeding weevil *Rhinocyllus conicus* Frölich (Curculionidae) released against spear thistle *Cirsium vulgare* (Savi) Ten. (Asteraceae) in South Africa, induced significantly fewer seeds in attacked capitula (Hodson *et al.*, 2003). Therefore, the release of a suite of agents, including those attacking reproductive parts could be effective against *S. madagascariensis* (Sheppard & Olckers, 2012).

It has been hypothesized that agent establishment and the successful control of the target weed are determined by the taxonomy of a candidate agent and its feeding guild (e.g., flower-feeder vs. stem borer). To guide agent prioritization for *S. madagascariensis*, an assessment was undertaken of agents released against weeds in the Asteraceae (Egli & Olckers, 2017). It was found that most releases involved Coleoptera (Curculionidae and Chrysomelidae), Diptera (Tephritidae) and Lepidoptera (Tortricidae). There was no significant relationship between agent feeding guild and the success of a biological control programme across the asteraceous weeds (Egli & Olckers, 2017). However, it was observed that root, stem and capitulum feeders had more successful establishments compared to foliage feeders.

Based on the studies undertaken in KwaZulu-Natal, four agents were short listed for fireweed (Egli & Olckers, 2015; 2020). These include a root-feeding flea beetle (Coleoptera: Chrysomelidae: Alticinae), a capitulum-feeding moth (Lepidoptera: Pyralidae), and two stem-boring insects that include a weevil (Coleoptera: Curculionidae) and a moth (Lepidoptera: Tortricidae). Studies on the seasonal abundance and field host range of stem-boring insects (Singh, 2019) and root-feeding flea beetles (Zuma, 2020) have already been undertaken. This study thus focussed on all capitulum-feeding insects including the pyralid moth *Homoeosoma stenotea* Hampson.

1.6. Aims of the study

The aims of this study were to investigate the biology, seasonal abundance and native host range of capitulum-feeding insects associated with *S. madagascariensis* to determine their potential for deployment as biocontrol agents in invaded countries like Australia. Objectives of the study included the following:

(1) Determining the relative abundance across seasons of all flower-feeding insects.

(2) Conducting host-range assessments from surveys on closely related *Senecio* species.

(3) Genetic barcoding of important flower-feeding insects recorded across *Senecio* species in the field to determine their host-plant relationships and identify species that are restricted to *S. madagascariensis*.

Chapter 2: Abundance of capitulum-feeding insects associated with *Senecio madagascariensis* across seasons

2.1. Introduction

Senecio madagascariensis Poir., commonly known as fireweed, is a short-lived perennial herb that is easily identified by small, yellow flower heads (capitula) that are typical of the family Asteraceae (Hilliard, 1977). The species is widely distributed in southern Africa and is abundant in KwaZulu-Natal province in South Africa (Hilliard, 1977). It has been introduced into several other countries and is invasive in Argentina, Australia, Japan, Kenya, and the USA (Hawaii) (Sindel *et al.*, 1998). Herbicides are effective against fireweed in the short term but are unsustainable over the long term (Sindel *et al.*, 1998). Biological control has the potential to be the most sustainable and effective management approach against fireweed invasion in livestock pastures (Sindel *et al.*, 1998; McFadyen & Morin, 2012).

Several properties of a biocontrol agent including its evolutionary history, feeding guild, seasonal abundance, and climatic adaptability influence its performance in the new range (Harris, 1973; Sheppard, 2003; Stiling & Cornelissen, 2005). All factors that affect the abundance of a biocontrol agent should be considered when evaluating its ability to reduce weed density. These include competition with native insects inhabiting the same parts of the plant, predation, and parasitism (Cullen & Sheppard, 2012; Hakizimana & Olckers, 2013); as well as climatic tolerance based on the agent's physiology (Cowie *et al.*, 2016).

Intensive reproductive output, among other features, has primarily been responsible for the success of most invasive plants (Wolfe, 2002). Since fireweed displays high floral output and is capable of extensive seed production (Sindel *et al.*, 1998), capitulum feeders may be effective biocontrol agents as they affect floral development and seed production. Insects

attacking reproductive structures have shown considerable success in biological control programs (McFadyen, 2000; Winston *et al.*, 2014). For example, the integration of the seed weevil *Rhinocyllus conicus* Froehlich (Curculionidae) and the capitulum gall fly *Urophora solstitialis* (L.) (Tephritidae), introduced for the biological control of nodding thistle *Carduus nutans* L. (Asteraceae), led to a significant reduction in the density of the weed in Canada, the USA, New Zealand, and Australia (Cullen & Sheppard, 2012; Winston *et al.*, 2014).

Successful control at the level of population density is influenced by the synchronization of the weed's phenology (e.g., flowering) with the agent's seasonal abundance, especially in flower- and seed-feeding agents (Sheppard *et al.*, 2005). For example, the introduction of *R. conicus* for *C. nutans* infestations in Australia was not as successful as recorded in the USA (Virginia State) and Canada (Cullen & Sheppard, 2012). This was because of the prolonged flowering time of *C. nutans* populations in Australia and the emergence of adult weevils not coinciding with the development of the capitula (Woodburn & Cullen, 1993).

Insects typically have short life cycles with seasonal peaks in abundance and sometimes spontaneous or periodic outbreaks (Williams & Simon, 1995; Danks, 2013). Yet, it is vital that during their peak abundance they inflict significant damage on weed populations (English & Olckers, 2018). It has been hypothesized that short-lived herbs are more susceptible to biocontrol as most invest heavily into seed production (Rea, 1998). There have also been successes in the biological control of weeds that flower almost throughout the year (McFadyen, 2000; Charudattan, 2005; Winston *et al.*, 2014). Fireweed flowers throughout the year, thus necessitating sustained agent impact across all seasons to limit its reproduction. Recent surveys for potential biological control agents in the South African native range

reported the incidence and relative abundance of several herbivorous insect species that include stem borers, capitulum feeders, and root feeders (Egli & Olckers, 2015; 2020).

The aim of this study was to investigate the seasonal abundance of capitulum-feeding insects to determine their potential for deployment as biocontrol agents of fireweed in invaded countries like Australia and Hawaii. *Senecio madagascariensis* was sampled monthly across all seasons at four sites in KwaZulu-Natal for a year. From previous studies (Egli & Olckers, 2020), flower-feeding lepidopteran larvae are of higher priority as candidate biocontrol agents, due to them causing more damage than the other flower-feeding insects.

2.2. Materials and methods

2.2.1. Study sites

Field collections of *S. madagascariensis* were conducted at four inland sites in the KwaZulu-Natal Midlands region that supported healthy populations of *S. madagascariensis* (Table 2.1). Monthly sampling was conducted from April 2017 to March 2018 to ensure sampling throughout all seasons.

Table 2.1: Details of four sites in the KwaZulu-Natal Midlands where *Senecio madagascariensis* populations were sampled monthly.

Site name	Co-ordinates (S,E)	Altitude (m a.s.l.)	Habitat
Cedara (Hilton)	29°53’96” 30°26’80”	1068	Paddock
Groundcover (Curry’s Post)	29°38’86” 30°17’50”	1280	Paddock
Ukulinga (Mkhondeni)	29°67’92” 30°50’84”	759	Paddock
Raptor Centre (Ashburton)	29°66’21” 30°40’48”	787	Pasture

2.2.2. Sampling procedure

On each monthly sampling occasion, five healthy fireweed plants were collected at each of the four sites and placed individually into a brown paper bag. In the laboratory, the inflorescences were removed from each plant and frozen until processing. The number of capitula (all flowering stages) were recorded for each plant and then inspected under a dissecting microscope to record the immature stages of all endophagous capitulum-feeding taxa. The larvae were recorded per plant and corresponding site, and identified as coleopteran, dipteran, or lepidopteran. Genetic barcoding of all larval lepidopteran and dipteran specimens was conducted (see Chapter 3) to precisely identify species that were collected, particularly those of Lepidoptera.

Three additional plants were collected on each sampling occasion to rear larvae to adults and facilitate the identification of the relevant taxa. The capitula (all flowering stages) were removed from each plant and separately placed in plastic emergence containers until adult emergence. Counts of adults were pooled from the three plants. Adults that emerged were either pinned for the reference collection or stored in 100% ethanol. Those stored in ethanol were sequenced so that larvae could be matched to adults.

2.2.3. Statistical analysis

Statistical tests were performed in IBM SPSS version 27. Generalized linear modelling was ideally suitable for the count data, but due to the absence of larvae in many samples across months and sites, the tests could not be performed. This was despite attempts to correct the data for conformity and adding a value of 1 to the data set to counteract the high number of zero values.

Data were standardized by calculating the number of larvae per 10 flowers for each sampled plant. The Kruskal-Wallis test was performed to test for differences in the number of larvae (pe 10 capitula) of Coleoptera, Lepidoptera, and Diptera recorded across the months and between the four sites. The non-parametric test could not test for any interaction between month and site. The Mann-Whitney U-test was used to test for post-hoc differences between months and sites for each of the three insect groups. A non-parametric Friedman's test was performed to determine whether total numbers of larvae collected across all seasons and between all sites differed between the three orders, Coleoptera, Lepidoptera, and Diptera.

2.3. Results

2.3.1. Seasonal abundance of coleopteran larvae

Based on adults reared (see below), capitulum-boring beetle larvae comprised an unidentified species of Nitidulidae. The mean abundance of beetle larvae recorded per 10 capitula differed significantly across the months ($H = 31.943$; $df = 11$; $p = 0.001$) and between the four sites ($H = 11.603$; $df = 3$; $p = 0.009$). Although larvae were recorded throughout most of the year (Fig. 2.1), none were recorded in September and February across all four sites, with only one and two individuals recorded in August and November in all capitula, respectively. Consequently, all months, except August and November, were significantly different from September and February ($p<0.05$). Six months (50%) supported total larval numbers greater than 10. March supported the highest total of larvae that accounted for 28% of total nitidulid larvae recorded across all seasons (Fig. 2.1). Late summer therefore appears to support a peak in nitidulid larval abundance (Fig. 2.1).

Larval numbers were influenced by site and Raptor Centre and Cedara supported the highest mean numbers of larvae. Cedara was significantly higher compared to Ukulinga ($p = 0.039$) and Groundcover ($p < 0.0001$) (Fig. 2.1). Numbers of larvae recorded at Groundcover were also significantly lower than at Raptor Centre ($p = 0.041$). Therefore, Raptor Centre and Cedara were the most suitable sites for supporting the nitidulid than Ukulinga and Groundcover.

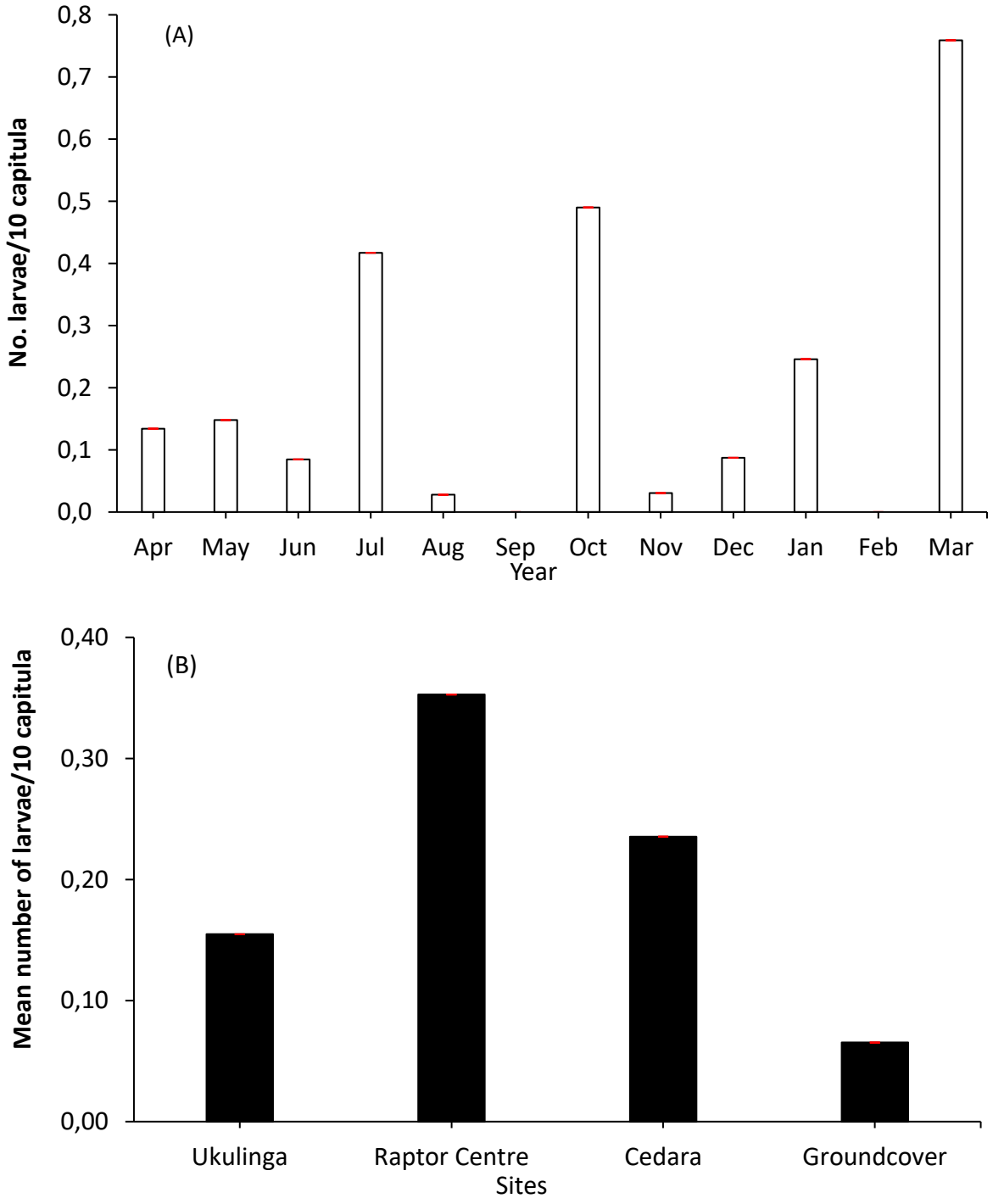


Figure 2.1. Mean numbers of Coleoptera larvae per 10 capitula of *Senecio madagascariensis* for all plant samples collected between the (A) different months, and (B) at each of the four sites.

2.3.2. Seasonal abundance of lepidopteran larvae

Based on adults reared (see below), capitulum-boring lepidopteran larvae comprised two species, namely *Homoeosoma stenotea* Hampson (Pyralidae) and an unidentified species of *Platyptilia* Hübner (Pterophoridae). The number of lepidopteran larvae collected differed significantly across months ($H = 26.302$; $df = 11$; $p = 0.008$). Lepidopteran larvae were far less abundant than the coleopteran larvae (Fig. 2.1) and during four months, no moth larvae were recorded at any of the four sites (Fig. 2.2). Most moth larvae were recorded in March, April, May and June (Fig. 2.2), corresponding to late summer and autumn. Larval numbers per 10 capitula were significantly higher for April compared to all the other months ($p < 0.05$) except March and May ($p = 0.256$).

Although most moth larvae were collected from Cedara and Groundcover, the effect of site was not significant ($H = 4.940$; $df = 3$; $p = 0.176$). Ukulinga recorded the lowest total number of larvae compared to the other sites (Fig. 2.2). Since moth larval abundance was highest during late summer (March) to autumn (April and May), there appeared to be an influence of season.

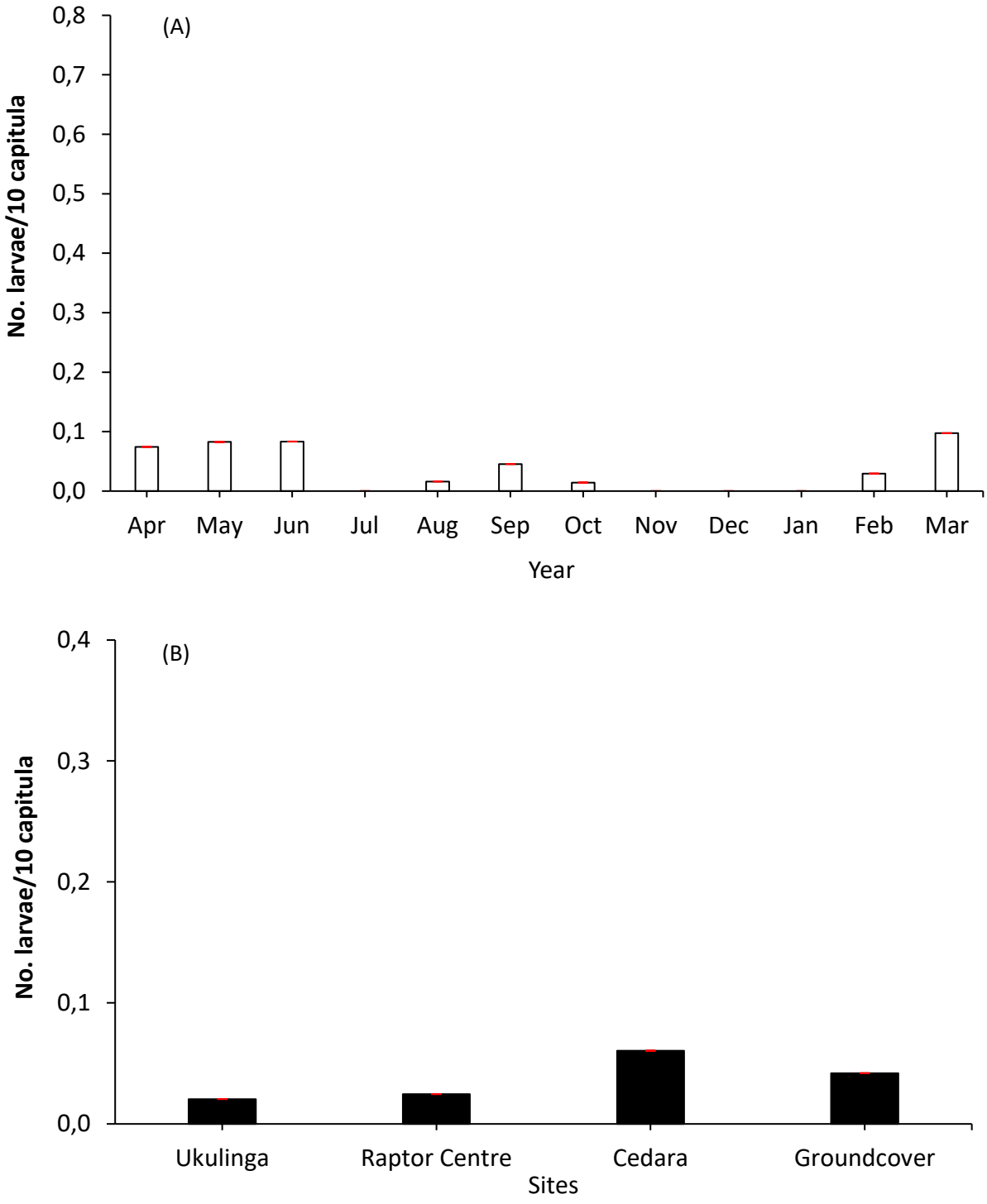
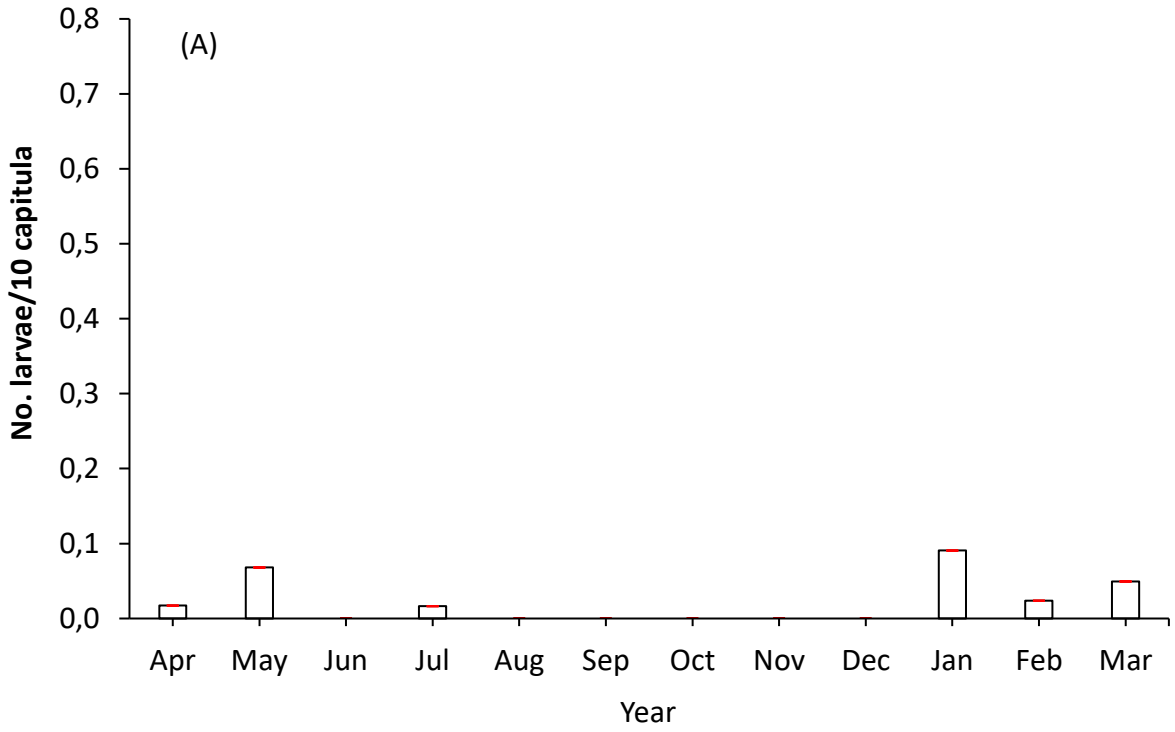


Figure 2.2. Mean numbers of Lepidoptera larvae per 10 capitula of *Senecio madagascariensis* for all plant samples collected between the (A) different months, and (B) at each of the four sites.

2.3.3. Seasonal abundance of dipteran larvae

Based on adults reared (see below), capitulum-boring dipteran larvae comprised four species, including an unidentified midge (Cecidomyiidae), two species of *Trupanea* Schrank (Tephritidae) and an unidentified fly (Agromyzidae). The number of dipteran larvae was not significantly influenced by month ($H = 18.364$; $df = 11$; $p = 0.074$). Dipteran larvae were the least abundant of all the flower feeders and during six months, no larvae were recorded at any of the four sites (Fig. 2.3). January and May supported the highest total number of larvae (Fig. 2.3). Larval numbers at each of the four sites were not dependent on season but were significantly influenced by site ($H = 9.953$; $df = 3$; $p = 0.019$). No larvae were collected from Ukulinga, and only two individuals were recovered from Groundcover. Thus, Raptor Centre and Cedara supported significantly higher fly larval numbers than Ukulinga ($p = 0.012$), but not significantly more than Groundcover ($p = 0.051$).



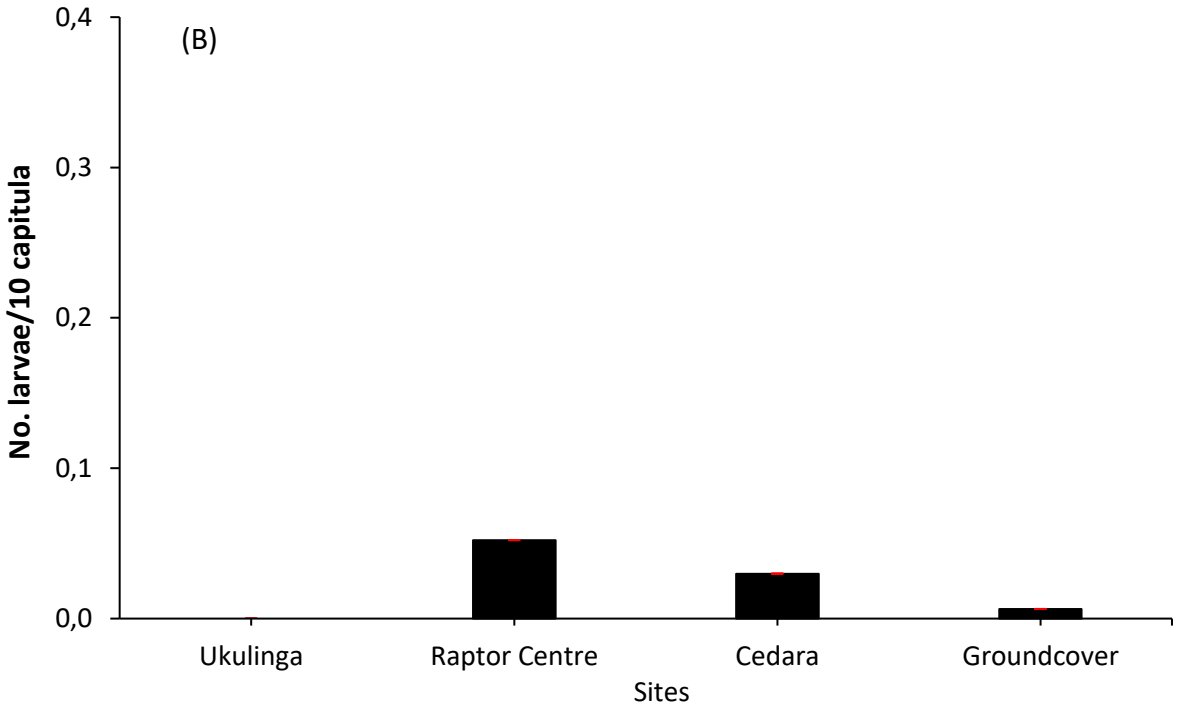


Figure 2.3. Mean numbers of Diptera larvae per 10 capitula of *Senecio madagascariensis* for all plant samples collected between the (A) different months, and (B) at each of the four sites.

2.3.4. Overall insect abundance across months and sites

2.3.4.1. Monthly larval loads

The total number of capitulum-boring larvae recorded across the 12 months and the four sites were compared between the three orders. There were significant differences in larval numbers between orders ($\chi^2 = 41.353$; df = 2; $p < 0.0005$), with more coleopteran (nitidulid) larvae collected (Fig. 2.4). Lepidoptera and Diptera had almost the same numbers (with mean ranks of 1.94 and 1.91, respectively). Multiple comparisons for Coleoptera and Lepidoptera indicated significantly higher numbers for the month of March against the lowest months. This suggests that herbivore loads in the flowers peak during late summer and towards autumn.

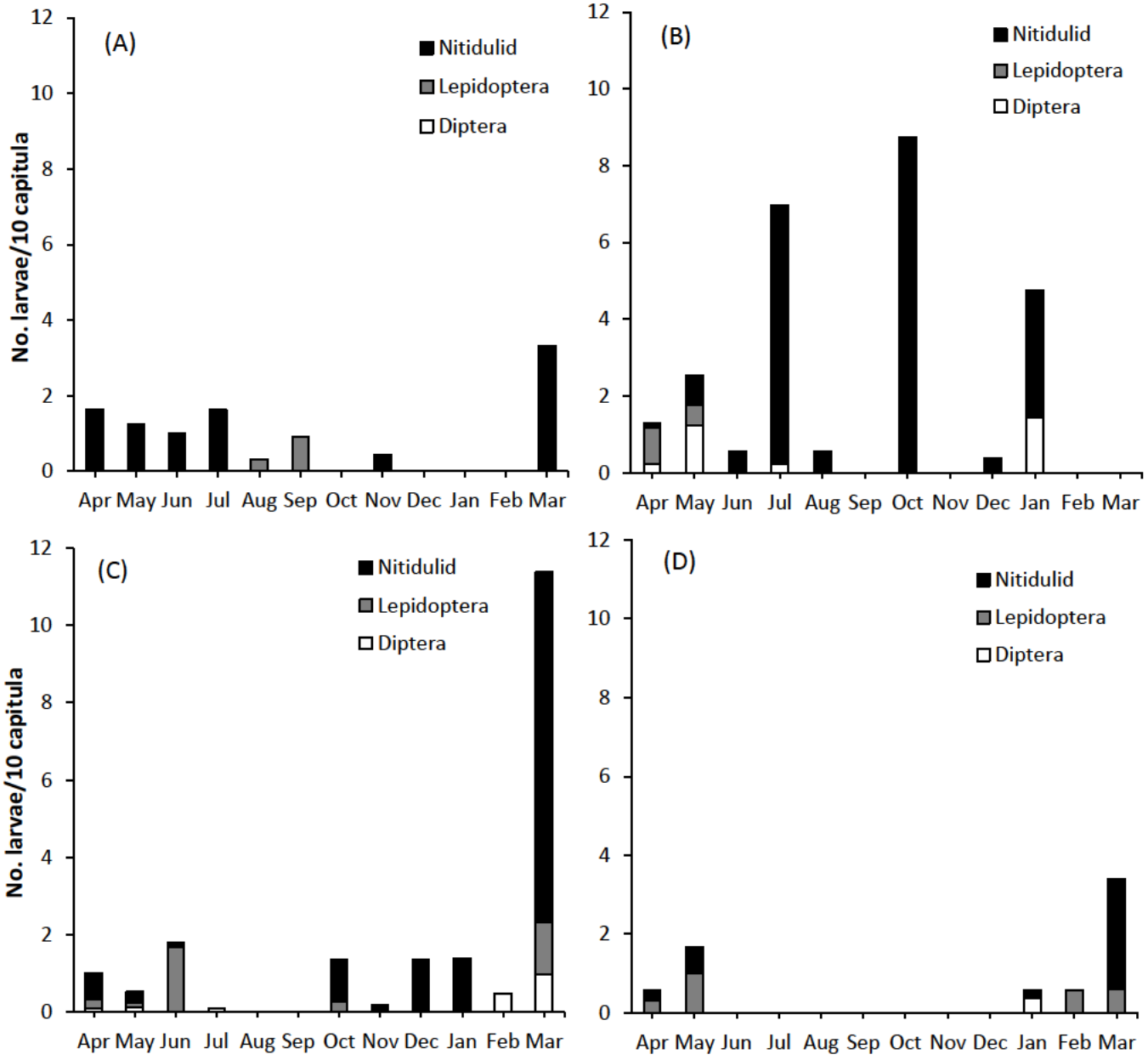


Figure 2.4. Monthly total numbers per 10 capitula of larvae from all insect taxa associated with the flowers of *Senecio madagascariensis* at the four sites, namely (A) Ukulinga, (B) Raptor Centre, (C) Cedara, and (D) Groundcover.

There were major differences in total larval loads between sites, with Cedara (Fig. 2.4C) followed by Raptor Centre (Fig. 2.4B) supporting more larvae than Ukulinga (Fig. 2.4A) and Groundcover (Fig. 2.4D). However, this pattern was more significant for Nitidulid, which were the most common of all taxa. No larvae were recorded in the flowers of *S. madagascariensis*

plants during several months, but this was not consistent across sites (Fig. 2.4). At Cedara, there were no larval recoveries in two months (August and September), compared with four months at Raptor Centre (September, November, February, and March) and Ukulinga (October, December, January, and February) and seven consecutive months (June to December) at Groundcover.

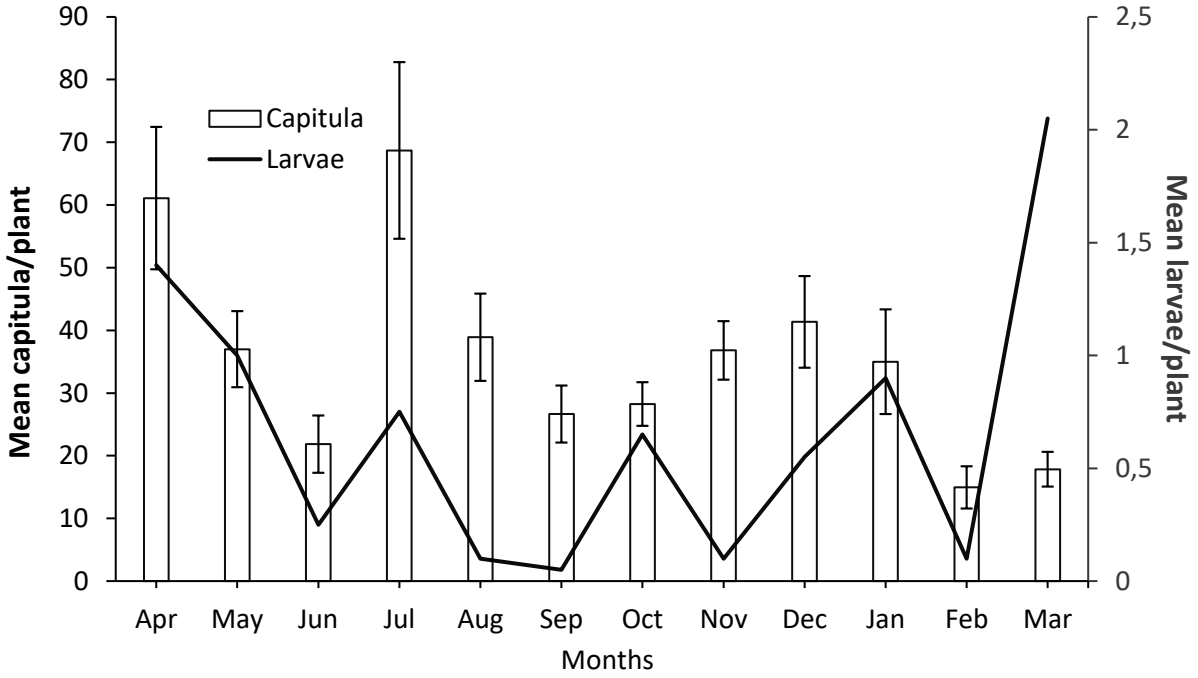


Figure 2.5. Monthly average (+SE) numbers of *Senecio madagascariensis* capitula per plant across all four sites. The line and the secondary axis (grey) represent the average numbers of capitulum-feeding larvae per plant recorded monthly at all sites.

The monthly average number of capitula per plant at all sites and the average number of capitulum-feeding larvae per plant at all sites were represented (Fig. 2.5) to determine whether larval numbers were influenced by floral abundance, or whether there were notable seasonal effects on the abundance of fireweed capitula, which are required for oviposition and larval development. There was no clear association between the abundance of capitula and the average number of capitulum-feeding larvae. Figure 2.5 illustrates that

fireweed flowers throughout the year with no clear seasonal peaks. Given that larval numbers were very low compared to flower numbers, there was no evidence of them being influenced by flower availability and the vast majority of the flowers were not infested by insect larvae (Fig. 2.5).

2.3.4.2. Adult stages reared from flowers

The immature stages of capitulum-feeding insects were reared to adulthood throughout the year for each site to indicate the taxa involved. Seven species were reared from *S. madagascariensis* capitula (Fig. 2.6), including one unidentified beetle (Nitidulidae), two moths (*Homoeosoma stenotea* Hampson (Pyralidae) and *Platyptilia* sp. Hübner (Pterophoridae) and four flies [Agromyzidae, Cecidomyiidae, and two species of *Trupanea* Schrank (Tephritidae)]. Five species were recovered at all four sites, namely the nitidulid, pyralid, agromyzid, cecidomyiid, and tephritid #1 (Fig. 2.6). The pterophorid and tephritid #2 were recovered at two sites. All seven species were recovered at the Raptor Centre, while six species were recovered at Ukulinga (all except the pterophorid) and Cedara (all except tephritid #2), with five species recovered at Groundcover (all except the pterophorid and tephritid #2) (Fig. 2.6).

Adult insects were consistently reared from the capitula across the 12 months at the four sites, although fewer specimens were reared from the material collected at Groundcover (Fig. 2.6D). Of the adults reared, the pterophorid and tephritid #2 were the least encountered. There were some differences between the sites in terms of the taxa that comprised most of the specimens reared. At Ukulinga, the most commonly reared adults comprised the agromyzid, tephritid #1 and pyralid, while at Raptor Centre these comprised the tephritid #1, pyralid and nitidulid (Fig. 2.6A, B). At Cedara, the most commonly reared adults comprised

the agromyzid, cecidomyiid and pterophorid, while at Groundcover these comprised the cecidomyiid and agromyzid (Fig. 2.6C, D).

There were several discrepancies between the monthly numbers of larvae dissected from the capitula and the numbers of adults reared from the capitula. For example, no insect larvae were recorded between June and December at the Groundcover site (Fig. 2.4D) while adults were reared from the material collected during the same time, albeit in low numbers (Fig 2.6D). Also, while nitidulid larvae were abundant at Cedara during March 2018 (Fig. 2.4C), no nitidulid adults were reared from the material collected during this month (Fig. 2.6C).

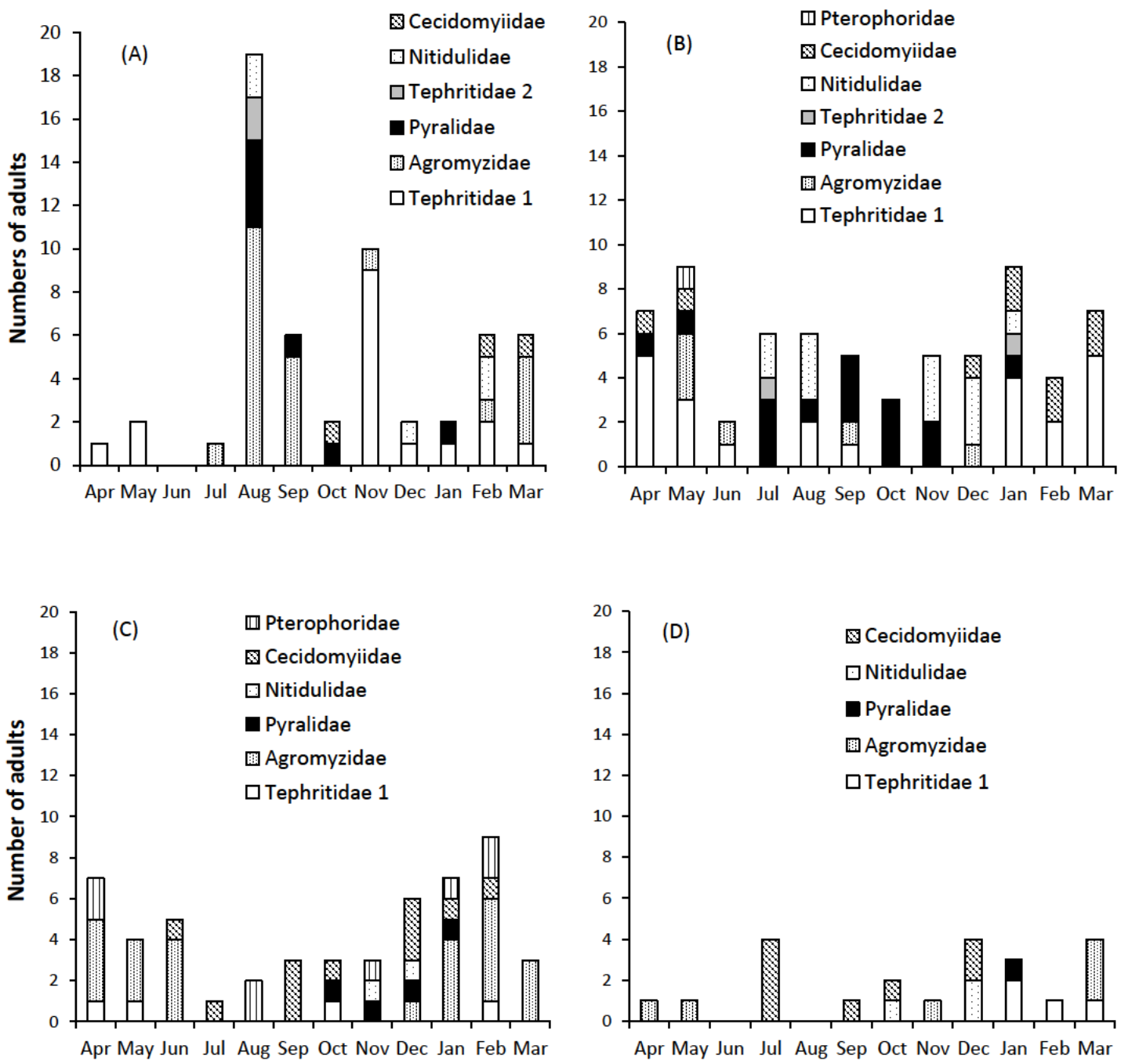


Figure 2.6. Numbers of adult individuals of capitulum-feeding taxa reared from *Senecio madagascariensis* throughout the year at the four sites, namely A) Ukulinga, B) Raptor Centre, C) Cedara, and D) Groundcover.

2.4. Discussion

Monthly sampling gave an indication of the incidence, abundance, and distribution of insect herbivores associated with the capitula of *S. madagascariensis* throughout all seasons. Such information is important for prioritisation of candidate biocontrol agents (Harris, 1973; Olckers, 1999; Sheppard, 2003). The incidence and abundance of all taxa was affected by season. This was shown in this study as a decline in larval numbers recorded in fireweed capitula across all sites during the winter months. The general influences of winter on larval abundance may be applied to the vast majority of insect taxa; hence the declining numbers that were evident in capitulum-feeding Coleoptera, Lepidoptera, and Diptera.

Frequency of occurrence of a potential biocontrol agent is crucial since it signifies the duration of feeding damage and therefore potential agent effectiveness and consequent success of the biological control program (Harris, 1973; McFadyen, 2003; McClay & Balciunas, 2005). Successful invasion in the majority of invasive weeds is attributed to prolonged flowering time (Pyšek & Richardson, 2008; van Kleunen *et al.*, 2010). As confirmed by this study, *S. madagascariensis* flowers throughout the year and thus requires sustained floral damage (Sindel *et al.*, 1998; Sheppard *et al.*, 2013). The incidence of endophagous larvae in floral material varied across insect orders, with Coleoptera (Nitidulidae) recorded the most at all sites. Lepidoptera and Diptera had substantially lower occurrences throughout the seasons compared to Nitidulidae. However, the Nitidulidae were ranked far lower than the Lepidoptera and Diptera as potential agents, due to their lower levels of damage inflicted (Egli & Olckers, 2020).

Abundance of a biocontrol agent in its native range is has been identified as an important ecological trait since it provides an estimate of population density achievable after release

into the invaded country (Cullen, 1995; McFadyen, 1998; 2003). Since the candidate biocontrol agents investigated in this study target the reproductive parts of fireweed, their impact on fireweed densities is determined by their abundance (McClay & Balciunas, 2005). Reduction in the reproductive output of the target weed in successful biological control programs has been linked to a density-dependent impact of the biocontrol agents infesting a significant proportion of flower buds, flowers, and seeds (Hoffmann & Moran, 1999; Story *et al.*, 2008; Cullen & Sheppard, 2012).

In this study, capitulum-feeding larval numbers were highest in autumn, declining in winter, and recovering from late spring to late summer, although this pattern was not depicted in the samples collected at the Groundcover site. It is likely that once average daily temperatures increase from late spring, insect development, mobility and dispersal improve thus leading to frequent adult mating and reproduction. Consequently, population sizes increase between late summer and autumn. The discrepancies between the monthly numbers of larvae dissected from the capitula and the numbers of adults reared from the capitula (see Results) suggest that actual larval numbers may have been higher than recorded for some taxa. The rearing of adults from samples in which no larvae were recorded suggest that the insects were present across more months than the larval data indicate. There are plausible explanations for such discrepancies. It is possible that immature stages were present as eggs in several samples, so that while no larvae were recorded during dissection of the frozen capitula, others were able to develop to adulthood in the emergence containers. Also, taxa that were abundant as larvae, notably those with longer development, may have suffered mortality in the emergence cages, leading to few or no specimens reared.

Although the absence of larval specimens during consecutive months may suggest that certain herbivores may not reach high densities after release, the rearing of adult specimens from plant material collected during some of these months suggests that the densities of capitulum-feeding insects were underestimated. Also, low abundance can be adequately compensated by the levels of damage inflicted on host populations (McClay & Balciunas, 2005). Such agent impact that is based on the magnitude or timing of damage, rather than on the agent's population size, is determined by the agent's capacity to exert extensive damage or lower levels of damage that are closely synchronized with the weed's most vulnerable life stage (Sheppard, 2003). However, fireweed does not seem to display periods of vulnerability in flowering since capitula were produced in all months of the year, but with lower numbers in late summer (Fig. 2.5). In addition, despite their low abundance, the lepidopteran larvae inflict the highest levels of damage on fireweed capitula (Egli & Olckers, 2020). This is because the developing larvae within the capitulum damaged the entire capitulum and destroyed the seeds (Egli & Olckers, 2020).

Agent damage that is adequately synchronised with the phenology of the weed increases the agent's overall control impact (Woodburn & Cullen, 1993; McClay & Balciunas, 2005; Sheppard *et al.*, 2005; Goolsby *et al.*, 2006). Phenological synchrony between herbivorous insects and their host plants is important for the intensity of the interaction. For flower-feeding agents, the degree of this synchrony is affected by variation in the timing of activity in the agent population and variation in the host plant's flowering time (Russell & Louda, 2004). Biological control of thistles by the capitulum weevil *Rhinocyllus conicus* has demonstrated the importance of phenological synchrony in improving the degree of agent damage on the target host (Woodburn & Cullen, 1993; Russell & Louda, 2004; Cullen & Sheppard, 2012). Due to fireweed being present throughout the year, potential capitulum-

feeding agents would adequately interact with the weed despite the substantial variation in their seasonal abundance.

The four sites also displayed differences in the numbers of capitulum-feeding insects. Although factors that might have contributed to this variation were not investigated in this study, it is possible that habitat or other differences across sites might have played a role. The four study sites were situated each at a different elevation (Table 2.1), although there was no consistent influence of elevation on larval densities and numbers of adults reared. The variable abundance and incidence of insects along an elevational gradient has been demonstrated in studies, but long-term sampling surveys have shown that there is also an influence of site disturbance and the interplay of other ecological processes (McCoy, 1990).

Changes in altitude also involve changes in environmental factors such as temperature, humidity, the presence of natural enemies, interspecific competition, and the abundance and quality of host plants. Therefore, variation in the abundance of capitulum-feeding insects associated with *S. madagascariensis* across the four sites could be explained by these variables. Although Groundcover, which is located at the highest altitude, displayed the lowest larval herbivore loads throughout all seasons, the trend was not consistent. Cedara, which is located at the second highest altitude, displayed higher or similar monthly larval herbivore loads than the two lowest altitude sites.

The variation in environmental conditions across field sites influences insect composition and abundance. *Trupanea* sp. 2 (Tephritidae 2) and *Platyptilia* sp. (Pterophoridae) were the least encountered species and were recovered in low numbers at only two sites. The unidentified nitidulid had the highest numbers of larvae collected across all four sites compared to the numbers of Lepidoptera and Diptera (Figure 2.4). These differences are presumably

determined by various factors that include the insects' habitat preferences, climatic tolerances and response to interspecific competition.

The taxonomy and feeding guild of an agent has been implicated in predicting its potential effectiveness, and this has been emphasized in literature on the criteria for agent selection (Harris, 1973; Crawley, 1989; McClay & Balciunas, 2005; Egli & Olckers, 2017). In a study on the effectiveness of biocontrol agents released against Asteraceae weeds, Egli & Olckers (2017) noted that Diptera and Lepidoptera provided most of the capitulum-feeding agents, with few Lepidoptera used. However, capitulum-feeding taxa like Curculionidae (not represented on fireweed) and Tephritidae (present on fireweed) were the most successful.

Although the unidentified nitidulid was the most common and abundant flower feeder, there are no precedents of Nitidulidae being used as biocontrol agents, possibly because of low levels of floral damage, such as observed on fireweed (Egli & Olckers, 2020). In contrast, the pyralid moth *H. stenotea*, despite the lack of biocontrol precedents, was present across several months and sites. Since the moth is particularly damaging to fireweed capitula, it was ranked as the most promising capitulum-feeding candidate (Egli & Olckers, 2020). The tephritid *Trupanea* sp. 1 also displayed a similar abundance and is sufficiently damaging (Egli & Olckers, 2020) to be considered as a potential agent.

Although this study has provided some insights into the insect herbivore fauna associated with fireweed capitula, it is acknowledged that native range studies for potential biocontrol agents cannot fully predict their performance after release into the new country (Sheppard, 2003; McClay & Balciunas, 2005). Aspects like the population size of the host target weed and natural enemies of the agents may be limiting the abundance and distribution of potential

biocontrol agents in their native range (Morin *et al.*, 2009) and thus underestimate their potential.

Several parasitoids that include species of Braconidae, Chalcidoidea, Eurytomidae and Ichneumonidae (all Hymenoptera) were consistently reared from fireweed capitula during the monthly surveys, providing some evidence of population suppression. There are examples of biocontrol agents that have performed well in their introduced range despite low abundance in their native range, as a result of escape from natural enemies (Winston *et al.*, 2014). Hence, native studies that demonstrate a low abundance and distribution of candidate agents should not necessarily be used to reject candidate agents. Native host range, which is the subject of the next chapter, is a more accurate predictor of agent suitability.

Chapter 3: Field host range of capitulum-feeding insects associated with *Senecio madagascariensis* revealed through DNA barcoding

3.1. Introduction

The biological control of weeds by arthropods and plant pathogens has been supported as an environmentally friendly and safe control method (McFadyen, 1998). Detailed preliminary studies in the weed's native country can be used to determine an agent's range of suitable host plants, improve risk assessments by predicting damage to non-target plants, and avoid the importation of unsuitable agents (Paynter *et al.*, 2018). Several reviews on biological control principles assert that the safety and release of a biocontrol agent is dependent on its host specificity (McFadyen, 1998; Paynter *et al.*, 2018).

Non-target effects have been the principal concern regarding the safety of the introduction of exotic species associated with this discipline (Louda *et al.*, 1997; Suckling & Sforza, 2014). Non-target plants that are of a greater concern are species closely related to the weed and that are of economic or ecological (e.g., endemic) importance (Wapshere, 1974). Hence, the host range of a biocontrol agent is tested by exposure to closely related non-target plants (test plants) to determine feeding, survivorship, and oviposition. Test plants may also include distantly related plants that occupy the same habitat as the target weed and are similar in morphology or overlap in the range of the potential agents hosted by the weed (Schaffner, 2001).

Host-specificity testing aims to establish the fundamental host range of an agent, particularly in light of evolutionary patterns in host selection or host shifting (McFadyen, 1998). In laboratory testing, test plants are exposed to the candidate agents in cages, in a series of scenarios (i.e., no-choice versus choice) to determine their survival and reproductive

performance on the different test plants relative to the target weed (McFadyen, 1998). It is predicted that non-target attacks are likely to occur in the field if the agent is able to complete its life cycle and reproduce on several non-target hosts (Blossey, 1995).

Despite the rigorous approach in determining the host range of a potential agent, the interpretation of laboratory host-range testing needs caution and cannot fully predict agent response in field conditions (Olckers *et al.*, 1995; Marohasy, 1998). This is because insects often display a broader host range during laboratory tests than is observed in the field. For instance, *Ceratapion basicorne* (Illiger) (Coleoptera: Curculionidae) was rejected for release against yellow star thistle *Centaurea solstitialis* L. (Asteraceae) in the USA because it developed on safflower during no-choice tests, although field experiments conducted in three countries have demonstrated that the agent shows no risk of non-target damage on safflower (Louda *et al.*, 2003; Cristofaro *et al.*, 2013).

The lack of discrete consistency between host selection during laboratory no-choice tests and that observed in the field is influenced by the patterns of insect host searching and selection behaviour in the field. In particular, the confining laboratory conditions promote feeding and oviposition on plants that are not natural hosts in the field, where the primary host (target weed) would be present (Marohasy, 1998; Schaffner *et al.*, 2018). Contrary to laboratory testing, field surveys and open-field tests have been proposed to prevent the rejection of agents that would be safe for release (Briese, 1999; Briese *et al.*, 2002). Open-field conditions allow the agent to exhibit its natural host selection behaviour, including the benefit of dispersal in locating the most preferred host (Briese *et al.*, 2002; Schaffner *et al.*, 2018).

Senecio madagascariensis (Poir.) (Asteraceae), known as fireweed, is native to southern Africa and Madagascar (Hilliard, 1977), but has been introduced to several other countries and has

become invasive (Sindel *et al.*, 1998). The weed has become a target for biological control in Australia and Hawaii (USA). In Australia, there is a diverse native *Senecio* flora (Sindel & Michael, 1992; McFadyen & Sparks, 1996), while in Hawaii, all species in the tribe *Senecio*neae are exotic and many are invasive (Ramadan *et al.*, 2011). Biological control of *S. madagascariensis* in Australia thus requires the release of highly host specific agents (Sheppard *et al.*, 2013), while the requirements for host specificity in Hawaii are less stringent (Ramadan et al., 2011).

Field surveys in the weed's native range and sampling of its closely related species can help elucidate agent-host associations (Goolsby *et al.*, 2006). Several native *Senecio* species were sampled to determine whether the insects associated with fireweed flowers also utilize additional *Senecio* species as hosts. The presence of immature stages confirms the use of a plant species as a host. However, it is often difficult to distinguish between the immature stages of different insect species collected on different host plants. DNA barcoding (Hebert *et al.*, 2003; Jurado-Rivera *et al.*, 2009) can be used in such cases to confirm species identifications.

DNA barcoding identifies species using short segments of DNA from regions on sequences (genes) that express low variation amongst individuals of the same species (Jurado-Rivera et al., 2009). It has been useful in determining insect-host affiliations (Egli *et al.*, 2020). This approach compares the DNA barcodes of insects collected across a range of host plants in the field and shows the occurrence of the same insect species on different host plants and can thus determine the host range of a potential agent (Egli *et al.*, 2020). Moreover, DNA barcoding is useful during host specificity testing in cases where a test plant is not a suitable host and thus larvae cannot complete development.

This study uses this method to assess the field host range of capitulum-feeding insects associated with *Senecio madagascariensis* populations in KwaZulu-Natal Province, South Africa.

3.2. Materials and methods

3.2.1. Study species and study sites

Senecio L. is a genus of Asteraceae, with 129 species identified in KwaZulu-Natal Province (Hilliard, 1977). Some species are morphologically similar and thus challenging to identify, but can be distinguished through their occurrence in different types of habitats (Egli *et al.*, 2020). Plants were identified as far as possible using the dichotomous key in Hilliard (1977), though not all plants could be identified. The study was conducted at various sites in KwaZulu-Natal that supported healthy populations of *S. madagascariensis* and of other *Senecio* species. Surveys were conducted on 35 *Senecio* species, including *S. madagascariensis*, and the related *Cineraria lyratiformis* Cron. between 2017 and 2020, while earlier samples collected in 2014-2015 were also analysed (Table 3.1).

Table 3.1. Plant species sampled for testing the field host range of capitulum-feeding insects, their sampling dates and sampling locations.

Species	Date	Site	GPS Coordinates	Habitat
Senecio adnatus DC.	25-11-2014	Vernon Crooks Nature Reserve	30°26'48''S 30°59'51''E	Grassland
	22-12-2014	Mount Gilboa	29°28'56''S 30°29'27''E	Rocky grassland
	05-03-2018	Mount Gilboa	29°28'57''S 30°29'28''E	Rocky grassland
	12-12-2019	Karkloof	29°26'31''S 30°34'53''E	Roadside
	28-01-2020	Groundcover	29°39'14''S	Roadside

			30°17'76''E	
Senecio affinis DC.	20-01-2014	Bellvue	29°63'56''S 30°43'47''E	Savanna
	08-01-2015	Ukulinga Research Farm	29°66'68''S 30°40'32''E	Grassland
	08-01-2015	Emanzini Private Reserve	29°46'85''S 30°37'10''E	Grassland
	27-03-2018	Cumberland Nature Reserve	29°51'87''S 30°51'45''E	Grassland
Senecio bupleuroides DC.	20-01-2014	Bellvue	29°63'56''S 30°43'37''E	Savanna
	12-11-2014	Camperdown, Ingomankulu	29°76'92''S 30°47'12''E	Grassland
	26-11-2014	Pietermaritzburg, Bishopstowe	29°57'63''S 30°43'07''E	Roadside
	29-11-2014	Sani Pass Hotel	29°66'75''S 29°45'83''E	Grassland
Senecio brevidentatus M.D.Hend	05-01-2015	Himeville	29°60'43''S 29°34'53''E	Roadside
Senecio conrathii N.E.Br	09-12-2014	Boston	29°76'13''S 30°13'74''E	Grassland
	05-12-2019	Mooi River	29°24'77''S 29°99'58''E	Grassland
Senecio coronatus (Thumb.) Harv.	12-11-2014	Ukulinga Research Farm	29°66'68''S 30°40'32''E	Grassland
	12-12-2014	Emanzini Private Reserve	29°48'48''S 30°36'45''E	Grassland
	13-12-2017	Ashburton	29°67'93''S 30°50'84''E	Grassland
	06-03-2018	Howick, Umngeni Valley	29°47'60''S 30°24'49''E	Grassland
Senecio decurrens DC.	02-12-2014	Karkloof	29°34’61’’S 30°29’21’’E	Grassland
Senecio glaberrimus DC.	22-11-2014	Giants Castle	29°04'85''S 29°42'52''E	Roadside
	22-12-2014	Mount Gilboa	29°28'56''S 30°29'27''E	Rocky grassland
	05-03-2018	Mount Gilboa	29°28'57''S 30°29'28''E	Rocky grassland

	12-12-2019	Karkloof	29°26'31''S 30°34'53''E	Roadside
	12-12-2019	Mount Gilboa	29°26'38''S 30°30'04''E	Rocky grassland
Senecio heliopsis Hilliard & B.L. Burtt	22-12-2014	Mount Gilboa	29°28'56''S 30°29'27''E	Rocky grassland
Senecio humidanus C.Jeffrey	05-01-2015	Himeville	29°64'63''S 29°42'96''E	Roadside
	13-01-2015	Curry's Post	29°42'15''S 30°18'19''E	Grassland
Senecio inaequidens DC.	22-11-2014	Giants Castle	29°04'95''S 29°40'42''E	Roadside
	02-02-2018	Impendle	29°52'31''S 29°64'61''E	Roadside
	02-02-2018	Impendle	29°45'25''S 29°76'53''E	Roadside
	02-02-2018	Nottingham Road	29°38'50''S 29°90'22''E	Roadside
Senecio inornatus DC.	18-02-2014	Emanzini Private Reserve	29°46'85''S 30°37'10''E	Savanna
	09-12-2014	Boston	29°76'13''S 30°13'74''E	Grassland
	22-12-2014	Mount Gilboa	29°28'56''S 30°29'27''E	Rocky grassland
	08-02-2015	Emanzini Private Reserve	29°46'85''S 30°37'10''E	Grassland
	20-02-2018	Fort Nottingham	29°40'31''S 29°86'44''E	Roadside
	20-02-2018	Himeville	29°64'63''S 29°54'61''E	Roadside
Senecio isatidioides Phill. & Sm.	12-12-2019	Karkloof	29°25'79''S 30°33'93''E	Roadside
	12-12-2019	Mount Gilboa	29°24'52''S 30°27'96''E	Rocky grassland
Senecio madagascariensis Poir.	21-01-2014	Hillcrest	29°75'85''S 30°78'29''E	Farmland
	21-01-2014	Summerveld	29°80'22''S 30°70'95''E	Roadside

	07-02-2014	Pietermaritzburg	29°62'62''S 30°39'66''E	Grassland
	13-02-2014	Nottingham Road	29°38'60''S 30°03'94''E	Grassland
	14-02-2014	Wedgewood Nougat	29°55'02''S 30°25'26''E	Cattle farm
	16-02-2014	Emanzini Private Reserve	29°46'85''S 30°37'10''E	Savanna
	15-04-2014	Wartburg	29°47'83''S 30°41'76''E	Cattle farm
	26-04-2014	Southbroom	30°91'96''S 30°32'45''E	Roadside
	24-09-2014	Hluhluwe	28°02'24''S 32°27'42''E	Roadside
	13-01-2015	Curry's Post	29°42'15''S 30°18'19''E	Grassland
	12-12-2019	Mooi River	29°24'77''S 29°99'58''E	Grassland
Senecio oxyriifolius DC.	09-12-2014	Boston	29°76'13''S 30°13'74''E	Rocky grassland
	22-12-2014	Mount Gilboa	29°28'56''S 30°29'27''E	Rocky grassland
Senecio panduriformis Hilliard	04-04-2018	Elandskop, Sevontein Prison	29°76'89''S 30°16'16''E	Grassland
Senecio polyanthemoides Sch. Bip.	21-01-2014	Hillcrest	29°75'85''S 30°78'29''E	Farmland
	22-01-2014	Ashburton Raptor Centre	29°67'54''S 30°51'35''E	Grassland
	27-01-2014	Port Edward, Izingolweni Rd	31°05'20''S 30°19'77''E	Roadside
	14-02-2014	Hilton, Wedgewood	29°55'02''S 30°25'26''E	Cattle farm
	27-04-2014	Palm Beach	30°99'21''S 30°26'17''E	Roadside
	13-01-2015	Curry's Post	29°42'15''S 30°18'19''E	Grassland
	16-01-2018	Curry's Post	29°38'86''S 30°17'51''E	Grassland

	06--03-2018	Howick, Umngeni Valley	29°47'60''S 30°24'49''E	Grassland
	13-03-2018	Hilton	29°50'82''S 30°30'89''E	Grassland
	04-04-2018	Elandskop, Sevontein Prison	29°76'43''S 30°14'58''E	Pine forest
	12-12-2019	Mount Gilboa	29°27'04''S 30°28'93''E	Rocky grassland
Senecio polyodon DC.	02-12-2014	Karkloof	29°34’61’’S 30°29’21’’E	Grassland
Senecio retrorsus DC.	20-01-2014	Bellvue	29°63'56''S 30°43'37''E	Savanna
	12-02-2015	Howick, Umngeni Valley	29°47'59''S 30°24'67''E	Grassland
	13-12-2017	Ashburton	29°67'93''S 30°50'84''E	Grassland
Senecio rhomboideus Harv.	05-01-2015	Himeville	29°60'43''S 29°34'53''E	Roadside
Senecio scitus Hutch. & Burtt Davy	29-11-2014	Sani Pass Hotel	29°66'75''S 29°45'83''E	Grassland
	04-01-2015	Garden Castle Reserve	29°75'54''S 29°22'93''E	Grassland
	05-01-2015	Himeville	29°60'43''S 29°34'53''E	Roadside
Senecio scoparius Harv.	12-02-2015	Howick, Umngeni Valley	29°47'59''S 30°24'67''E	Grassland
Senecio serratuloides DC.	13-03-2018	Hilton	29°50'82''S 30°30'89''E	Marshy area
	04-04-2018	Elandskop, Sevontein Prison	29°75'64''S 30°15'63''E	Marshy area
Senecio skirrhodon DC.	13-04-2014	Mthwalume	30°49'96''S 30°62'97''E	Edge of beach sand
	01-02-2015	Hibberdine	30°57'47''S 30°57'53''E	Roadside
	06-02-2018	Mthwalume	30°49'12''S 30°63'34''E	Sand dunes
Senecio sp. nr. *adnatus* DC.	16-01-2018	Curry’s Post	29°39'18''S 30°17'78''E	Roadside
	02-02-2018	Impendle	29°45'25''S	Roadside

			29°76'53''E	
Senecio sp. nr. *adnatus 2*	04-01-2015	Garden Castle Reserve	29°75'54''S 29°22'93''E	Grassland
	05-01-2015	Garden Castle Reserve	29°75'54''S 29°22'93''E	Grassland
	05-01-2015	Himeville	29°60'43''S 29°34'53''E	Roadside
Senecio sp. nr. *affinis* DC.	06-03-2018	Howick, Umngeni Valley	29°47'95''S 30°24'77''E	Grassland
Senecio sp. nr. *conrathii* N.E.Br	16-01-2018	Curry's Post	29°39'35''S 30°17'43''E	Grassland
	13-03-2018	Hilton	29°50'32''S 30°31'03''E	Roadside
Senecio sp. nr. *hastatus* L.	29-11-2014	Sani Pass Hotel	29°66'75''S 29°45'83''E	Grassland
Senecio sp nr *serratuloides* DC.	13-03-2018	Hilton	29°50'82''S 30°30'89''E	Marshy area
	04-04-2018	Elandskop, Sevontein Prison	29°74'63''S 30°15'47''E	Marshy area
Senecio striatifolius DC.	02-12-2014	Karkloof	29°34'61''S 30°29'21''E	Grassland
Senecio urophyllus Conrath.	05-01-2015	Himeville	29°62'83''S 29°40'59''E	Roadside
Senecio sp. *1*	12-11-2014	Camperdown Ingomankulu	29°76'92''S 30°47'12''E	Grassland
Senecio sp. *2*	29-11-2014	Sani Pass Hotel	29°66'75''S 29°45'83''E	Grassland
Senecio sp. *3*	12-02-2015	Howick Umngeni Valley	29°47'59''S 30°24'67''E	Grassland
Cineraria lyratiformis Cron.	13-02-2014	Nottingham Road	29°38'60''S 30°03'94''E	Grassland
	29-01-2020	Mooi River	29°24'77''S 29°99'58''E	Grassland

3.2.2. Sampling procedure

On each sampling occasion, five to 13 healthy plants per species were collected and individually placed into a brown paper bag and frozen until processing. One voucher plant specimen was pressed per species for identification and preservation at the John Bews Herbarium (NU). In the laboratory, the inflorescences were removed from each plant (see Table 3.2) and inspected under a dissecting microscope to record the immature stages of all endophagous capitulum-feeding taxa. The larvae were placed in glass vials containing 100% alcohol and the number of coleopteran, dipteran, and lepidopteran larvae per host plant and corresponding site were recorded. Larvae collected from the monthly fireweed samples and adults reared from the flowers (Chapter 2) were stored in 100% alcohol and were included in the analysis (see below).

Table 3.2. Number of plants sampled and total number of capitula inspected for each of the *Senecio* species and *C. lyratiformis* throughout the sampling periods (2017-2020).

Species	**Number of plants**	**Total capitula inspected**
Senecio adnatus DC.	22	1088
Senecio affinis DC.	11	506
Senecio bupleuroides DC.	9	278
Senecio brevidentatus M.D.Hend	1	3
Senecio conrathii N.E.Br	9	170
Senecio coronatus (Thumb.) Harv.	23	273
Senecio decurrens DC.	9	50
Senecio glaberrimus DC.	23	740
Senecio heliopsis Hilliard & B.L. Burtt	1	67

Senecio humidanus C.Jeffrey	2	96
Senecio inaequidens DC.	18	354
Senecio inornatus DC.	31	1511
Senecio isatidioides Phill. & Sm.	10	637
Senecio madagascariensis Poir.	89	3002
Senecio oxyriifolius DC.	12	386
Senecio panduriformis Hilliard	5	198
Senecio polyanthemoides Sch. Bip.	43	2150
Senecio polyodon DC.	10	281
Senecio retrorsus DC.	9	309
Senecio rhomboideus Harv.	1	42
Senecio scitus Hutch. & Burtt Davy	3	105
Senecio scoparius Harv.	3	196
Senecio serratuloides DC.	12	741
Senecio skirrhodon DC.	8	123
Senecio sp. nr. *adnatus* DC.	11	915
Senecio sp. nr. *adnatus* 2	5	342
Senecio sp. nr. *affinis* DC.	6	325
Senecio sp. nr. *conrathii* N.E.Br	11	118
Senecio sp. nr. *hastatus* L.	11	48
Senecio sp. nr. *serratuloides* DC.	10	418
Senecio striatifolius DC.	10	74
Senecio urophyllus Conrath.	1	142
Senecio sp. 1	12	69

Senecio sp. 2	4	139
Senecio sp. 3	5	94
Cineraria lyratiformis Cron.	15	305

3.2.3. Genetic barcoding

An image of each insect specimen (for all life stages) was taken using stereo imaging (equipment by Leica Microsystems) before DNA extraction and sequencing. These images were later uploaded to the Barcode of Life Database (BOLD). Insect specimens were loaded into a 96-well microplate with 100% alcohol and then sent to the Canadian Centre for DNA Barcoding, at the University of Guelph, Canada, for DNA extraction, amplification, and Sanger sequencing of the cytochrome oxidase I (COI) gene using standardized protocols (Hajibabaei *et al.*, 2005). All larval specimens of Lepidoptera and Diptera collected from all *Senecio* plants and across all sites were prepared and sent for barcoding. Larvae of Nitidulidae (Coleoptera) were not sent for barcoding due to their low status as potential agents (Egli & Olckers, 2020). COI barcode compliant sequences that were generated (n = 74) were deposited in BOLD (see Table 3.3).

Table 3.3. BOLD accession numbers for sequences of the COI gene from specimens of the two insect orders.

	Lepidoptera			**Diptera**	
#	**Sample ID**	**BOLD Accession number**	**#**	**Sample ID**	**BOLD Accession number**
1	Lep001	SABCI514-20	1	Dip002	SABCI482-20
2	Lep002	SABCI515-20	2	Dip003	SABCI483-20
3	Lep003	SABCI516-20	3	Dip004	SABCI484-20
4	Lep005	SABCI518-20	4	Dip005	SABCI485-20
5	Lep006	SABCI519-20	5	Dip007	SABCI487-20

6	Lep009	SABCI522-20	6	Dip009	SABCI489-20
7	Lep010	SABCI523-20	7	Dip010	SABCI490-20
8	Lep011	SABCI524-20	8	Dip011	SABCI491-20
9	Lep012	SABCI525-20	9	Dip013	SABCI492-20
10	Lep013	SABCI526-20	10	Dip014	SABCI493-20
11	Lep014	SABCI527-20	11	Dip015	SABCI494-20
12	Lep016	SABCI529-20	12	Dip021	SABCI499-20
13	Lep017	SABCI530-20	13	Dip022	SABCI500-20
14	Lep018	SABCI531-20	14	Dip023	SABCI501-20
15	Lep019	SABCI532-20	15	Dip024	SABCI502-20
16	Lep021	SABCI534-20	16	Dip028	SABCI503-20
17	Lep022	SABCI535-20	17	Dip029	SABCI504-20
18	Lep023	SABCI536-20	18	Dip033	SABCI505-20
19	Lep024	SABCI537-20	19	Dip036	SABCI506-20
20	Lep028	SABCI540-20	20	Dip037	SABCI507-20
21	Lep029	SABCI541-20	21	Dip039	SABCI508-20
22	Lep030	SABCI542-20	22	Dip041	SABCI509-20
23	Lep031	SABCI543-20	23	Dip048	SABCI510-20
24	Lep032	SABCI544-20	24	Dip049	SABCI511-20
25	Lep033	SABCI545-20	25	Dip058	SABCI512-20
26	Lep034	SABCI539-20	26	Dip059	SABCI513-20
27	Lep035	SABCI546-20			
28	Lep036	SABCI547-20			
29	Lep037	SABCI548-20			
30	Lep038	SABCI549-20			

31	Lep039	SABCI550-20
32	Lep043	SABCI554-20
33	Lep044	SABCI555-20
34	Lep045	SABCI556-20
35	Lep047	SABCI558-20
36	Lep048	SABCI559-20
37	Lep049	SABCI560-20
38	Lep050	SABCI561-20
39	Lep051	SABCI562-20
40	Lep052	SABCI563-20
41	Lep055	SABCI564-20
42	Lep056	SABCI565-20
43	Lep057	SABCI566-20
44	Lep058	SABCI567-20
45	Lep060	SABCI568-20
46	Lep061	SABCI569-20
47	Lep062	SABCI570-20
48	Lep063	SABCI571-20

The COI sequence data for the Lepidoptera and Diptera data sets were downloaded from BOLD and each aligned using CLUSTALW (version 2.1) in BioEdit 7 (Hall, 1999). Final alignments excluded insertion/deletion polymorphisms (indels). Phylogenies were first inferred using the maximum likelihood approach using GARLI 2.0 (Zwickl, 2006). The best-fit model of nucleotide substitution was selected using the corrected Aikaike Information Criterion (AICc) in jModelTest2 (Darriba *et al.*, 2012). Selected models were GTR + I + G for

Lepidoptera and GTR + I for Diptera. Branch support for maximum likelihood was estimated using 1000 bootstrap replicates and the consensus tree was created in Phylip 3.69 (Falsenstein, 2005). Bootstrap values were annotated onto the most likely phylogeny.

Phylogenies were also inferred using Bayesian inference performed in MrBayes 3.2.2 (Ronquist *et al.*, 2012). Four Markov Chain Monte Carlo (MCMC) chains were run for 20 million generations to estimate the posterior distribution. Trees were sampled every 300 generations. For each of the two insect orders, two runs were conducted simultaneously. After 20 million generations, the convergence of chains was determined by using Tracer 1.7 (Rambaut *et al.*, 2018). Convergence was considered to have occurred when Effective Sample Size (ESS) values were all above 200. The first 2 million trees were discarded as burn-in and the trees in the remaining posterior distribution were used to estimate a 50% majority rule consensus tree in Phylip 3.69. Branch support (posterior probabilities) were also calculated from the posterior distribution.

Trees generated using the two optimality criteria (maximum likelihood and Bayesian inference) were compared for consistency and thereafter, all branch support values (bootstrap values and posterior probability values) were annotated onto the most likely phylogenetic tree using FIGTREE 1.4.4 (Rambaut, 2009). For maximum likelihood, percentages below 60% were not considered and Bayesian probabilities below 0.7 were excluded as branch support. All phylogenies were mid-point rooted.

Lepidoptera and Diptera specimens were assigned to Barcode Index Numbers (BINs) through the clustering algorithm implemented in BOLD. BINs functionally represent operational taxonomic units (OTUs) as they contain grouped specimens based on sequence similarity and are thus representative of distinct species. Hence, in the absence of taxonomic identity, BIN

clustering is important for classification of specimens to species level using the DNA barcode data (Ratnasingham & Hebert, 2007).

3.3. Results

3.3.1. Capitulum-feeding Lepidoptera

The most likely phylogenetic tree (Fig. 3.1) constituted a total of 48 lepidopteran specimens that were successfully sequenced, and these represented all the lepidopteran specimens recorded from monthly samples (Chapter 2) and host range samples. A total of 36 *Senecio* species were sampled, with capitulum-feeding Lepidoptera recorded from eight species, namely *Senecio affinis*, *S. inaequidens*, *S. inornatus*, *S. madagascariensis*, *S. polyanthemoides*, *S. polyodon*, *S.* sp. nr. *conrathii*, and *Senecio* sp. 3. *Senecio madagascariensis* supported the most lepidopteran specimens, followed by *S. inaequidens*. A total of eight distinct genetic lineages (BINs) were recorded, with most specimens belonging to two main BINs (labelled BIN 1 and BIN 4 in this study).

The first lineage (BIN 1) included 23 lepidopteran specimens collected from four *Senecio* species (Fig. 3.1) that were mostly found on *S. madagascariensis* (13) and *S. inaequidens* (6), but also *Senecio* sp. *3* (3) and *S. polyanthemoides* (1). Five of the 23 specimens of BIN 1 were adults and the rest were larvae. The five adults were identified as *Homoeosoma stenotea* Hampson (Pyralidae). BIN 2 was restricted to *S. madagascariensis* and was represented by three adult specimens while BIN 3 was a single adult lepidopteran collected on *S. madagascariensis*.

BIN 4 was the second most recorded lineage and was represented by 12 specimens that were associated with only two *Senecio* species, principally *S. madagascariensis* and *S. polyodon* (Fig. 3.1). Three of the 12 insect specimens were adults recorded from fireweed and were

identified as belonging to the genus *Platyptilia* Hübner (Pterophoridae). The remaining four lineages were represented by only a few specimens (Fig. 3.1). BIN 5 comprised only four larvae, all restricted to *S. madagascariensis*. BIN 6 was represented by only two larval specimens that were both recovered from *S. affinis*. BIN 7 was a single specimen collected on *S.* sp. nr*. conrathii*. Based on branch support on the phylogeny (Fig. 3.1), BIN 5, BIN 6, and BIN 7 form a monophyletic lineage and could represent closely related taxa. BIN 8 was represented by two larval specimens, one from *S. inaequidens* and another from *S. inornatus*.

Senecio madagascariensis hosted a total of five out of the eight lepidopteran lineages derived from this study. *Senecio inaequidens* was a host to two lineages, BIN 1 and BIN 8, with BIN 8 not recorded on fireweed. The other six *Senecio* species, namely *S. affinis*, *S. inornatus*, *S. polyanthemoides*, *S. polyodon*, *Senecio* sp. 3, and *S.* sp. nr. *conrathii*, each hosted a single lepidopteran lineage, with two lineages (BIN 1, BIN 4) shared with *S. madagascariensis*.

3.3.2. Capitulum-feeding Diptera

The phylogeny (Fig. 3.2) includes a total of 26 dipteran specimens that were successfully sequenced. Capitulum-feeding larvae and adults were recorded from only two out of the 36 *Senecio* species sampled, namely *S. madagascariensis* and *S. polyanthemoides*. With the exception of one larval specimen recorded from *S. polyanthemoides*, all specimens originated from fireweed. A total of three dipteran lineages were derived from the sequences. The first dipteran lineage (BIN 1), which was identified a species of Agromyzidae, was represented by 20 specimens with 19 recorded from fireweed and one from *S. polyanthemoides*.

The remaining two lineages were identified as species in the genus *Trupanea* Schrank (Tephritidae). BIN 2 was recorded from *S. madagascariensis* and was represented by five adult specimens that were reared from this species during the seasonal surveys. Similarly, BIN 3

was represented by a single specimen reared from fireweed. During the host range survey, all dipteran larvae recorded were agromyzids and no tephritids were recorded on any of the *Senecio* species.

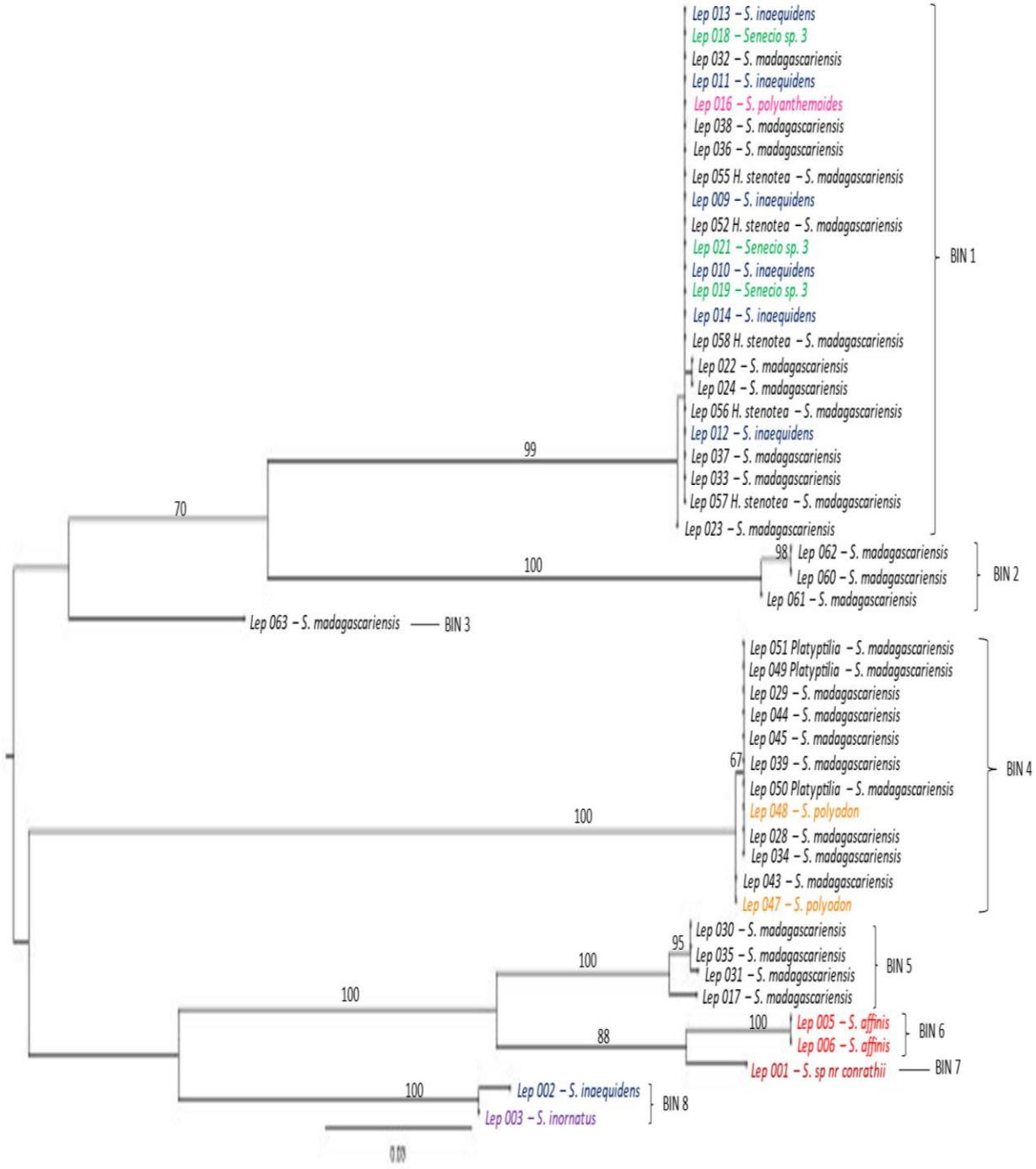


Figure 3.1. Mid-point rooted maximum likelihood COI phylogeny for capitulum-feeding Lepidoptera. Branch values represent bootstrap values and Bayesian posterior probabilities. Only bootstrap values greater than 65% and probability values greater than 0.5 are presented. Branch lengths are proportional to the number of substitutions, which are indicated by the scale bar. *Senecio* host plants are indicated at the end of the branches. Different Barcode Index Numbers (BINs) indicate different lineages (likely species).

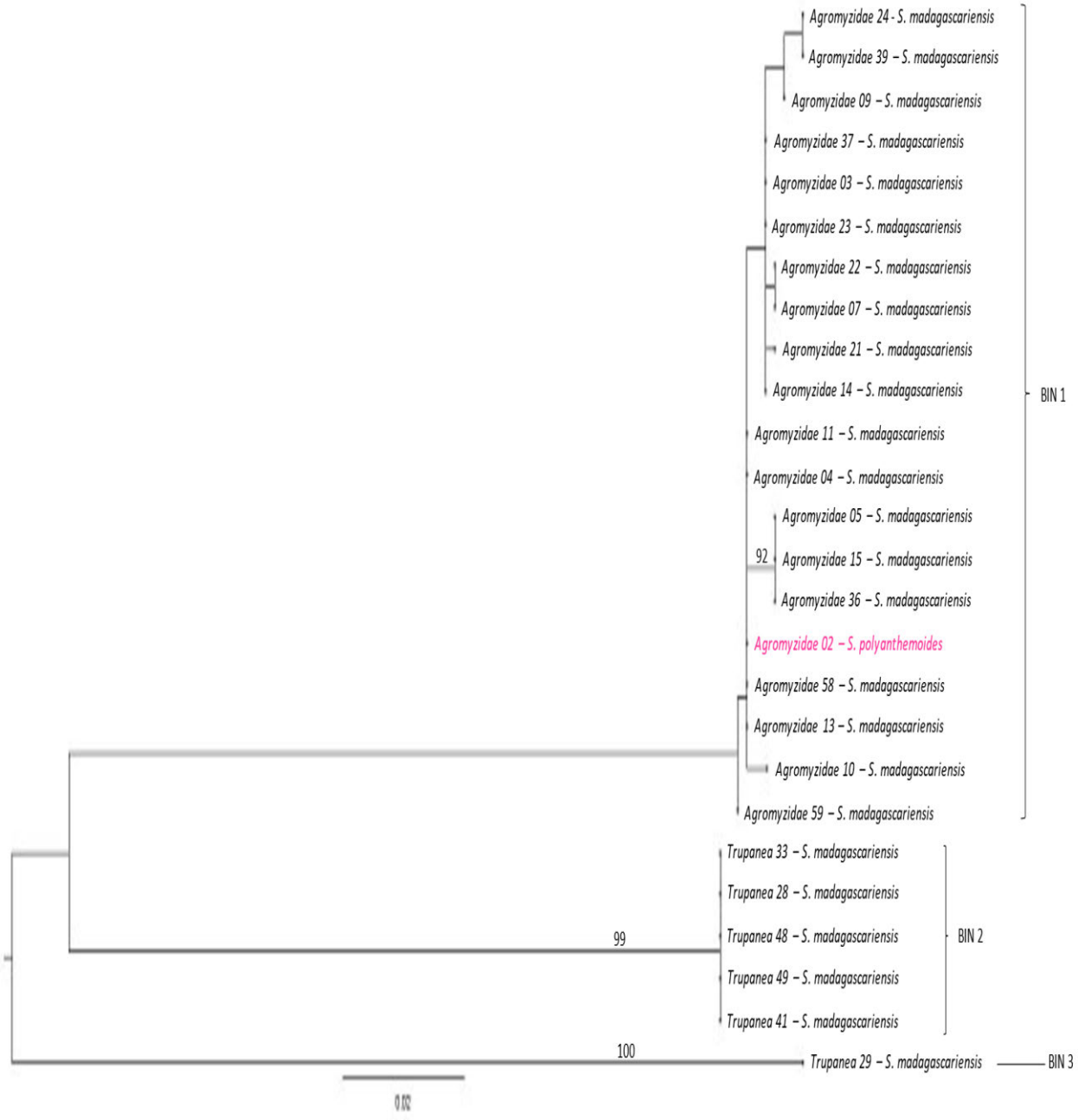


Figure 3.2. Mid-point rooted maximum likelihood COI phylogeny for capitulum-feeding Diptera. Branch values represent bootstrap values and Bayesian posterior probabilities. Only bootstrap values greater than 65% and probability values greater than 0.5 are presented. Branch lengths are proportional to the number of substitutions, which are indicated by the scale bar. *Senecio* host plants are indicated at the end of the branches. Different Barcode Index Numbers (BINs) indicate different lineages (likely species).

3.4. Discussion

It is imperative that a biological control agent is host-specific to avoid attacks on non-target host plants. Phylogenetic analysis on capitulum-feeding Lepidoptera collected from a range of *Senecio* species demonstrated that these potential agents are not strictly host specific and therefore may be unsuitable for release into countries with a diverse *Senecio* flora. The capitulum-feeding moth *H. stenotea* (BIN 1) was found on four *Senecio* species. *Homoeosoma stenotea* has been prioritised for possible host-range testing due to the extensive damage inflicted on the capitula by the developing endophagous larvae (Egli & Olckers, 2020). *Senecio madagascariensis* and *S. inaequidens* are closely related, therefore the recovery of *H. stenotea* on *S. inaequidens* was not unexpected. However, the recovery of *H. stenotea* on two more distantly related *Senecio* species (*S. polyanthemoides* and *Senecio* sp. 3) (Fig. 3.1) suggests that its host range is not confined to the *S. madagascariensis* species complex. Despite this*, H. stenotea* may be suitable for release in Hawaii due to the absence of *Senecio* natives.

BIN 2 was restricted to *S. madagascariensis*, although this does not necessarily conclude its host specificity, given the few specimens collected. However, BIN 2 could not be confidently identified to species level and thus further consideration would require the rearing of additional adult specimens for identification by expert taxonomists to elucidate its taxonomic status. Nevertheless, genetic barcoding assists in the taxonomic identification of larvae, which is more important in determining the host range of an insect, since the collection of adult specimens, which often show high mobility and dispersal, may not necessarily indicate host usage (Gaskin *et al.*, 2011). BIN 3 was represented by a single specimen recovered only from fireweed and this suggests low incidence and abundance. These factors are equally significant

in the prioritization of insect species that could be potential biocontrol agents against fireweed, considering the flowering phenology of the weed.

A genetic lineage from the genus *Platyptilia* (Pterophoridae) (BIN 4) was also recovered from the distantly related *Senecio polyodon*. The genus *Platyptilia* has provided biocontrol agents that inflict extensive impact on their target weeds (Ireson & McLaren, 2012; Winston *et al.*, 2014; Egli & Olckers, 2017), and the unidentified species was prioritised as a potential agent against fireweed based on its appreciable damage as both a capitulum feeder and a stem borer (Egli & Olckers, 2020). Provided that this species does not utilize a range of native *Senecio* species, the suitability of *Platyptilia* as a biocontrol agent could be limited by its seasonal abundance, as it was recovered in relatively low numbers at only two study sites (Chapter 2). A potential flower-feeding biocontrol agent for fireweed must be present throughout the year in synchrony with the year-round flowering of the target plant. However, the lower incidence of *Platyptilia* sp. should not restrict further consideration, since native surveys on agent populations, which are subject to natural enemy regulation, cannot be fully predictive of agent population densities after release. However, recovery from distantly related *Senecio* species indicates that host specificity could also be a concern.

Upon determining a potential biocontrol agent's field host range, host ranking is an integral part of determining its preferred hosts (Schaffner, 2001; Sheppard *et al.*, 2005). The pyralid moth, the most promising of the capitulum-feeding insects recovered in this study, was mainly recovered in *S. madagascariensis* relative to the other *Senecio* species, suggesting that fireweed is the primary host. However, although fireweed may be the primary host, the extent to which it will be attacked is dependent on the relative densities of all related *Senecio* host plants in a plant community. Therefore, in the absence or scarcity of the target weed,

agent populations will attack non-target plants that are secondary hosts (Marohasy, 1996), particularly if they support oviposition, feeding, and larval development, as did the other *Senecio* species from which *H. stenotea* was recovered. Additionally, the inclusion of more distantly related *Senecio* species in the field host range of *H. stenotea* indicates that *Senecio* species in Australia may be at risk for direct non-target impacts. In Hawaii, however, any risks of non-target attacks would include *Senecio* species which are all exotic and thus of no consequence.

In relation to capitulum-feeding Diptera, there is no previous use of capitulum-feeding Agromyzidae as biocontrol agents, and the unidentified species associated with fireweed was designated low status as a potential agent (Egli & Olckers, 2020). However, since this species was recorded on only one other *Senecio* species, it appears to have a narrow host range. Although agromyzids were seasonally abundant and frequent on fireweed (Chapter 2), their damage inflicted on the capitula is minimal (Egli & Olckers, 2020). The level of damage inflicted is vital in determining the effectiveness of a biocontrol agent, since it quantifies the level of agent impact on weed populations (Sheppard, 2003; Goolsby *et al.*, 2006).

Two unidentified tephritid species in the genus *Trupanea* were restricted to *S. madagascariensis*. However, since these were collected only during the seasonal surveys and not the host-range surveys, their host specificity was not concluded, although Egli *et al.* (2020) found that both were restricted to the *S. madagascariensis* complex. Tephritidae have provided successful biocontrol agents for invasive weeds in the Asteraceae (Egli & Olckers, 2017). However, in Australia, native species of Agromyzidae and Tephritidae have been recovered on *S. madagascariensis*, having expanded their host ranges from native *Senecio*

species (Harvey *et al.*, 2015; Egli & Olckers, 2020). Consequently, the capitulum-feeding Diptera were deemed to have low biocontrol potential for Australia (Egli & Olckers, 2020).

Coleoptera, represented by unidentified Nitidulidae were seasonally abundant (Chapter 2), but were not considered for host range testing since they were given low priority as biological control agents (Egli & Olckers, 2020). Moreover, Nitidulidae were also recovered in high numbers across the majority of the *Senecio* species sampled for field host range testing, suggesting a broad host range. Also, they inflict minimal damage in the capitula of fireweed. Host specificity and high levels of damage are emphasised in weed biological control to ensure safety and effectiveness (McClay & Balciunas, 2005), thus disqualifying insect herbivores whose host ranges include several species, even within the same genus.

The use of genetic barcoding to determine the field host range of capitulum feeders associated with fireweed has been advantageous in this study. The identification of genetically distinct lineages has confirmed the host plant affiliations of insects collected across the different *Senecio* species sampled. Genetic identification of immature stages recovered from host plants in the field aids in determining the suitability of a plant for female oviposition, larval feeding and development, which are important factors for defining the host range of an insect (McFadyen, 1998). Furthermore, field sampling adopted in this study allowed for a more precise estimate of the host specificity of the capitulum feeders associated with fireweed, since their natural host selection behaviour remained unaltered (Briese et al., 2002; Schaffner et al., 2018).

In conclusion, while the capitulum-feeding insects reported in this study may be unsuitable for Australia, they may be suitable for release in Hawaii. The absence of any native species in the tribe *Senecio*neae in Hawaii (Ramadan *et al.*, 2011) negates the risk of non-target attacks

on closely related native plants. Therefore, the relatively restricted host ranges displayed by the capitulum-feeding insects of fireweed may suggest potential for biological control in countries with a limited *Senecio* flora. Further genetic studies and field collections might be required to confirm the host ranges and biocontrol potential of certain of these insects.

Chapter 4: General Discussion and Conclusions

4.1. Introduction

The success of weed biological control has been assessed on the basis of agent establishment, agent impact, and the level of reduction in weed population densities (McFadyen, 2000; Moran *et al.*, 2005; Cullen *et al.*, 2011; Winston *et al.*, 2014), with other analyses applying more rigorous approaches in quantifying agent impact and overall success (Moran *et al.*, 2021). Successful control has varied amongst countries and regions, with some countries achieving relatively higher rates of success, and some regions reporting agent impact as highly extensive (Schwarzländer *et al.*, 2018). The overall rate of successful weed biocontrol on a global scale has been estimated to be 65.7% from the analysis of Schwarzländer *et al.* (2018). Due to the economic impact of alien invasive plants, the economic returns from the biological control of weeds have been considered as a key factor in determining success (Page & Lacey, 2006; van Wilgen & De Lange, 2011).

Potential agents for the biological control programme against *Senecio madagascariensis* Poir. in Australia were previously investigated and prioritised based on their broader seasonal abundance, ability to inflict damage, and host specificity (Egli & Olckers, 2020; Egli *et al.*, 2020). This study explored the seasonal abundance of capitulum-feeding insects on fireweed populations at different sites and on a finer (monthly) scale. Capitulum-feeding insects have been considered due to their potential to reduce the plant's extensive rates of seed production. The most damaging capitulum-feeding insects were the lepidopterans *Homoeosoma stenotea* (Pyralidae) and an unidentified species of *Platyptilia* (Pterophoridae), with dipterans that included two unidentified species of *Trupanea* (Tephritidae) also having some potential (Figure 4.1). Although Coleoptera have a good history of weed biocontrol

success globally (Winston *et al.*, 2014; Egli & Olckers, 2017; Schwarzländer *et al.*, 2018), the only capitulum-feeding beetles recorded were unidentified Nitidulidae (Coleoptera). These, along with unidentified capitulum-feeding Agromyzidae, were of low priority in this study, since they were the least damaging to the capitula (Egli & Olckers, 2020).

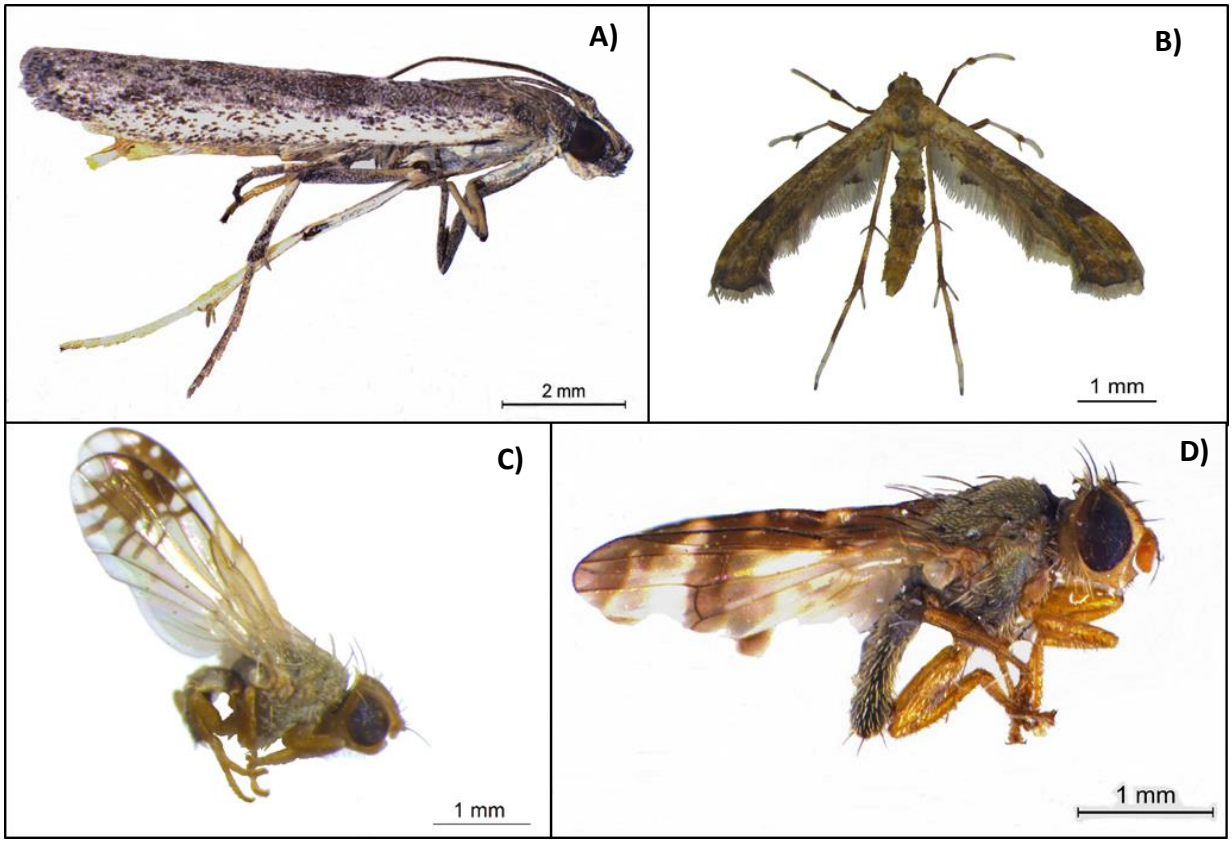


Figure 4.1. Adult lepidopterans and dipterans that represent potential capitulum-feeding agents for *S. madagascariensis* in countries without, or with few, native *Senecio* species. A) *Homoeosoma stenotea* (Pyralidae); B) *Platyptilia* sp. (Pterophoridae); C) *Trupanea* sp. 1) (Tephritidae); *Trupanea* sp. 2) (Tephritidae). Images by D. Egli.

4.2. Potential capitulum-feeding agents based on their seasonal abundance

The phenology of the interaction between the biocontrol agent and its host weed in the introduced range has been emphasised as vital in determining the outcome of biocontrol. Effective control necessitates a consistent and damaging agent population throughout the

seasons of growth of the weed. For capitulum-feeding agents, presence throughout the flowering period of the weed is essential to reduce seed production and thus reduce the spread of the weed. Capitulum-feeding Lepidoptera and Diptera were only occasionally encountered on fireweed and with low records of abundance in consecutive months (Chapter 2). The insects of least interest, namely the Agromyzidae and Nitidulidae, were the most abundant.

The abundance of insect herbivores on their host plant is influenced by an interplay of various factors that are not often measurable during field sampling for the prioritization of potential biocontrol agents (McClay & Balciunas, 2005; Goolsby *et al.*, 2006). These include factors that may be influencing the seasonal occurrence of a potential agent such as predation, parasitism, or variations in the quality of the host plant, which were not quantified during this study. Indeed, populations of herbivorous insects are generally much lower in their native range due to their suppression by natural enemies and other ecological factors.

The numbers of insect herbivores recovered monthly from the capitula of fireweed built up in late summer and peaked in autumn (Chapter 2), and this coincided with the flowering time of *S. madagascariensis* in Australia, which peaks in autumn and spring (Sindel, 2009). If the duration of this synchrony with the flowering phenology of fireweed is sustained, the most damaging capitulum-feeders are likely to contribute to the suppression of the weed's spread. This is particularly likely as it seems that the naturalization of fireweed populations in the invaded countries is principally credited to its seed production (Sindel *et al.*, 1998; Sindel *et al.*, 2008). Generally, the use of biocontrol agents that attack the floral components or seeds of their target weeds is important for restricting weed spread and this has led to the release of such agents against highly invasive weeds like thistles and legumes (Sheppard, 2003).

However, it is understood that the effectiveness of these agents is determined by the population dynamics of the weed and its synchrony with the agents (Sheppard, 2003).

The establishment and proliferation of a biological control agent after release follows the pattern that is typical of exotic species invasion (e.g., escape from natural enemies). However, the factors that would permit successful 'invasion' (i.e., agent establishment and dispersal) are often unpredictable during native range studies (McClay & Balciunas, 2005). Since agent abundance in the native range is often considered to be predictive of agent effectiveness, this may suggest that potential agents that exhibit low abundance are less likely to be suitable and effective as agents. However, agent abundance does not necessarily translate into damage, highlighting that impact on weed populations is also dependent on the timing of damage, and the vulnerability of the plant at a certain growth or reproductive stage or the particular part/tissue that is attacked.

McClay & Balciunas (2005) listed several biocontrol agents that successfully established and became widely abundant on their target weeds but have not displayed any considerable impact on populations of their host plants. In South Africa, the seed beetle *Acanthoscelides macrophthalmus* (Schaeffer) (Chrysomelidae) has become widely established as a biocontrol agent of the invasive tree *Leucaena leucocephala* (Lam.) de Wit (Fabaceae). However, its variable levels of seed damage, that are less evident at high levels of seed availability, and its interaction with several native parasitoids have rendered it insufficient to severely restrict seed production in leucaena (Sharratt & Olckers, 2012; 2019). Capitulum-feeding lepidopterans, which largely comprised *H. stenotea*, but also *Platyptilia* sp*.,* severely damaged the capitula and their developing seeds (Egli & Olckers, 2020), and despite their low seasonal abundance, have the potential to halt the continued spread of fireweed. As shown in other

reviews (Sheppard, 2003; Morin *et al.*, 2009), low abundance in the native range does not predict agent proliferation after introduction into the introduced range. However, higher numbers caused by favourable climatic conditions in the new range and an escape from native natural enemies would be needed to facilitate this.

4.3. Potential capitulum-feeding agents based on their field host range

Agent host range is a critical aspect of safe and effective biological control against invasive weeds. The host range of capitulum-feeding insects associated with *S. madagascariensis* was assessed by field sampling and genetic sequencing of the immature stages associated with their various *Senecio* host plants. Field host-range surveys are an effective procedure for determining the susceptibility of non-target plants to biocontrol agent attack, as they allow insects to display their natural responses and thereby locate and select their preferred host plant (Marohasy, 1998; Briese *et al.*, 2002). In this study, insect taxa recovered from the capitula were not restricted to *S. madagascariensis*.

A total of seven genetic lineages of lepidopteran herbivores were recovered from eight *Senecio* species (Chapter 3, Fig. 3.1), including *S. madagascariensis* and some species that are distantly related. Neither of the two lepidopterans prioritised as potential biocontrol agents for fireweed displayed rigorous host specificity. *Homoeosoma stenotea* utilised four *Senecio* species including fireweed and *Platyptilia* sp. was recovered from two species including *S. madagascariensis*. This suggests that there are likely to be non-target attacks on other *Senecio* species during more rigorous host-range testing in quarantine. Even though host finding and selection in the field may be site dependent, in that an insect deprived of its principal host plant may resort to feeding and oviposition on less preferred host plants (Marohasy, 1998), the utilization of non-target *Senecio* species under field conditions is of

great concern for countries like Australia. Although most specimens of capitulum-feeding lepidopterans were recovered from fireweed, even in densely infested regions in the invaded countries, non-target hosts could potentially be attacked.

The revelation of distinct genetic lineages restricted to *S. madagascariensis* (BIN 2 and BIN 5) (Chapter 3, Fig. 3.1) may indicate other lepidopteran species or cryptic lineages that are host specific. These BINs represent unidentified lepidopterans that have not yet been matched with adult specimens. Rigorous host-range tests to clarify host specificity would be compulsory before release in Australia, due to the wide diversity of native *Senecio* species in that country (Sheppard *et al.*, 2013). In contrast, Hawaii lacks native or economically important *Senecio* species and may thus be able to accept agents like *H. stenotea* that are not strictly host specific (Ramadan *et al.*, 2011).

Capitulum-feeding Agromyzidae were not only recovered from fireweed, but also from the distantly related *S. polyanthemoides*. Furthermore, in another study, it was reported that their host range included several other *Senecio* species (Egli *et al.*, 2020). Capitulum-feeding Tephritidae are more damaging than Agromyzidae (Egli & Olckers, 2020), but their host range could not be concluded based on the results of this study. However, Egli *et al*. (2020) revealed that the host range of unidentified capitulum-feeding *Trupanea* species was restricted to the *S. madagascariensis* species complex. However, capitulum-feeding Tephritidae associated with Australian native *Senecio* species were discovered on fireweed in Australia (Harvey *et al.*, 2015), which may indicate reduced biocontrol potential for introduced South African tephritids as well as susceptibility to Australian parasitoids.

Capitulum-feeding Coleoptera recovered from *S. madagascariensis* all comprised unidentified Nitidulidae. The nitidulids were not quantified or sent for genetic barcoding as they were

deemed the least damaging of the capitulum-feeding insects of fireweed (Egli & Olckers, 2020). Importantly, their host range was not restricted to *S. madagascariensis*, as a significant number of larvae were recovered from the capitula of distantly related *Senecio* species during the processing of the host-range samples. Lack of host-specificity and reduced potential to inflict damage are grounds for rejecting biological control agents, confirming that the nitidulid should not be considered as a candidate biocontrol agent for Australia or Hawaii.

4.4. Comparisons with other capitulum-feeding agents released against Asteraceae

Biological control of invasive Asteraceae has shown high success in agent establishment, although agent impact has been lower (Egli & Olckers, 2017). Coleoptera, Lepidoptera, and Diptera have featured regularly in the selection of biocontrol agents that have been released against asteraceous weeds (Winston *et al*., 2014; Egli & Olckers, 2017). All three orders have shown considerable success in the establishment of agent species, although agent impact has varied, with biocontrol agents from Coleoptera being more successful than Lepidoptera and Diptera (Egli & Olckers, 2017).

Capitulum feeders have frequently been used against asteraceous weeds with most derived from Coleoptera (51%) and Diptera (46%) (Egli & Olckers, 2017). Lepidoptera represented only 2% of agents for the various weeds, and this constituted a single species from the family Gelechiidae. Agent establishment was highly successful with 78% establishment across all agent releases. Similar rates of successful establishment have been reported in comparison with other feeding guilds (Egli & Olckers, 2017). 5% and 18% are the proportion of agent introductions that have yielded extensive and considerable impacts respectively, although there have been more negligible (44%) impacts from the various agent taxa. Curculionidae have been the most successful of the capitulum-feeding agents, their releases providing

extensive (8%) and considerable (28%) impacts on their target weeds. Considerable impact has also been provided by another coleopteran agent from the family Brentidae. Surveys from this study indicated potential coleopteran agents to be of less priority. Nitidulidae have not been used against invasive Asteraceae and one nitidulid agent released against *Echium plantagineum* L. (Boraginaceae; Paterson's curse) has had no impact on the weed (Winston *et al*., 2014).

Capitulum-feeding dipteran agents of Asteraceae have largely featured Tephritidae, compared to other families (Egli & Olckers, 2017). The impact of tephritid agents has been considerable in some programmes, with most dipteran capitulum-feeders providing negligible impact. However, tephritids were not prioritized as potential biocontrol agents for fireweed. There are also no capitulum-feeding species from the Agromyzidae that have been used as biocontrol agents. Agromyzidae associated with fireweed were the least damaging of all potential agents and thus had the lowest priority.

The only capitulum-feeding lepidopteran agents that were released against Asteraceae were from the family Gelechiidae and comprised a single agent species that was released against *Centaurea* L. species, but had a negligible impact (Winston *et al*., 2014; Egli & Olckers, 2017). Pyralidae have featured in the biocontrol of Asteraceae, but not as capitulum-feeding agents (Egli & Olckers, 2017). The use of Lepidoptera as agents against asteraceous weeds has been significant with high rates of successful establishment from the different families (Egli & Olckers, 2017). Extensive impact has been achieved by four lepidopteran agents from the two families, namely Pterophoridae and Tortricidae, although these were stem-boring and not capitulum-feeding species (Egli & Olckers, 2017). The success of Lepidoptera as agents against

Asteraceae warrants the prioritization of *H. stenotea* as a capitulum-feeding agent against *S. madagascariensis*, but only for certain countries.

4.5. Conclusion and recommendations

Senecio madagascariensis has been designated as a "Weed of National Importance" in Australia (Sheppard *et al.*, 2013). The distribution of fireweed is predicted to extend to climatically suitable regions in Australia, Japan, and other invaded countries (Sindel *et al.*, 1998; Sindel *et al.*, 2008). Due to the ecological, economic, and social impacts of fireweed, the potential for biological control has been considered, with recent investigations in KwaZulu-Natal, South Africa for potential agents. The challenge for success in countries like Australia is a diverse native *Senecio* flora, necessitating that biocontrol agents should exhibit a high degree of host specificity (Sheppard *et al.*, 2013). In Hawaii, the absence of native *Senecio* species increases the likelihood that a suitable agent will be found (Ramadan *et al.*, 2011).

Although other studies have focused on root-feeding and stem-boring insects of fireweed (Singh, 2019; Zuma, 2020), this study focused on capitulum feeders. The most promising capitulum-feeding agent is *Homoeosoma stenotea* (Pyralidae) due to the high levels of damage inflicted on the capitula and its developing seeds. Another potential agent is the unidentified species of *Platyptilia* (Pterophoridae), despite its scarcity in the field. Moreover, Lepidoptera have achieved success as biocontrol agents of invasive Asteraceae, although not as capitulum feeders (Egli & Olckers, 2017). However, both *H. stenotea* and *Platyptilia* sp. are more likely suitable for release in Hawaii than in Australia, due to the recovery of larvae from *Senecio* species outside the *S. madagascariensis* complex.

The presence of genetically distinct lepidopteran lineages that may exhibit restricted host ranges (BIN 2 and BIN 5) (Chapter 3, Fig. 3.1) has shown genetic barcoding to be vital in elucidating agent-plant affiliations and revealing possible host-specific cryptic species or host races. Furthermore, field host-range surveys prevent the importation of unsuitable agents for quarantine studies. Rigorous laboratory host-specificity testing may then be conducted on genetic lineages with more restricted host ranges to confirm their host specificity. Also, the identity of BIN 5 should be determined, as it may represent a rare species with biocontrol potential. Since the host-range surveys did not conform or refute the reported narrow host range of the two species of Tephritidae (Egli *et al.*, 2020), more studies on this are required.

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