

**Ant diversity, assemblage composition and other arthropod activities in
relation to the invader *Parthenium hysterophorus* L. (Asteraceae)
and its biological control agent**

By

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Preface

The work contained in this thesis was completed by the candidate in the School of Life Sciences of the College of Agriculture, Engineering and Science, University of KwaZulu-Natal, Pietermaritzburg, under the supervision of Dr Caswell Munyai and co-supervised by Lorraine Strathie (Agricultural Research Council). The research was financially supported by the Agricultural Research Council - Plant Health and Protection (ARC-PHP) and the Department of Forest, Fisheries, and the Environment (DFFE). The contents of this work are my own and have not been submitted in any form to another university. Where use has been made of the work of others, it is acknowledged in the text. The thesis is formatted according to South African Journal of Botany.



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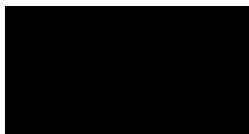
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DECLARATION 2: PUBLICATIONS/ CONFERENCE PROCEEDINGS

Details of publications/ conference proceedings that form part and/or include research presented in this dissertation

1. Hlabisa F.S., Strathie, L., Munyai T.C. Ant diversity and assemblage composition in relation to the invader *P. hysterothorus* and its biological control in South Africa. National Symposium on Biological Invasions, Online Symposium, 5-7 May 2021. Virtual Presentation by F. S Hlabisa.
2. Hlabisa F.S., Strathie L., Munyai T.C. Ant diversity, assemblage composition and arthropod activity in relation to the invader *Parthenium hysterophorus* (Asteraceae) and its biological control, 22nd Hybrid Congress of the Entomology Society of Southern Africa, Online Conference, 28 June- 1 July 2021. Virtual Presentation by F.S Hlabisa.

Abstract

Invasive alien plants have the potential to alter ecosystem function. While a growing number of studies have focused on the effects of invading plants on native biodiversity and the underlying community dynamics, there is still a lack of studies that detail the impact of invasive plants, such as *Parthenium hysterophorus* L. (Asteraceae), at higher trophic levels. This study investigated whether *P. hysterophorus* and its biological control agent, the stem-boring weevil *Listronotus setosipennis* (Hustache) Coleoptera: Curculionidae, affected ant diversity, assemblages and arthropod activities in the KwaZulu-Natal and Mpumalanga provinces of South Africa. The objectives of this study were to assess the impacts of *P. hysterophorus* invasion and presence of *L. setosipennis* on the diversity and assemblage composition of ants, a dominant terrestrial group. Additionally, whether vegetative variables in habitats invaded by *P. hysterophorus* affected ant assemblages, was examined. Lastly, the study investigated the impacts of the presence and absence of *L. setosipennis* on other arthropod activities. To study ant diversity and assemblage composition, ants were collected using pitfall traps, over five sampling periods from December 2019 to March 2020, in nine locations around KwaZulu-Natal and Mpumalanga provinces. At each site, three treatments were sampled; viz. *P. hysterophorus* invaded habitat, *P. hysterophorus* invaded habitat with *L. setosipennis* present, and habitat without *P. hysterophorus*. Species richness and abundance were compared between treatments using ANOVA and the Post-hoc Turkey test. Assemblage composition was analyzed using non-metric multidimensional scaling (NMDS). A Canonical Correspondence Analysis (CCA) was also used to correlate the best environmental variable with ant diversity and assemblage composition. Some 16 463 ant specimens that were collected were identified from four subfamilies, 27 genera, and 55 species. Results indicated that the presence of *P. hysterophorus* and its biological control agent *L. setosipennis* did not significantly alter ant diversity, as indicated by species richness and abundance, and assemblage composition, although some differences occurred across locations. The results also showed that vegetative variables (*P. hysterophorus* height, flowering and cover) did not significantly influence ant assemblages. A separate experiment at six locations in KwaZulu-Natal and Mpumalanga provinces, arthropod activities on *P. hysterophorus* plants were studied during timed observations at 07h00, 10h00 and 12h00 during monthly sampling from December 2020 to March 2021. Although some arthropod groups were less common visitors to *P. hysterophorus* than others, this study showed that the presence of *L. setosipennis* had no significant effect on overall arthropod activities. However, results demonstrated greater activity for some arthropod

groups, specifically Hymenopterans, in both treatments, accounting for 60% of all arthropods recorded. Other studies have reported either a positive or negative impact on arthropods by invasive alien plants. These plants may provide a better food resource for native insects and other arthropods, causing them to be attracted to them. However, invasive alien plants have also been linked to a decline in invertebrate species diversity or changes in the composition of populations. This study contributed to growing knowledge on the impacts of invasive alien plants and on terrestrial arthropods, the most prominent group of invertebrates, that are also known to be significant indicators of biological change.

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Table of Contents

Preface	ii
Declaration 1: Plagiarism.....	iii
Declaration 2: Publications/ Conference Proceedings.....	iv
Abstract.....	v
Acknowledgements.....	vii
Table of Contents.....	ix
List of Tables	x
List of Figures	xi
Chapter 1: General Introduction	1
1.1 Background of the study	1
1.1.1 Known factors influencing arthropod assemblages	2
1.1.2 Arthropod visitations to invasive alien plants	3
1.2 Study species and its impacts	4
1.3 Justification of the study	5
1.4 Aim and objectives.....	7
References.....	8
Chapter 2: Ant diversity and assemblage composition in relation to <i>Parthenium hysterophorus</i> L. (Asteraceae) and a biological control agent <i>Listronotus setosipennis</i> (Hustache) (Coleoptera: Curculionidae) in South Africa.....	14
Abstract.....	14
Introduction.....	15
Materials and Methods.....	17
Statistical Analysis.....	18
Results.....	20
Discussion.....	29
References.....	33
Chapter 3: Activities of native arthropods associated with <i>Parthenium hysterophorus</i> L. (Asteraceae) and the influence of the biological control agent <i>Listronotus setosipennis</i> (Hustache) (Coleoptera: Curculionidae).....	38
Abstract.....	38
Introduction.....	39
Materials and Methods.....	40
Statistical Analysis.....	42

Results.....	42
Discussion.....	52
References.....	56
Chapter 4: General Summary and Recommendations	59
4.1 Introduction	59
4.2 Revisiting aims and objectives	59
4.3 Contributions of the study	60
4.4 Limitations and future recommendations.....	60
4.5 Summary conclusions	60
References.....	62
Appendices.....	63

List of Tables

Table 2.1: Ant species richness and species abundance in habitats invaded by <i>Parthenium hysterophorus</i> , with and without <i>Listronotus setosipennis</i> , and adjacent uninvaded habitats at nine study sites in KwaZulu-Natal and Mpumalanga provinces.....	21
Table 2.2: Ant abundance, species richness, and diversity measure indices (Dominance_D, Simpson_1-D, Shannon_H, Evenness_e ^H /S) in habitats invaded by <i>Parthenium hysterophorus</i> , with and without <i>Listronotus setosipennis</i> , and adjacent uninvaded habitats at nine study sites in KwaZulu-Natal and Mpumalanga provinces.....	23
Table 2.3: Overall similarity of ant species composition in habitats invaded by <i>Parthenium hysterophorus</i> , with and without <i>Listronotus setosipennis</i> , and uninvaded habitats at nine study sites in KwaZulu-Natal and Mpumalanga provinces.....	25
Table 3.1: The total number (N) of arthropod visitors per taxonomic group, identified to Order and proportion (%) of total visits observed on <i>Parthenium hysterophorus</i> plants at six study sites with and without <i>Listronotus setosipennis</i> in KwaZulu-Natal and Mpumalanga provinces during December 2020 to March 2021.....	43

List of Figures

Figure 2.1: Individual-based and rarefaction (solid line segments) and extrapolation (dotted line segments) sampling curves with 95% confidence intervals for ant assemblage data of three treatments by diversity order: $q = 0$ (species richness), at nine study sites in (a) KwaZulu-Natal and (b) Mpumalanga provinces.....	24
Figure 2.2: Nonmetric Multidimensional Scaling (nMDS) of ant assemblages in three treatments at study sites in (a) KwaZulu-Natal and (b) Mpumalanga provinces. The circles represent No <i>Parthenium hysterophorus</i> (No_P), squares represent <i>P. hysterophorus</i> (P), and triangles represent <i>P. hysterophorus</i> with <i>Listronotus setosipennis</i> (P+L) treatments.....	26
Figure 2.3: Canonical Correspondence Analysis ordination plot of the habitat structure variables (mean <i>Parthenium hysterophorus</i> plant height, total number of <i>P. hysterophorus</i> flowers and proportion of <i>P. hysterophorus</i> cover) that explained significant amounts of variation between ant assemblages and treatments in sites in (a) KwaZulu-Natal and (b) in Mpumalanga. See Appendix 3a and 3b for details of ant names.....	28
Figure 3.1: Total number of arthropods observed visiting <i>Parthenium hysterophorus</i> plants with and without <i>Listronotus setosipennis</i> at three times of the day monthly during December 2020 to March 2021.....	44
Figure 3.2: Number of arthropods per order as a proportion of the total number of arthropods that visited <i>Parthenium hysterophorus</i> with and without <i>Listronotus setosipennis</i> at six study sites in KwaZulu-Natal and Mpumalanga provinces during monthly sampling from December 2020 to March 2021.....	45
Figure 3.3: Total number of visitors, per order, to <i>Parthenium hysterophorus</i> plants with and without <i>Listronotus setosipennis</i> for (a) Coleopterans ($F = 4.294$, $p > 0.05$); (b) Hemipterans ($F = 2.497$, $p > 0.05$), and (c) Hymenopterans ($F = 14.040$, $p < 0.05$). The arthropod visitors were analyzed per group within each order and compared using Turkey HSD test. Crosses and small circles indicating the means ($p < 0.05$).....	47

Figure 3.4: Linear regression indicating the number of arthropod visitors (per flower, per plant, per plot) in relation to the number of <i>Listronotus setosipennis</i> eggs per flower (per plant per plot); $R^2 = 0.648$, $p < 0.05$	49
Figure 3.5: Visitation frequency of arthropods to <i>Parthenium hysterophorus</i> flowers with and without <i>Listronotus setosipennis</i> eggs (Chi-square = 41.855, df= 1, $p > 0.05$).....	50
Figure 3.6: Proportion (%) of resting, foraging and exploratory activities by arthropod visitors on <i>Parthenium hysterophorus</i> plants with and without <i>Listronotus setosipennis</i> eggs.....	51

Chapter 1: General Introduction

1.1 Background of the study

Invasive alien organisms from key taxonomic groupings have impacted ecosystems worldwide (Chamier et al., 2012). Most previous concerns focused on the impact of invasive alien organisms on conservation areas, and other areas of natural vegetation e.g., forests, grasslands, and agricultural output (Le Maitre et al., 2000; Hartley et al., 2010; Bajwa et al., 2019). Following habitat loss in the twenty-first century, plant invasion poses a significant danger to species diversity worldwide (Shiferaw et al., 2018). Many alien plants are introduced to countries around the world, but only a few of them become problematic (Shiferaw et al., 2018). Invasive plants have several characteristics, including prolific seeding and first reproduction at a young age, unpalatable foliage, and the ability to quickly establish in deteriorated settings (Kacheche and Mzuza, 2021). This makes them strong competitors among other plant species, allowing for their survival and wide dispersal (Kacheche and Mzuza, 2021).

The considerable extent and consequences of alien trees and shrubs and the fact that just a few alien grasses have caused substantial damage are notable traits of plants invading South Africa (Dean et al., 2000; Richardson et al., 2000). Most alien plant research in South Africa has been conducted at small spatial scales (communities or plots), and much of the research on impacts has been conducted in the fynbos biome. At the scale of small plots, this research has revealed that thick stands of alien trees and shrubs in fynbos can rapidly lower the abundance and diversity of native plants (Richardson and van Wilgen, 2004). New methodologies are emerging for depicting and assessing the effects of invasive species and translating these effects into monetary terms (Pyšek and Richardson, 2010).

The impact of invasions by invasive species is recognized worldwide, and multiscale programmes have been implemented to decrease current and future effects (Pyšek and Richardson, 2010). With South Africa's long history of problems with invasive alien species and of research and management on them, the national 'Working for Water' programme was started in 1995 to coordinate and manage alien plant species across South Africa (Dean et al., 2000; Richardson and Wilgen, 2004). The programme initially focused mainly on riparian and watershed areas but now runs management initiatives for alien plants in all semi-natural and natural systems. This programme has developed into one of the world's most prominent programmes to control invasive alien species (Richardson and Wilgen, 2004).

The threats posed by invasive alien species to biodiversity is only second to habitat degradation in terms of importance (Singh, 2005). Invasive species cause biodiversity losses, including the extinction of species, and changes in ecosystem function and hydrology (Singh, 2005). Invasive plants also significantly impact catchment hydrology; watershed areas with dense stands of alien species have been shown to have 30%-70 % decreased water runoff (Kunwar, 2003). Most of the effects are harmful to the supply of essential ecosystem services. Reduced streamflow negatively influences aquatic biota (Kunwar, 2003). Moreover, changes in soil structure, profile, decomposition, nutrient content, moisture availability, and other factors may result from differences in the requirements of native and exotic plant species, resource acquisition and consumption modes. Invasive species are thus a barrier to biodiversity conservation and sustainable use, with considerable negative consequences for ecosystem goods and services (Singh, 2005). Although there is no doubt that dominant and widespread invasive plants can have a negative impact on ecosystem features when present in large numbers, there is less evidence that their presence alone causes negative changes in the health of the recipient ecosystem (Panetta and Gouden, 2017).

1.1.1 Known factors influencing arthropod assemblages

Arthropod assemblages are influenced by biotic and abiotic factors at both the local and landscape scales. It is still debateable which of the factors have the most significant impact on species composition (Schaffers et al., 2008). Studies (e.g. Philpott et al., 2014) have shown that habitat factors, such as vegetation diversity, leaf litter depth, tree number and size, influence arthropod abundance, richness, and composition. In general, vegetation is regarded as one of the most critical variables, not only because it serves as a physical habitat for most arthropod species but also because some insects have been linked to a small number of plant species (Schaffers et al., 2008). Moreover, nutrients, particularly nitrogen, have also been shown to directly impact insect community structure and abundance (Perner et al., 2005). In most terrestrial ecosystems, arthropods are a dominant component of the carnivore, herbivore, and detritivore trophic hierarchy (Perner et al., 2005).

Various hypotheses predict that arthropod abundance will be altered by alien plant species (Perner et al., 2005). These include i) “Resource concentration hypothesis” which states that “specialist herbivore insects should be more abundant in large patches of host plants” (Stephens and Myers, 2012), and ii) “Natural-enemy hypothesis” which states that “invasive alien plants spread rapidly because they are liberated from their co-evolved natural enemies” (Lui and Stiling, 2006). Moreover, natural and anthropogenic disturbances may also potentially reduce

arthropod population numbers in the short term (Perner et al., 2005). In general, physical environment characteristics and management techniques tend to be more important drivers of arthropod assemblages, which may confound any effect of plant production and variety on arthropod abundance (Perner et al., 2005).

1.1.2 Arthropod visitations to invasive alien plants

Due to the ecological and financial effects of plant invasions, research concentrating on interactions between invasive plants and native insects has arisen (Sunny et al., 2015). Native insects that come into contact with invasive plants may display varying degrees of adaptation to the defensive compounds of the plant. New compounds found in invasive plants may deter native specialized and generalist insects from ovipositing, impact the survival of the native insect and ability to reproduce, and ultimately result in a decline in native insect populations (Bezemer et al., 2014, Sunny et al., 2015). Nectar from several plant species contains alkaloids that are toxic to bees (Witt and Belgeri, 2019). For example, nectar from California buckeye (*Aesculus californica* (Spach.) Nutt.; Sapindaceae) and jimson weed (*Datura stramonium* L.; Asteraceae) has been reported to be toxic to both brood and adult bees (Witt and Belgeri., 2019). Native insects might also be attracted to the compounds that invading plants emit. This might lead to a variety of different interactions with the invasive plants, which could ultimately determine whether native insect populations survive or decline. Whether an invasive plant is used for feeding, laying eggs, or for supporting the growth and development of the progeny will determine how interaction with it turns out (Sunny et al., 2015).

In many different systems, alien plants interact significantly with flower visitors/ pollinators. For example, in scattered ecosystems where native plants are few, alien plants are particularly likely to dominate these interactions (Williams et al., 2011). Some introduced plants are referred to as magnet species because their numerous attractive or bright flowers with large amounts of pollen and nectar attract flower visitors (Gibson et al., 2013; Ojija et al., 2019). Due to limited visits to native plants by pollinators, alien invasive plants also grow extensive monospecific patches with many flowers, causing them to outcompete native flowering plants (Ojija et al., 2019). Entomophilous alien plants rely heavily on native pollinators for reproduction and population increase since invasive plants must form mutualistic associations with pollinators already existing in the environment to establish and invade (Nienhuis et al., 2008). Previous research (Lopezaraiza-Mikel et al., 2007) has discovered increased pollinator richness and abundance as a result of making it easier to visit native plants. However, a meta-analysis (Morales and Traveset, 2009) found that flowering alien plants have detrimental

effects on native plant pollination and reproduction, potentially reducing native plant and pollinator diversity. Invasive plant species are therefore considered a significant threat to biodiversity worldwide for this and other reasons. Invasive plants can directly impact native insect herbivores and their natural enemies because they are a new resource and are the principal producers that support the majority of terrestrial food webs (Bezemer et al., 2014). However, through effects on the quality, quantity, or diversity of native plants or the design of their habitat, invasive plants can also indirectly change the abundance and performance of native insects on native plants (Bezemer et al., 2014).

1.2 Study species and associated impacts

Parthenium hysterophorus L. (Asteraceae: Heliantheae) is an annual herbaceous plant that can grow to a maximum height of 2 m in good soils (Adkins et al., 2019). It flowers within four to six weeks of germination, and can yield up to 30,000 seeds per plant (Strathie et al., 2011). *Parthenium hysterophorus* is a recognized invasive plant species in South Africa and is classified as a category 1b weed, which requires compulsory control, according to the National Environmental Management: Biodiversity Act (NEMBA), 2004. It is a weed of global importance, occurring as an alien invader in nearly 50 countries (Mao et al., 2021). *Parthenium hysterophorus* forms dense infestations, causing numerous detrimental impacts on native communities, crop and animal production, and human and animal health (Adkins and Shabbir, 2014, Strathie, 2015). A considerable amount of literature is available on the impacts of *P. hysterophorus* (Nigatu et al., 2010; Patel, 2011; Adkins and Shabbir, 2014; Ojija et al., 2019). Most of these studies have focused on its effects on natural ecosystems (Ayele, 2007; Adkins and Shabbir, 2014), human health; causing diseases such as asthma, dermatitis, and bronchitis (Towers and Subba Rao, 1992; Wiesner et al., 2007; Allan et al., 2018), and livestock; tainting milk and meat (Bajwa et al., 2019; Duguma et al., 2019). It has invaded a wide range of croplands, in particular essential crops such as wheat (*Triticum aestivum* L.), sorghum (*Sorghum bicolor* L.), and maize (*Zea mays* L.) which are important crops used for human and animal consumption (Adkins and Shabbir, 2014). *P. hysterophorus* has the ability to interfere with these crops under various geographic and climatic conditions. (Adkins and Shabbir, 2014). Furthermore, *P. hysterophorus* cause vast economic losses to the agricultural sector in many countries including South Africa. These economic losses aggravate the stability of households (causing income reduction) and farming communities (causing difficulties marketing stock produced in invaded croplands.) (Bajwa et al., 2019)

The aggressive expansion of *P. hysterophorus* in ecosystems has been reported worldwide (Kija et al., 2013). For example, studies in India indicated that *P. hysterophorus* occupies new surroundings and often replaces native plant species, resulting in extensive damage to biodiversity (Patel, 2011; Abdulkarim-Ute and Legesse, 2016). Ayele et al. (2013) also reported that *P. hysterophorus* led to a loss in the species richness and diversity of native plant species and their seed banks. In addition, *P. hysterophorus* acts as a reservoir host for insect pests of crops and plant pathogens as well as vectors of human and animal diseases for example mosquitos, with implications on disease e.g. avian and human malaria. (Guyana and Paraguay, 2014; Ayele et al., 2013; Benelli, 2015).

1.3 Justification of the study

Exotic plants and biological control agents may have unpredictable and potentially indirect effects on native species, which is a challenging but debatable problem (De Clercq et al., 2011). Species community and population metrics such as richness, abundance, and composition can provide useful indicators of changes in animal diversity in areas invaded by invasive alien plants (Garcia and Clusella-Trullas, 2017). The direction and degree of the effects of alien plant invasions on animal communities can vary depending on several factors, including the scale of the plant invasions (extent and density), the stage of invasion, and the region and taxonomic group (Schirmel et al., 2016).

The impacts of alien plant invasions on arthropods may have extreme ecological consequences, and several studies have reported that invaded areas had lower arthropod diversity and abundance relative to uninvaded sites (Simao et al., 2010; Tang et al., 2012). Arthropods are intimately associated with native vegetation, and any alteration in the physical characteristics of habitats is expected to negatively impact indigenous fauna (Mgobozi et al. 2008). Alien plant invasions have been reported to reduce the diversity and abundance of invertebrates (mainly insects), which are essential in the food webs in terrestrial ecosystems (Yoshioka et al. 2010). Studies have reported the impacts of invasive plants on arthropod assemblages (Yoshioka et al, 2010; Tang et al., 2012); however, very few studies have examined the impacts of *P. hysterophorus* on native arthropods in South Africa

Parthenium hysterophorus was initially recorded in South Africa in 1880 in Inanda, KwaZulu-Natal, and is now widespread in KwaZulu-Natal, Mpumalanga, North West and Limpopo provinces, as well as neighbouring countries of eSwatini, Zimbabwe, Botswana and Mozambique (McConnachie et al., 2011; Terblanche et al., 2016; Chetty et al., 2021). Biological control efforts on *P. hysterophorus* using selected introduced natural enemies began

in South Africa in 2003, under the sponsorship of the then Working for Water 'Emerging Weeds Programme' (Strathie et al., 2011). Several biological control agents were prioritized for introduction, based on their impact on the plant and their likely compatibility with the local climate. Those agents that had been prioritised were evaluated for suitability for release and later released. These agents included the leaf-feeding beetle *Zygogramma bicolorata* Pallister (Coleoptera: Chrysomelidae), the stem-boring weevil *Listronotus setosipennis* (Hustache) (Coleoptera: Curculionidae), and the summer rust fungus *Puccinia xanthii* var. *parthenii-hysterophorae* Seier, H.C. Evans & Á. Romero (Pucciniaceae) (Strathie et al., 2011). Additionally, the winter rust fungus *Puccinia abrupta* var. *partheniicola* (H.S. Jacks.) Parmelee (Pucciniaceae) was already present without deliberate introduction, and the seed feeding weevil *Smicronyx lutulentus* Dietz (Coleoptera: Curculionidae) was introduced later (Strathie et al., 2021). The subsequent establishment of these agents has been relatively localized and in some cases limited (Strathie et al., 2021).

This study focused on only one agent, *L. setosipennis* as the weevil had established fairly readily, whereas *Z. bicolorata* was not well established and *S. lutulentus* had been introduced only more recently (Strathie et al., 2021). *Listronotus setosipennis* was initially used to good effect as a biological control agent in Queensland, Australia, and later released in South Africa from 2013 onwards (Strathie et al., 2011). The weevil deposits single eggs in flowers of *P. hysterophorus*, and hatched larvae tunnel within the stems as they feed, making their way towards the base of the plant, before exiting the plant to pupate in the soil, followed by adult eclosion with the onset of rainfall (Strathie et al. 2011). Some biocontrol agents, such as *L. setosipennis*, typically have slow dispersal rates, while other agents may disperse readily. However, possible causes for restricted establishment of any biocontrol agent could also be caused by predation and parasitism by native arthropods. Considerable ant activity had been observed on *P. hysterophorus* field populations, leading to consideration of the association of the weed, its biocontrol agents and native arthropods (L. Strathie, pers. comm.).

Parthenium hysterophorus has strong allelopathic effects (Lalita, 2018). Several studies have demonstrated the impacts of chemical components of *P. hysterophorus* on arthropods. For example, Sreekanth (2013) reported that methanol extracts from *P. hysterophorus* showed superior insecticidal effects against the paddy brown plant hopper *Nilaparvata lugens* Stål (Hemiptera: Delphacidae). Raipat (2010) also reported that *P. hysterophorus* not only adversely impacted the population growth of earthworms (*Eisenia fetida* (Savigny) (Opisthopora) but also decreased their biomass. The active compound parthenin, present in *P. hysterophorus*, was shown to have insecticidal activity against termites, cockroaches,

and *Melanoplus sanguinipes* (Fabricius) (Orthoptera: Acrididae) (migratory grasshoppers) (Rajiv et al., 2014). Benelli (2015) reviewed the effectiveness of *P. hysterothorus* plant-borne ovicides against mosquito vectors of veterinary and medical significance. The selective efficiency of *P. hysterothorus* leaf extracts was the most effective oviposition deterrent against these mosquitoes. Another recent study investigated the insecticidal activities of *P. hysterothorus* extracts and parthenin against *Plutella xylostella* L. (Lepidoptera: Plutellidae) (diamondback moth) and *Aphis craccivora* Koch (Hemiptera: Aphididae) (aphid) and demonstrated that parthenin had repellent activity to the diamondback moth and toxicity to the larvae of aphids (Reddy et al., 2018). Abdulkarim-ute and Legesse (2016) indicated that an increase in level of *P. hysterothorus* infestations caused a rapid decrease in plant and animal diversity and populations in ecosystems in Ethiopia.

Arthropods make up 84 percent of all known animal species and are essential to the functioning of terrestrial ecosystems. Thus, understanding their diversity and composition is critical (Dossey, 2010; Simao et al., 2010). However, the impact of invasive alien plants on native arthropod species is less clear, and to date, few studies have been conducted to assess arthropods associated with native and alien plants (Ernst and Cappucino, 2005). The current study used arthropods as a model set of organisms to investigate the effects of *P. hysterothorus* invasions and the presence of an established biological control agent, the stem-boring weevil *L. setosipennis*, selected due to its perceived potential vulnerability to egg predation and slow population expansion.

1.4 Aims and objectives

This study aimed to determine the impacts of *P. hysterothorus* and its biological control agent *L. setosipennis* on ant diversity, assemblage composition, and overall arthropod activities.

The specific objectives of this study were to:

1. Assess the impacts of *P. hysterothorus* and the biocontrol agent *L. setosipennis* on the diversity and assemblage composition of ants at selected sites in South Africa.
2. Examine arthropod activities in relation to *P. hysterothorus* and the biological control agent *L. setosipennis* to determine which arthropods visited the plants, their range of activities on the plants, and whether the presence of *L. setosipennis* influenced these aspects.

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Chapter 2: Ant diversity and assemblage composition in relation to *Parthenium hysterophorus* L. (Asteraceae) and its biological control agent *Listronotus setosipennis* (Hustache) (Coleoptera: Curculionidae) in South Africa

Abstract

Parthenium hysterophorus L. (Asteraceae) is a severe terrestrial invader in South Africa, with impacts on floral and faunal biodiversity. The impacts of this invasive plant on native arthropods have received little attention in most of its invaded range. This study, therefore, aimed to investigate the effects of the presence of *P. hysterophorus* and the stem-boring weevil *Listronotus setosipennis* (Hustache) (Coleoptera: Curculionidae), on the diversity and assemblage composition of ants, a dominant terrestrial faunal group. Pitfall traps were used to sample ants in *P. hysterophorus* invaded habitats, with and without *L. setosipennis* established, and in uninvaded habitats. Ants were collected during five monthly sampling periods from December 2019 to March 2020, and was disrupted by Covid-19 pandemic travel restrictions, but resumed for a final assessment in October 2020 at nine study sites in KwaZulu-Natal and Mpumalanga provinces. A total of 16 463 ant specimens were collected and identified, from four subfamilies, 27 genera, and 55 species. As indicated by species richness and abundance, ant diversity did not differ significantly across habitats with *P. hysterophorus* with and without *L. setosipennis*, and habitats without *P. hysterophorus*. The three treatments also had similar ant species assemblage compositions. This study therefore did not present significant impacts of *P. hysterophorus* and *L. setosipennis* on ant diversity and assemblage composition. Vegetative variables, namely *P. hysterophorus* height, flowering, and cover, were also found not to influence ant species diversity and assemblage composition. Future studies should investigate the effects of *P. hysterophorus* on habitat and food requirements for ant assemblages, which the current study did not investigate. Furthermore, evaluation of changes in vegetation, litter, and soil properties related to *P. hysterophorus* invasions could improve understanding of the processes that drive the effects of alien plant invasions on arthropod communities.

Keywords: Ants, biodiversity, biological control, community ecology, impacts, invasive plants, Parthenium weed

Introduction

Invasive plants change natural ecosystems worldwide, seriously threatening native species (Fork, 2010). There is a link between the introduction of alien plant species into natural ecosystems and some anthropogenic factors such as habitat degradation, which may cause changes in plant or animal populations (Marshall and Buckley, 2009). Understanding how significant changes in vegetation species composition have affected animal communities is vital in terms of quantifying the impacts caused by non-native plants. In response to novel vegetation, animals that have evolved with another pre-existing vegetation type may change their behaviour, distribution, or abundance (Fork, 2010). Since many organisms need particular plants for food or reproduction sites, changes in the composition caused by plant invasions can be particularly harmful to arthropods (Cord, 2011; Litt et al., 2014). While alien plant invasions have been linked to severe ecological changes in a range of ecosystems, their impact on native invertebrate fauna has received far less attention (French and Major, 2001; Bultman and DeWitt, 2008).

Invertebrates make up most of the diversity in terrestrial ecosystems, and arthropods are the most abundant group, with insects thought to account for 80-90% of all species in the world (Dossey, 2010). The impacts of invasive plants on arthropod structure, diversity, and abundance tend to be sporadic (Litt et al., 2014). Plant quantity and type influence arthropod community composition (Schaffers et al., 2008). Given that exotic plant invasions can drastically alter vegetation structure by displacing native herbs and tree seedlings, it is not unexpected that these invasions might also change arthropod populations, resulting in altered diversity and abundance (Tang et al., 2012). Some studies reported that invaded environments had lower arthropod abundance and diversity (Samways et al., 1996; Ernst and Cappucino, 2005; Lindsay and French, 2006; Florens et al., 2010; Roberson et al., 2010; Simao et al., 2010). In contrast, other studies reported that invaded ecosystems had higher arthropod diversity or abundance (Simao et al., 2010; Lescano and Farji-Brener, 2011; Emery and Doran, 2013). Knowing whether plant invaders modify the structure and function of recipient ecosystems in predictable ways requires knowledge of generally applicable invasion effects on arthropod groups (Tang et al., 2012).

Ants, grouped within the Order Hymenoptera and Family Formicidae, constitute a significant component of arthropod groups and terrestrial ecosystems, in terms of the numbers of individuals, species, or biomass (Andersen, 2019). They are easily identifiable and their diversity can provide essential insights into environmental change (Litt et al., 2014). Because

of their sensitivity to environmental changes, ants have recently gained much interest as a possible indicator group (Ostoja et al., 2009; Andersen, 2019). Many ant species are susceptible to even small changes in their environments; therefore, modifications to the environment caused by plant species can negatively impact on ant species richness, colony density, and foraging behavior (Lenda et al., 2013). However, the impacts of invasive alien plant species on ants may differ spatially. For example, the areas in the centre of the habitat patch are usually different (e.g., in air temperature, humidity, soil moisture) from those at the habitat patch boundaries, which may result in the establishment of an edge effect, with ant species richness varying between the centre and the edge of the habitat patch (Gehlhausen et al., 2000; Lenda et al., 2013).

Several physical and biological factors may influence the diversity and abundance of ant communities in specific habitats (Ríos-Casanova et al., 2006; Radnan et al., 2018). Ramos et al. (2018) stated that energy-related variables are the key drivers of ant diversity across various environmental gradients. Physical factors such as solar radiation, temperature, and water could have a crucial influence in defining ant variety in an ecosystem because ants are small-bodied, with high surface-to-volume ratios, making them susceptible to desiccation. According to Vasconcelos et al. (2008), significant variation in tree, shrub, and grass cover can be detected over relatively short distances. Such diversity affects the associated ant fauna. However, most studies have stated that plant biomass, richness, and cover appeared to negatively impact ant diversity (Dean and Milton, 1995; Boulton et al., 2005).

Several studies have linked non-native plant species to a decline in invertebrate species diversity or a change in population composition (Fork, 2010). The impacts of invasive alien plants on native fauna have received limited attention, although environmental changes can be used to predict their impacts. Assessing arthropod populations may provide valuable information about the effects of plant invasion on animal communities (Fork, 2010). Because of the small spatial distribution, this current study examined ants and plants because ecological changes could be observed over short distances.

Parthenium hysterophorus is renowned for its numerous impacts on native biodiversity, agricultural production, and the health of humans and animals (Adkins and Shabbir, 2014). Due to the severity of invasion in numerous countries, *P. hysterophorus* has been subjected to management interventions, including biological control using selected, introduced host specific natural enemies in several countries (Strathie et al., 2011). Nine insect agents and two rust fungi have been introduced and established in Australia, which has the most advanced

biological control programme for *P. hysterothorus* (Dhileepan et al., 2019). Although the realised impacts of *P. hysterothorus* invasions have caused widespread concern, only a few systematic studies, such as those by Ayele *et al.* (2013) and Ojija *et al.* (2019), have investigated the ecological impacts of this plant on native flora and fauna. Therefore, this study set out to investigate whether ant diversity and assemblage composition are altered by the occurrence of *P. hysterothorus* and one of its established biological control agents, *L. setosipennis*. I hypothesized that ant diversity and assemblage composition will be altered by the occurrence of *P. hysterothorus* and *L. setosipennis*.

Materials and Methods

Study site and data collection

Ant diversity and assemblage composition were studied at four locations in KwaZulu-Natal province and five in Mpumalanga province (refer to Appendix 1 for site locality details). The selected sites included locations adjacent to riparian habitats, cultivated or abandoned croplands, within game reserves and along roadsides. The study took place at sites at which *L. setosipennis* had been released previously and was known to have established (Strathie et al., 2021). The study was conducted on five sampling occasions during the growing season of *P. hysterothorus* (as it is an annual plant), from December 2019 to March 2020, thereafter disrupted by Covid-19 pandemic travel restrictions, but resumed for a final assessment in October 2020. Sites in KwaZulu-Natal province were sampled on consecutive days, followed at a two-week interval by consecutive sampling of sites in Mpumalanga province, so that all study sites were sampled on a monthly basis.

The temperatures during the entire study period were recorded using an iButton® (Maxim Integrated San Jose, CA, USA), placed at a height of 1m in the shade at each site (Refer to Appendix 2 for mean minima and maxima).

At each study site, three patches that were approximately 100m apart from each other were selected, for three treatments. The selected treatments were: *P. hysterothorus* invaded site (referred to as *P. hysterothorus* only), *P. hysterothorus* invaded site with presence of *L. setosipennis* (referred to as *P. hysterothorus* + *L. setosipennis*), and site without *P. hysterothorus* (referred to as No *P. hysterothorus*). The presence of *L. setosipennis* was confirmed by characteristic black frass spots that covered *L. setosipennis* eggs laid within *P. hysterothorus* flowers, or by wilted shoot tips caused by feeding within stems by the larvae. If the areas selected were inadvertently sprayed with herbicide or mechanically cleared by

landowners during the course of the study, nearby suitable patches were selected as replacements.

Ant sampling

Pitfall trapping

Each 15m transect had five pitfall traps placed 1 m apart. Pitfall traps consisted of clear plastic jars (64 mm diameter, 110 mm height and 120 ml volume). Traps were placed from the 5m to 10m mark, to avoid any possible edge effects. Pitfall traps were dug into the soil at the centre of each 0.5 m x 0.5 m quadrat. The rim of each trap was placed level with the soil surface and the soil was pushed up adjacent to the trap jars so that ants could easily fall into the traps. The traps were half-filled with propylene glycol, which neither attracts nor repels ants, and left undisturbed for 48 hours during each sampling occasion. Traps were collected and returned to the lab, where ant specimens were washed in water and stored in 70% alcohol. All ant specimens were identified to genus level and valid species names were confirmed using AntWiki (<http://www.antwiki.org>) and AntWeb (<http://antweb.org>); otherwise, those ants that were unidentifiable below genus level were assigned to morphospecies.

Plant and biocontrol agent population variables

Plant (*P. hystrophorus* and other vegetation) and biocontrol agent (*L. setosipennis*) variables were assessed within each of the five 0.5 m x 0.5 m quadrats placed along the 15 m transect in each treatment. Within each quadrat, the following variables were recorded: the proportion (percentage) of *P. hystrophorus*, other herbs, grasses, and bare soil cover was estimated and the number of *P. hystrophorus* plants were counted. To determine average height, the height of 10 *P. hystrophorus* plants (two tallest, two shortest, and six of intermediate height) per quadrat was measured using a wooden folding ruler. The total number of inflorescences was counted on the 10 plants selected per quadrat. Later, the average number of flowers per inflorescence on ten plants was counted, to facilitate calculation of the total number of flowers available. The number of *L. setosipennis* eggs laid in flowers (as indicated by the black frass capping over each egg inserted into the flowerheads) was counted on the ten plants to assess the abundance of the weevil.

Statistical Analysis

Using IBM SPSS Statistics 20 software, an Analysis of Variance (ANOVA) was conducted to compare ant species richness between the three treatments after the data was tested for normality. To compare abundance of ants between treatments, the number of individuals collected in each sampling cycle was summed for each treatment replicate (five pitfall traps) and compared between treatments using an ANOVA, followed by the Post-hoc Tukey's test. The pitfall trap abundance data were log transformed to help meet the ANOVA assumptions.

Simpson's Diversity Index ($1/D$), Evenness Index (e^H/S) and Shannon's Diversity Index (H' , using \log_e) and Dominance were used to compare ant diversity in the three treatments, using PAST (PAleontological STatistics) software Version 4.03 (Anu and Sabu, 2007). Although there are several indices that can be used to show diversity, one of the fundamental difficulties in all fields of biodiversity assessment is the over-dependence of these indices on sampling effort (Anu and Sabu, 2007).

iNEXT online (Chao and Hsieh, 2016) was used to compute individual-based rarefaction curves. Interpolation and extrapolation of ant assemblages were based on Hill's numbers and were obtained at 95% confidence intervals by bootstrap method based on 100 replicates. This was useful in determining sampling efficiency.

To examine treatment effects on ant species composition, a non-metric multidimensional scaling analysis (nMDS) with a Bray-Curtis distance scale in Primer Version 6 was used (Clarke and Gorley 2006). The analysis of similarities (ANOSIM) method was used to compare treatments and similarity of percentage (SIMPER) to determine dissimilarities between the three treatments. ANOSIM detects differences in species assemblages to differentiate between two or more groups.

The best (most representative) vegetative variable (*P. hysterothorus* height, cover and flowering score) was used to correlate ant diversity and assemblage composition using CANOCO Version 4.5 software. The default options for the Canonical Correspondence Analysis (CCA) used were focus scaling on interspecies distance, scaling type = bi-plot scaling. No data were transformed, although the option to down-scale the weighting of rare species was selected.

A forward selection of vegetative variables was performed using the Monte Carlo Permutation test under a complete model to determine the vegetative variables that explained the majority of the variation in ant species diversity and composition. Using 499 Monte Carlo permutations,

the importance of the CCA was investigated in relation to the complete model and each vegetative variable.

Results

Ant species diversity

A total of 16 463 individual ants were collected over five sampling periods in nine locations in the two provinces. Four subfamilies, 27 genera and 55 species were recorded. Myrmicinae was the most abundant and diverse subfamily with 29 species, nine genera and 85% of the total abundance, followed by the Formicinae subfamily (15 species and nine genera), and Ponerinae (nine species and seven genera) within KwaZulu-Natal and Mpumalanga provinces.

The differences in ant species richness and abundance among all three treatments within KwaZulu-Natal and within Mpumalanga sites were not significant. At the KwaZulu-Natal sites, the highest ant species abundance (51% of the total abundance) was recorded in the *P. hysterophorus* invaded sites, followed by those sites with no *P. hysterophorus* (27% of the total abundance). Sites with *P. hysterophorus* and *L. setosipennis* had the lowest number of individuals (22% of the total abundance). However, there were no statistically significant differences in abundance among the three treatments in KwaZulu-Natal sites ($F = 0.575$; d.f. = 2; $p > 0.05$). In KwaZulu-Natal, *P. hysterophorus* invaded sites with *L. setosipennis* had the highest number of genera (19) and species (40) followed by *P. hysterophorus* invaded sites (18 genera and 39 species) (Table 2.1).

At the sites in Mpumalanga province, the highest ant species abundance was recorded in the sites with no *P. hysterophorus* (35% of the total abundance of all treatments), followed by *P. hysterophorus* invaded sites (33% of the total abundance) and *P. hysterophorus* invaded sites with *L. setosipennis* (32% of the total abundance). However, there were no statistically significant differences in abundance among the three treatments at Mpumalanga sites ($F = 0.006$; d.f. = 2; $p > 0.05$).

In Mpumalanga, *P. hysterophorus* invaded sites had the highest number of genera (18) and species (33). However, there were no significant differences in ant species richness between the three treatments at the Mpumalanga study sites ($F = 0.294$; d.f. = 2; $p > 0.05$). KwaZulu-Natal sites had a higher diversity of ants, with a higher number of species recorded in all treatments as compared to Mpumalanga province (Table 2.1).

In summary, there were no statistically significant differences in ant species abundance among the three treatments at sites within KwaZulu-Natal ($F = 0.575$; $df = 2$; $p > 0.05$) and within Mpumalanga provinces ($F = 0.006$; $df = 2$; $p > 0.05$). There were also no significant differences in ant species richness between the three treatments at the KwaZulu-Natal study sites ($F = 0.782$; $d.f. = 2$; $p > 0.05$) or at the Mpumalanga study sites ($F = 0.294$; $d.f. = 2$; $p > 0.05$).

Table 2.1: Ant species richness and species abundance in habitats invaded by *Parthenium hysterophorus*, with and without *Listronotus setosipennis*, and adjacent uninvaded habitats at nine study sites in KwaZulu-Natal and Mpumalanga provinces.

Treatment	No. of Genera	No. of Species	No. of Specimens	Abundance (%)
KwaZulu Natal				
No <i>P. hysterophorus</i>	15	38	2308	27
<i>P. hysterophorus</i>	18	39	4388	51
<i>P. hysterophorus</i> + <i>L. setosipennis</i>	19	40	1945	22
Mpumalanga				
No <i>P. hysterophorus</i>	18	29	2745	35
<i>P. hysterophorus</i>	18	33	2545	33
<i>P. hysterophorus</i> + <i>L. setosipennis</i>	17	30	2532	32

In KwaZulu-Natal, Shannon's diversity index recorded high diversity in No. *P. hysterophorus* treatment and equal diversity in *P. hysterophorus* and *P. hysterophorus* + *L. setosipennis* treatments. Simpson's diversity index recorded higher diversity in No. *P. hysterophorus* treatments and equal diversity in *P. hysterophorus* and *P. hysterophorus* + *L. setosipennis* treatments. Evenness was high in No. *P. hysterophorus* treatments and equal evenness in *P. hysterophorus* and *P. hysterophorus* + *L. setosipennis* treatments.

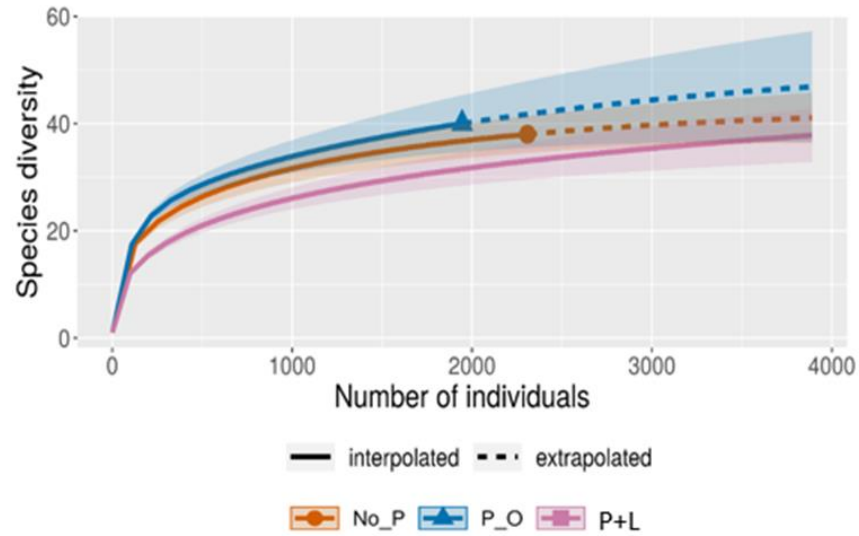
In Mpumalanga, Shannon's diversity index recorded high diversity in No. *P. hysterophorus* treatment and lower diversity in *P. hysterophorus* + *L. setosipennis* treatment (Table 2.2). Simpson's diversity index recorded higher diversity in No. *P. hysterophorus* treatments and lower diversity in *P. hysterophorus* + *L. setosipennis* treatment. Evenness was high in No. *P. hysterophorus* treatments and similar lower evenness in *P. hysterophorus* + *L. setosipennis* treatment (Table 2.2).

Table 2.2: Ant abundance, species richness, and diversity measure indices (Dominance_D, Simpson_1-D, Shannon_H, Evenness_e^H/S) in habitats invaded by *Parthenium hysterophorus*, with and without *Listronotus setosipennis*, and adjacent uninvaded habitats at nine study sites in KwaZulu-Natal and Mpumalanga provinces.

Treatment	Dominance_D	Simpson_1-D	Shannon_H	Evenness_e ^H /S
KwaZulu Natal				
No <i>P. hysterophorus</i>	0.184	0.816	2.258	0.252
<i>P. hysterophorus</i>	0.439	0.561	1.656	0.131
<i>P. hysterophorus</i> + <i>L. setosipennis</i>	0.352	0.648	1.644	0.133
Mpumalanga				
No <i>P. hysterophorus</i>	0.281	0.718	1.93	0.238
<i>P. hysterophorus</i>	0.343	0.657	1.812	0.186
<i>P. hysterophorus</i> + <i>L. setosipennis</i>	0.448	0.552	1.465	0.144

The extrapolation curves demonstrated the highest species diversity in *P. hysterophorus* invaded habitats and lowest in habitats with *P. hysterophorus* and *L. setosipennis* in KwaZulu-Natal study sites (Fig. 2.1a). However, at the Mpumalanga study sites, species diversity was the same for those two habitats (Fig. 2.1b). In both regions, diversity was higher in *P. hysterophorus* invaded habitats than uninvaded habitats.

(a)



(b)

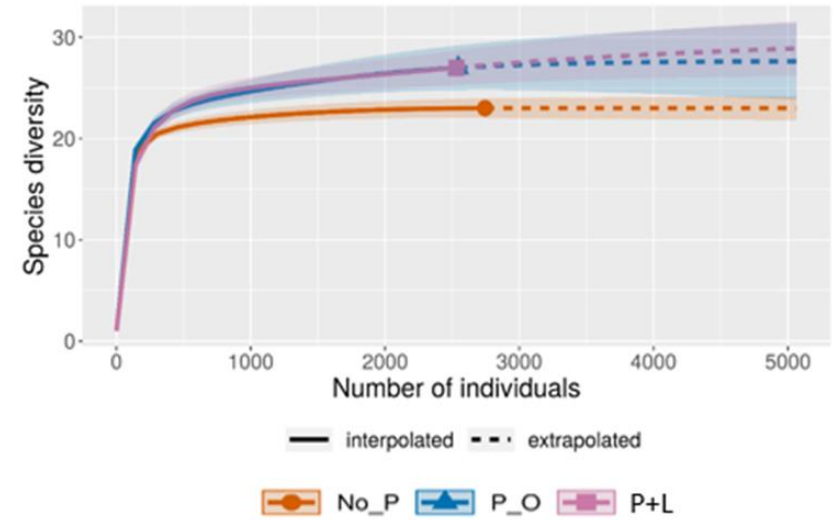


Figure 2.1: Individual-based and rarefaction (solid line segments) and extrapolation (dotted line segments) sampling curves with 95% confidence intervals for ant assemblage data of three treatments by diversity order: $q = 0$ (species richness), at nine study sites in (a) KwaZulu-Natal and (b) Mpumalanga provinces. The reference samples are represented by symbols: circle = No *P. hysterothorus*, triangle = *P. hysterothorus* and a square = *P. hysterothorus* with *L. setosipennis*.

Assemblage composition of ants

Species composition was most similar in the *P. hysterothorus* and *P. hysterothorus* with *L. setosipennis* treatments in KwaZulu-Natal and Mpumalanga provinces (Table 2.3). However, ANOSIM showed that there were no significant differences in species composition of ants between the three treatments within KwaZulu-Natal ($p > 0.05$; $R = 0.065$) and within Mpumalanga ($p > 0.05$; $R = 0.085$).

Table 2.3: Overall similarity of ant species composition in habitats invaded by *Parthenium hysterothorus*, with and without *Listronotus setosipennis*, and uninvaded habitats at nine study sites in KwaZulu-Natal and Mpumalanga provinces.

Treatment comparison	Overall similarity (%)	
	KwaZulu-Natal	Mpumalanga
No <i>P. hysterothorus</i> vs <i>P. hysterothorus</i>	25.7	63.5
No <i>P. hysterothorus</i> vs <i>P. hysterothorus</i> + <i>L. setosipennis</i>	62.9	46
<i>P. hysterothorus</i> vs <i>P. hysterothorus</i> + <i>L. setosipennis</i>	85.7	83.3

The non-metric multidimensional scaling (nMDS) plot showed similarities in ant species composition in the no *P. hysterothorus* and *P. hysterothorus* only treatments in KwaZulu-Natal (Fig 2.2a). Treatments with *P. hysterothorus* only and *P. hysterothorus* with *L. setosipennis* had similar species composition in Mpumalanga (Figure 2.2b).

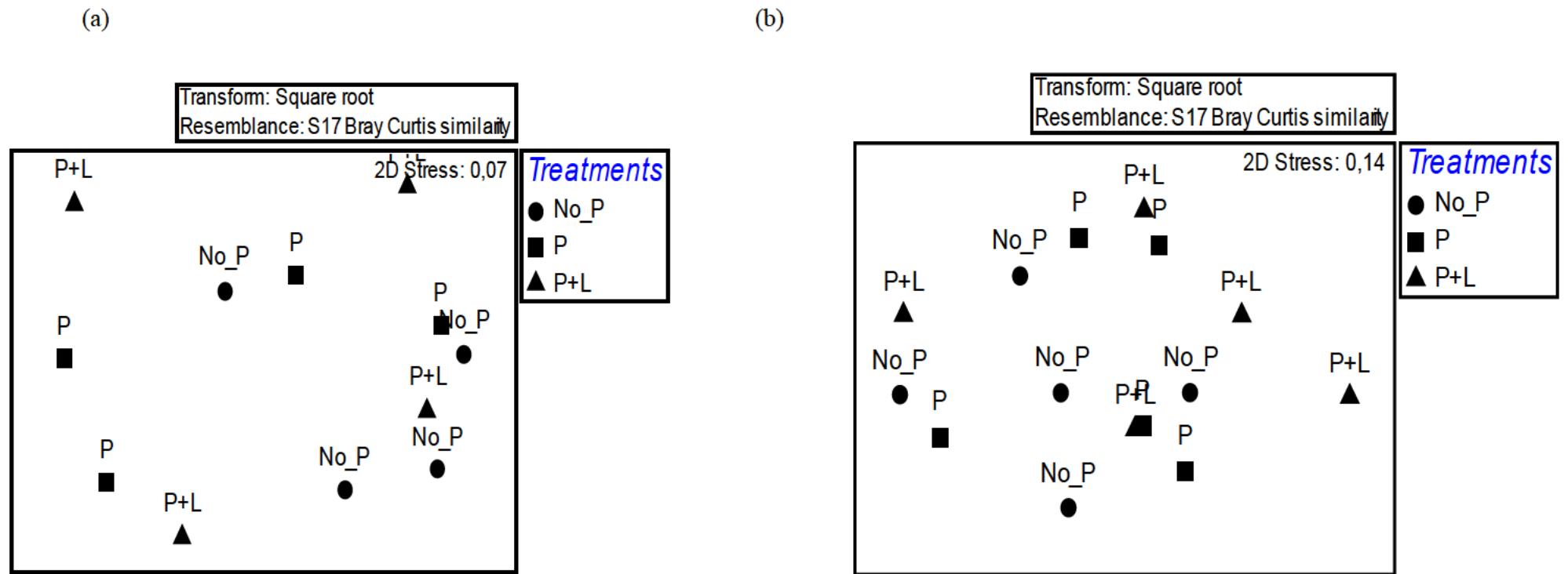


Figure 2.2: Nonmetric Multidimensional Scaling (nMDS) of ant assemblages in three treatments at study sites in (a) KwaZulu-Natal and (b) Mpumalanga provinces. The circles represent No *P. hysterophorus* (No_P), squares represent *P. hysterophorus* (P), and triangles represent *P. hysterophorus* with *L. setosipennis* (P+L) treatments

The Canonical Correspondence Analysis results showed spatial distribution of variables (Fig. 2.3). Ant species were most highly correlated with the second (horizontal) ($r= 0.831$) and first (vertical) ordination ($r= 0.723$) axis in KwaZulu-Natal and in Mpumalanga, respectively. In KwaZulu-Natal, axes one and two accounts for 16.1% and 26.6% of the total variability of the species data set and 49.8% and 82.2% of the variability in the environmental data set (*P. hysterothorus* height, cover and flowering score) respectively. In Mpumalanga, axes one and two account for 10.1% and 15.2% of the total variability of the species data set and 51.8% and 78.4% of the environmental data set (*P. hysterothorus* height, cover and flowering score), respectively. In KwaZulu-Natal, the ordination diagram (Fig. 2.3a) shows that the second ordination axis (horizontal) was closely correlated with all measured variables. Along the direction of the horizontal axis, the left side represents the *P. hysterothorus* + *L. setosipennis* treatment. It corresponds to ant species dominated by *Pheidole* UKZN_02 (*megacephala* gp.), *Carebara vidua*, *Cardiocondyla* UKZN_02 (*elegans* gp.), *Polyrhachis* UKZN_02 (*schistacea* gp) and *Parasyscia* UKZN_03. In Mpumalanga, the ordination diagram (Fig. 2.3b) shows that the first ordination axis (vertical) was closely correlated with all variables. Along the vertical axis, the right side represents the *P. hysterothorus* and *P. hysterothorus* + *L. setosipennis* treatments and is dominated by *Bothroponera kruegeri*, *Camponotus* UKZN_05 (*maculatus* gp.), *Pheidole* UKZN_02 (*megacephala* gp), *Lepisiota crinata*, *Plectroctena mandibularis*, and *Cardiocondyla* UKZN_02.

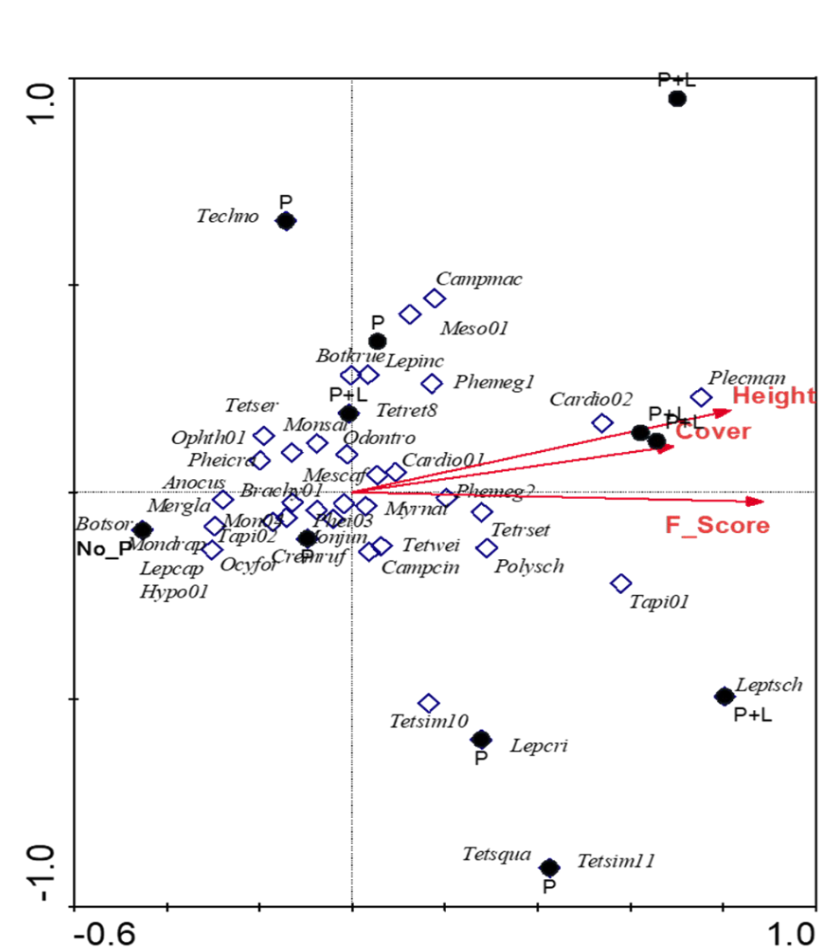
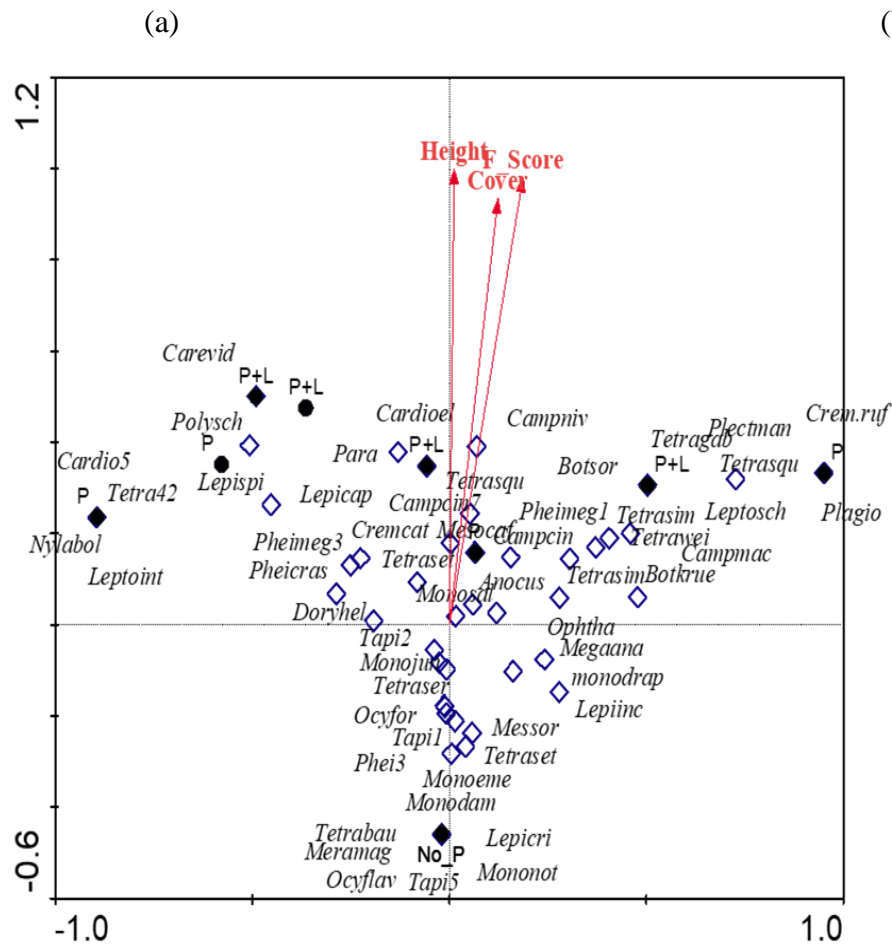


Figure 2.3: Canonical Correspondence Analysis ordination plot of the habitat structure variables (mean *P. hysterothorus* plant height, total number of *P. hysterothorus* flowers and proportion of *P. hysterothorus* cover) that explained significant amounts of variation between ant assemblages and treatments in sites in (a) KwaZulu-Natal and (b) in Mpumalanga. Refer to Appendix 4a and 4b for details of ant names.

Discussion

Alien plant invasions influence not only native plant communities but also the diversity and structure of associated arthropod communities. The current study indicated that the presence of *P. hystrophorus* and its biological control agent *L. setosipennis* had no significant influence on ant diversity and assemblage composition, although there were some differences in total ant abundance and diversity across treatments in both KwaZulu-Natal and Mpumalanga regions. Comparisons with other studies are difficult due to the lack of studies investigating the impact of *P. hystrophorus* on ant communities. However, studies such as Parr et al. (2010) reported that the invasion of *Andropogon gayanus* Kunth (Poaceae), a different invasive plant to that studied here, similarly had no effect on ant species diversity and abundance across the tropical Australian savannah.

In general, very few studies have investigated the impacts of invasive alien plants on ant assemblages. However, studies such as French and Major (2001) reported some variations in the composition of ant assemblages caused by the invasion of the alien plant *Acacia saligna* (Labill.) H.L. Wendl. (Fabaceae) in South African fynbos. Fork (2010) also reported that the abundance of Hymenoptera, including ants, decreased as the numbers of the invasive plant *Baccharis pilularis* de Candolle (Asteraceae), increased. Studies such as that of Mgobozi et al. (2008), Bezemer et al. (2014), and Simao et al. (2016) have reported that by interacting with or displacing native host plants, invasive plants such as *Chromolaena odorata* R. King and H. Robinson (Asteraceae), *Vincetoxicum rossicum* (Kleopov) Barbarich (Apocynaceae) and *Microstegium vimineum* (Trinius) A. Camus (Poaceae) may significantly impact on native arthropod populations. And therefore causing detrimental impacts on the diversity and abundance of native insects. Other studies, for example, Gerber et al. (2008), have shown that removing invasive plants such as *Fallopia japonica* var. *japonica* (Houtt) (Polygonaceae) increases native insect abundance and diversity, or contributes to a complete recovery of the native insect population in riparian habitats.

Studies such as those by Simao et al. (2010), Bemezer et al. (2014), Litt et al. (2014), Schirmel et al. (2016) found both positive and negative impacts on native insects caused by invasive alien plant species. For example, these studies determined that plant invasion correlated with increases and decreases in abundance, composition, and diversity of arthropod communities. However, considering that alien plant invasions can drastically change vegetation structure, it is expected that they could consequently lead to a change in the diversity and abundance of arthropods (Tang et al., 2012). Invasive alien plants often outcompete native plants, essential

food sources for animals, or alter ecosystem features, rendering them unsuitable for specific species. To manage these novel ecosystems, it is critical to understand how native insect assemblages respond to these changes.

Since many arthropods are known to specialize by associating with specific host plants for foraging and reproduction, the lack of noticeable differences between the arthropods associated with native and non-native plant species, as observed in this current study, may be surprising. Additionally, the assessment of ant assemblages was limited to soil dwelling ants. It is possible that *P. hysterophorus* invasion did affect the species richness and abundance of non-soil dwelling arthropods and this was not investigated in the study. However, many arthropods are generalist predators or scavengers unaffected by plants. Other factors such as variation in rainfall may have played a role in some of the results of this study for certain groups of ants that might have been less affected by the changes in standing vegetation. For example, non-arid specialist species appeared unaffected by non-native grass invasion. In contrast, those limited to arid environments declined in regions invaded by European grasses in the San Diego National Wildlife Refuge (Wolkovich et al., 2009). In other studies, species richness is associated with temperature; for example, in warmer regions, ant assemblages were more diverse than in colder regions on a global scale (Gibb et al., 2015).

Individual-based and rarefaction and extrapolation curves demonstrated that the species evenness was particularly sensitive to sampling effort. The function of rarefaction is to standardize an unequal number of samples. In this case, the differences were not sufficiently distinct to detect changes in ant diversity between the sites. Additionally, comparing species distribution equity among assemblages may be misleading, especially if the number of samples is different. Ants live in colonies varying in size (Leponce et al., 2004). Therefore, it can not be concluded that the species correspond to distinct treatments.

The current study also considered vegetative variables. The results showed that plant and biocontrol agent population variables (*L. setosipennis* eggs) did not significantly influence ant assemblages. The study plots had a wide range of native vegetation cover, with *P. hysterophorus* cover ranging from 5% to 90%, cover of other herbaceous species varied from 5% to 70%, and bare soil varied from 0.5% percent to 85% during this study. This change resulted from seasonality and growth of plants, which was not considered in the analyses of this study. However, geographical variation in plant vegetation cover poorly explained the observed variance between treatments in ant diversity. Cross et al. (2016) found declines in overall ant species richness, total ant abundance, and the abundance of many common ant

species with increased vegetation cover in peri-urban coastal dunes. As the amount of natural insulation on the ground provided by vegetation cover decreases, the thermal environment for thermophilic organisms becomes less suitable (Cross et al., 2016).

Some studies reported that the richness and abundance of invertebrates differed between native and non-native species. The observed impact of several taxa depended on which microhabitat of the plant was sampled. For example, Simao et al. (2010) reported that tick survival was reduced due to *Microstegium vimineum* invasion resulting in higher temperatures and lower humidity in the invaded habitat. Studies such as Wielgoss et al. (2010), Gibb et al. (2019) and Pereda-Gomez et al. (2020) have associated factors such as temperature, rainfall, and relative humidity with ant diversity and abundance in habitats infested by invasive alien plants. These may account for some significant variations in aspects such as diversity and composition.

Understanding how native insect assemblages respond to these changes in vegetation is critical in managing these novel ecosystems. Multiple studies such as Richardson et al. (2007) and McCary et al. (2016) have linked alien plant invasions and biological control to increases and decreases in diversity and abundance of native plants and animals. Changes in ant assemblages with *P. hysterophorus* infestations and biocontrol agent establishment have not been recorded historically, so a change or loss of vulnerable species may not be apparent. Despite these caveats, the findings of this study showed no significant variations in ant species diversity and assemblage composition whether *P. hysterophorus* was present, with or without *L. setosipennis*, or absent. Few studies (for example Topp et al., 2008; Watts et al., 2015; Kajzer-Bonk et al., 2016), have examined changes in animal assemblages, especially invertebrate assemblages, in weed-invaded sites, so this study has contributed to this aspect.

Further research could explore potential allelopathic elements of *P. hysterophorus* on ant communities, another factor that may influence ant diversity and assemblage composition. Possible explanations for the lack of significant results on the impacts of *P. hysterophorus* and its biological control agent *L. setosipennis* on ant diversity and assemblage composition, could include that sites with no *P. hysterophorus* may not have been sufficiently distinct or distant from *P. hysterophorus* invaded sites, or that *L. setosipennis* was not sufficiently abundant or established for long enough, as a consequence of its limited establishment, to have significantly influenced ant communities. It is however, still recommended that *P. hysterophorus* be managed to ensure native plant productivity and community stability. Furthermore, the findings of this study have added to growing evidence demonstrating the impact of ant assemblages as important indicators of local species richness. As native insects and alien plants

have little or no evolutionary background, native insects are not evolved to use these novel plants for food or reproduction (Mgobozi et al., 2008). As a result, arthropod population dynamics, including diversity and abundance, could heavily influence plant invasions (Spafford et al., 2013). However, this current study could not demonstrate or verify this association.

Information from studies such as this one could be important in determining prioritization of restoration projects, such as responding to the presence of a specific invasive alien plant species in an area of conservation significance. The use of ants as study subjects can yield valuable information as they represent various functional groups and are relatively easy to identify. Ants are essential seed dispersers and predators (Litt et al., 2014), and their effects on seed removal should be carefully considered for planned seeding and restoration efforts.

Although the extent of invaded regions, patch connectivity, and distance to native vegetation were not considered in this study, these parameters could be crucial indicators of invasive plant effects on arthropods such as ants. Furthermore, gathering and incorporating extensive information about litter and soil features related to alien plant invasions could increase understanding of the mechanisms that drive the effects of plant invasions on arthropods, for example, on different life stages of ants and their activities. Quantifying changes in the interaction of species (e.g. pollination networks, food webs) and population-level variables (e.g. reproduction, survival) might also reveal complex effects that are not captured by presence and abundance information alone.

An increasing number of studies has demonstrated significant changes to native invertebrate communities where alien invasive plants have invaded (Harris et al., 2004). Given that alien plant invasions can dramatically alter vegetation structure, it is not surprising that these invasions also can change arthropod communities, through declines in abundance and diversity (Simao et al., 2010; Yoshioka et al., 2010). This study also examines the impacts of *P. hysterophorus* and *L. setosipennis* on other native arthropods.

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Chapter 3: Activities of native arthropods associated with *Parthenium hysterophorus* L. (Asteraceae) and the influence of the biological control agent *Listronotus setosipennis* (Hustache) (Coleoptera: Curculionidae)

Abstract

The invasion of alien plant species will likely change the native faunal and floral communities in the invaded area. These changes may be especially detrimental or beneficial to some arthropods as many species rely on specific plants for food and reproduction. This study investigated the arthropods that visited *Parthenium hysterophorus* plants, with and without the biological control agent *Listronotus setosipennis*, and examined their activities on the plants. Visitations to *P. hysterophorus* plants by arthropods were quantified in timed observations conducted at three-time monthly intervals from December 2020 until March 2021 at six sites in KwaZulu-Natal and Mpumalanga provinces. The total number of arthropod visitors and their visitation frequency did not vary between the treatments with and without *L. setosipennis*. A diverse range of arthropod visitors, including representatives of Coleoptera, Diptera, Hymenoptera, Hemiptera as the major groups were recorded on *P. hysterophorus*, but Hymenoptera, and particularly ants, were the most common. When all arthropods, as measured by the number of individuals, were taken into account in the current study, there were no significant differences in arthropod activity noted between *P. hysterophorus* plants with and without the biological control agent *Listronotus setosipennis*. This study also showed that the exploratory, feeding, and resting activities of arthropods on *P. hysterophorus* were not significantly impacted by the presence or absence of *L. setosipennis*. In terms of land management, it is reassuring that even heavily invaded landscapes can sustain diverse arthropod populations. Non-native species are not always associated with decreased arthropod assemblages. Arthropod communities do not seem to respond strongly to plant origin. Assessing arthropods at higher taxonomic level (as one currently) does not provide a good indication of the ecological impacts of plant invasions and their management.

Keywords: biological control, impact, invertebrates, parthenium weed, visitations

Introduction

Invasive alien plants have the potential to change habitat structure due to their effects on vegetation variety and composition, ecosystem processes and biotic interactions (Galle et al., 2015). Although there are exceptions, an increasing number of studies have shown that where invasive plant species have replaced native plants, there is an influence on native invertebrate assemblages (Standish et al., 2004). While alien plant invasions have been linked to severe ecological damage in various ecosystems, their impact on native arthropod fauna has received less attention. While it is difficult to generalise due to the limited information, invasive plants are known to significantly influence arthropods that forage on plants directly or indirectly (Bultman and DeWitt, 2008). Unless these invasive alien plants are closely related or chemically similar to native plant species, alien plants are unlikely to be palatable to most native herbivores (Ernst and Cappucino, 2005).

Many alien plants establish dense monospecific stands that flower profusely, potentially introducing significant volumes of exotic pollen and nectar into the native communities (Larson et al., 2006). Plants convey information to animals in two ways: signals that benefit the plant and incidental cues that animals detect. These plant-animal communications are crucial for plant-animal interactions, including pollination, herbivory and frugivory (Stewart et al., 2021). Changes in native plant seed set, pollen deposition, pollinator numbers, and variety, or pollinator fauna composition could result from such integration (Gibson et al., 2012). The impacts of invasive plants on arthropod abundance, composition, and diversity have been the subject of several studies, with evidence of impact in both negative and positive directions (Maceda-Veiga et al., 2016).

This study examined arthropods visiting *P. hysterothorus* plants, their activities on the plant, and the impact of the presence and absence of a biological control agent, *L. setosipennis*, used in the control of *P. hysterothorus*, in two South African provinces. The biological control programme on *P. hysterothorus* in South Africa has focused on the importation, host-specificity evaluation, mass-rearing, release and post-release evaluation of three insect agents, including *L. setosipennis*, and a rust fungus. More than 45 000 adults of *L. setosipennis*, reared by ARC-PPH Cedara and the South African Sugarcane Research Institute Weed Biocontrol Unit, have been released since 2013, at approximately 150 sites in KwaZulu-Natal and Mpumalanga provinces, with releases at new sites continuing (Strathie et al. 2021). *Listronotus setosipennis* has established at multiple sites, and survived severe drought conditions during

2015/2016. Although the weevil disperses slowly, larval feeding causes structural damage to the plant and results in localised impact on *P. hysterothorus* (Strathie et al., 2021).

Non-native plant introductions can introduce additional non-native species, such as biological control agents, which may alter the composition of non-native plant communities (Sunny et al., 2015) and, as a result, the insects that depend on them. Arthropod activities may be influenced by the invasive plant positively, negatively, or neutrally, as recorded in systems with other invasive plants such as *Opuntia stricta* (Haw.) Haw. (Cactaceae) (Robertson et al., 2011), *Solidago* sp. L. (Asteraceae) (Lenda et al., 2013), and *Chromolaena odorata* (Mgobozi et al., 2008). Invasive plants may provide a sufficient niche at first, but native insect fitness may deteriorate with time, resulting in a decline in native insect populations (Sunny et al., 2015). Assuming that the plant species complex within a habitat remains relatively consistent over time, insects may evolve responses that allow them to choose patches or sites that contain sufficient resources while avoiding patches that do not (Bezemer et al., 2014).

The objectives of this study were to (i) identify the arthropods that visited *P. hysterothorus*, with and without *L. setosipennis* eggs on plants, during the day and (ii) investigate the type of activities that were undertaken by the arthropods on *P. hysterothorus* plants, with and without *L. setosipennis*. It was postulated that the presence of *P. hysterothorus* and one of its biological control agents, *L. setosipennis*, may affect the surrounding faunal activities by attracting or deterring arthropods to the plants. Therefore, it was hypothesized that populations of the biological control agent, *L. setosipennis*, may increase the number of arthropod visitors and their visitation frequency to plants and flowers with eggs of the weevil.

Materials and Methods

Study sites

The study was carried out at three sites in KwaZulu-Natal province and three sites in Mpumalanga province. In these provinces, *P. hysterothorus* is most invasive and at sites at which *L. setosipennis* had been released previously and had established. The selected study sites were located along roadsides, banks of dams and rivers, and adjacent to cultivated croplands, most of which are frequently disturbed either by humans or animals (Refer to Appendix 5 for site details).

Plant visitation activities

At each site, there were two treatments, namely (i) *P. hysterothorus* with *L. setosipennis* present and (ii) *P. hysterothorus* present but without *L. setosipennis*. *Listronotus setosipennis* adults hide, especially during the day, so are difficult to detect and larvae are internal stem feeders so can only be quantified by destructive sampling. So eggs (identifiable by distinctive black marks on flowers, where frass covers eggs inserted into the flowerhead) as the most commonly observed life stage, were used to indicate and quantify *L. setosipennis* presence. Within each treatment at each site, a 15 m transect was laid out using a 50 m measuring tape. The transects were selected within approximately 100 m of each other at each site.

Four quadrats (0.5 m x 0.5 m) were placed at 5 m intervals along each 15 m transect. The number of flowers and *L. setosipennis* eggs per plant were counted on all plants in each quadrat. Observations of visitations and activities of arthropods on *P. hysterothorus* plants were conducted monthly at all sites from December 2020 to March 2021. All arthropod visitors to *P. hysterothorus* plants within each quadrat were recorded by visual observation at three set times (at 07:00 am, 10:00 am, and 12:00 pm) of the same day per site, for each sampling occasion. Observations were undertaken at three time intervals to accommodate for variations in the type of arthropod visitors and their possibly variable levels of activity during the day. Each quadrat was observed by two observers for five minutes, with quadrats observed consecutively along each transect and each treatment, at each set time. Sites within each province were sampled on consecutive days every month, with two weeks between sampling in each province. For the most part, the study was conducted under conditions with no rain and wind speeds less than 8 m/s.

For each arthropod visitor observed on *P. hysterothorus* plants within quadrats, their taxonomic group, the part(s) of the plant visited (reproductive, vegetative or both reproductive and vegetative structures), their type of activity on the plant, and the duration of their visit to the plant were recorded. Visitors to *P. hysterothorus* plants were identified to the taxonomic level of Order. The number of arthropods that visited the plants was recorded per taxonomic group. Activities were categorised as (i) exploratory (activity directed towards acquiring information about the plant), (ii) foraging (searching for/collecting food on the plant), or (iii) resting (a period of immobility, individuals unresponsive to the plant). The number of visits made to individual flowers with and without *L. setosipennis* eggs were recorded, as well as whether these visits were to feed on flower nectar or "other", to represent another potential activity such as collecting pollen or predation of *L. setosipennis* eggs.

Statistical analysis

The visitation frequency and the number of visitors to the plants were compared across all sites because the data were not normally distributed and comprised of many zeros (no visitations occurred). The flower visitation frequency was calculated as the number of visits of each group per flowerhead per quadrat in each treatment to avoid the bias of unequal numbers of flowers between replicates. The number (per taxonomic group) of plant visitors, their visitation frequency and their foraging behaviour on the plant were analysed using a one-way ANOVA (generalized linear model) with the number of quadrats as the units of replication and the treatments (with or without *L. setosipennis*) as the categorical predictor. The visitor taxonomic groups were compared between the treatments. Normality and variance homogeneity were tested using the Shapiro-Wilk and Levene's tests, respectively using IBM SPSS Statistics 20 software.

Results

A total of 11373 arthropod visitors were observed during the study period. A total of 622 visitors were observed on treatments with only *P. hysterothorus*, while treatments with *P. hysterothorus* and *L. setosipennis* had a total of 515 visitors (Table 3.1). The visitors belonged to 10 arthropod orders, of which the five most abundant orders were Hymenoptera (677 individuals), Hemiptera (55), Diptera (202), Araneae (84) and Coleoptera (65); these groups were used for further analyses. The remaining orders, in low numbers, were grouped into a category named "Other" (54) and included Orthoptera (30), Lepidoptera (13), Ixodida (5), Thysanoptera (4), and Odonata (2).

Table 3.1: The total number (N) of arthropod visitors per taxonomic group, identified to Order and proportion (%) of total visits observed on *Parthenium hysterophorus* plants with and without *Listronotus setosipennis* during fixed-time observations at six study sites in KwaZulu-Natal and Mpumalanga provinces during December 2020 to March 2021.

Visitor common name	Order	<i>P. hysterophorus</i>		<i>P. hysterophorus</i> + <i>L. setosipennis</i>	
		N	%	N	%
Spiders	Araneae	54	9	30	6
Ladybird beetles	Coleoptera	24	3	28	6
Weevils	Coleoptera	7	1	6	1
Flies	Diptera	94	15	108	21
Truebugs	Hemiptera	54	9	1	0
Bees	Hymenoptera	1	0	0	0
Ants	Hymenoptera	358	58	316	61
Wasps	Hymenoptera	1	0	1	0
Ticks	Ixodida	4	1	1	0
Butterflies	Lepidoptera	1	0	1	0
Moths	Lepidoptera	5	1	6	1
Dragonflies	Odonata	2	0	0	0
Crickets	Orthoptera	0	0	3	1
Grasshoppers	Orthoptera	15	3	10	2
Locusts	Orthoptera	1	0	1	0
Thrips	Thysanoptera	1	0	3	1
Total visits		622	100	515	100

Data are summed for the entire study period

There was no significant difference ($df = 1$, $F = 4.996$, $p > 0.05$) in the number of arthropod visitors to *P. hysterophorus* plants with and without *L. setosipennis*, at different times of the day over the study period (Fig. 3.1).

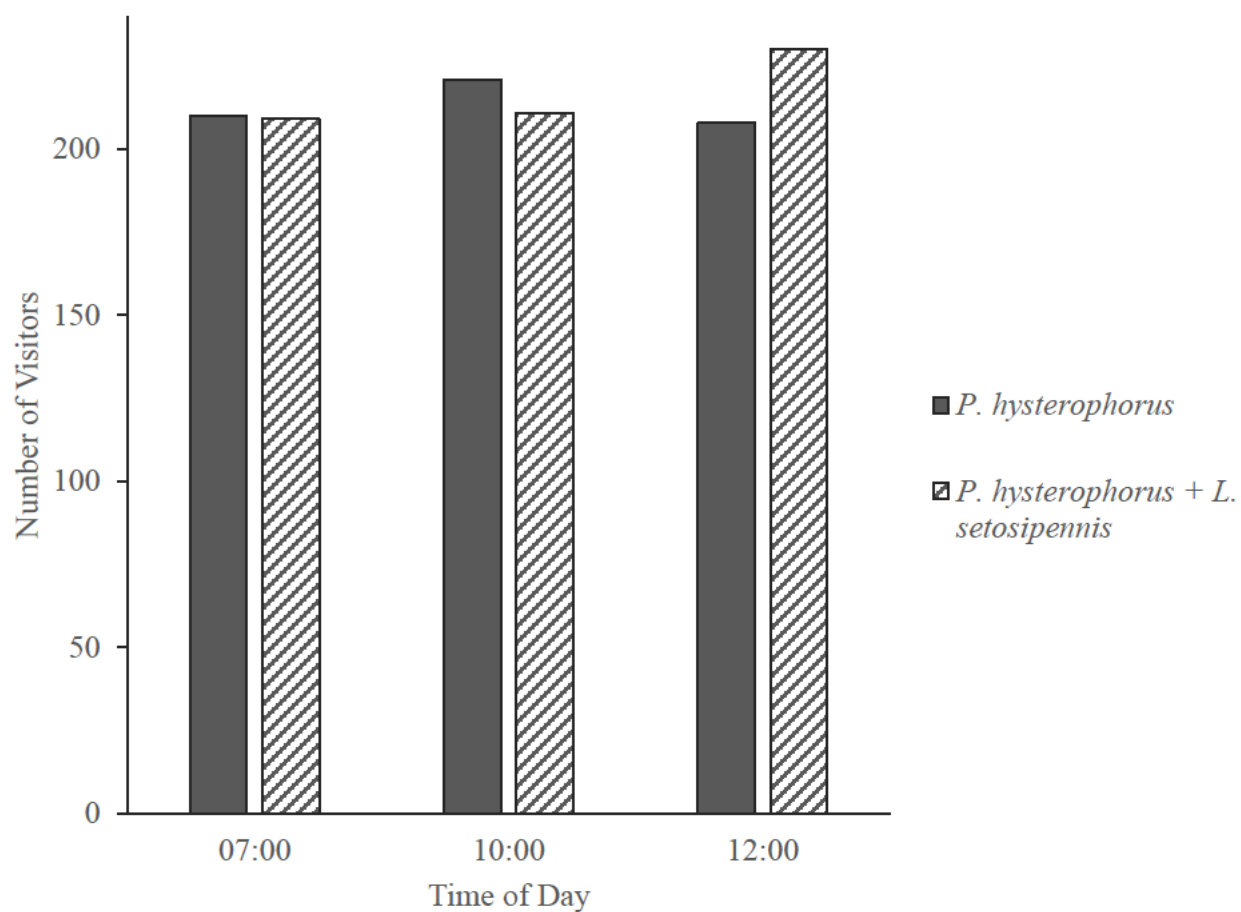


Figure 3.1: Total number of arthropods observed visiting *Parthenium hysterophorus* plants with and without *Listrionotus setosipennis* at three times of the day, assessed monthly during December 2020 to March 2021 at six study sites in KwaZulu-Natal and Mpumalanga provinces.

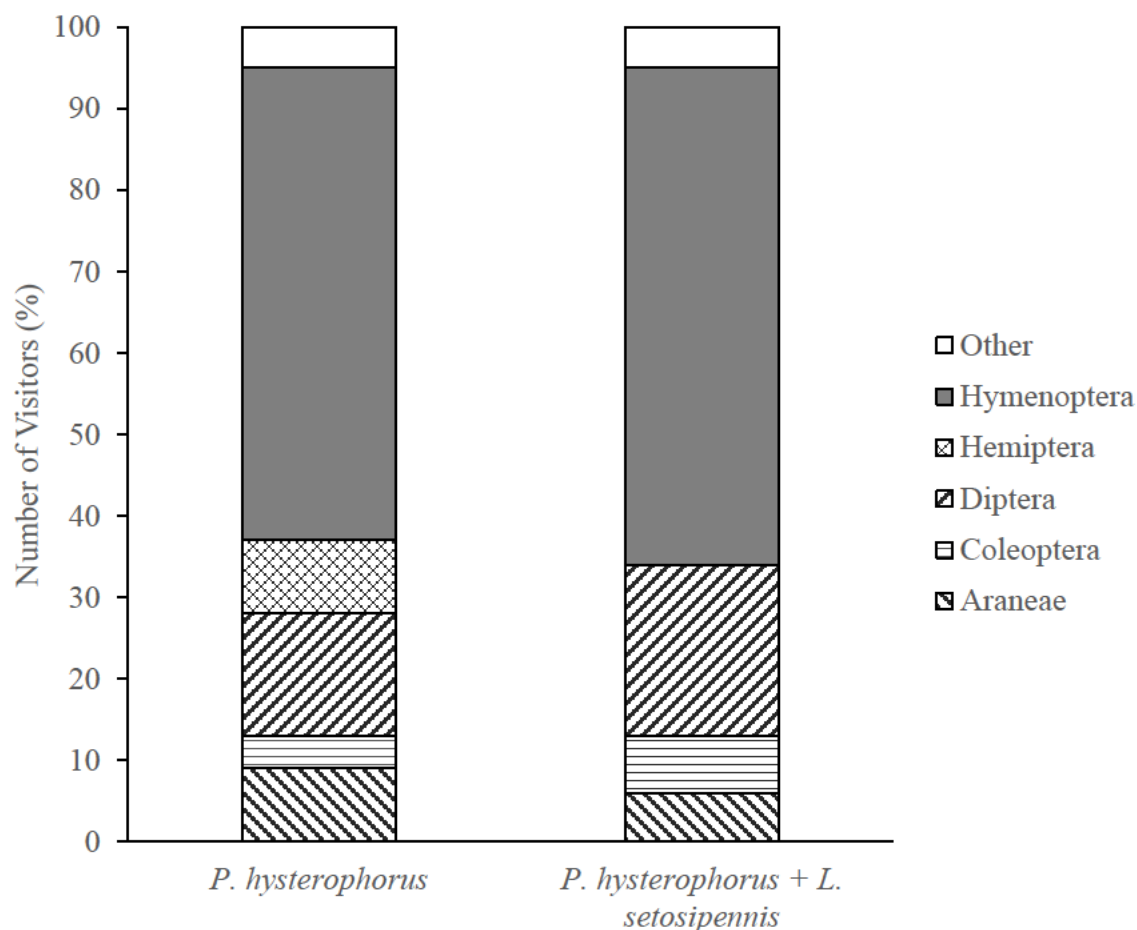
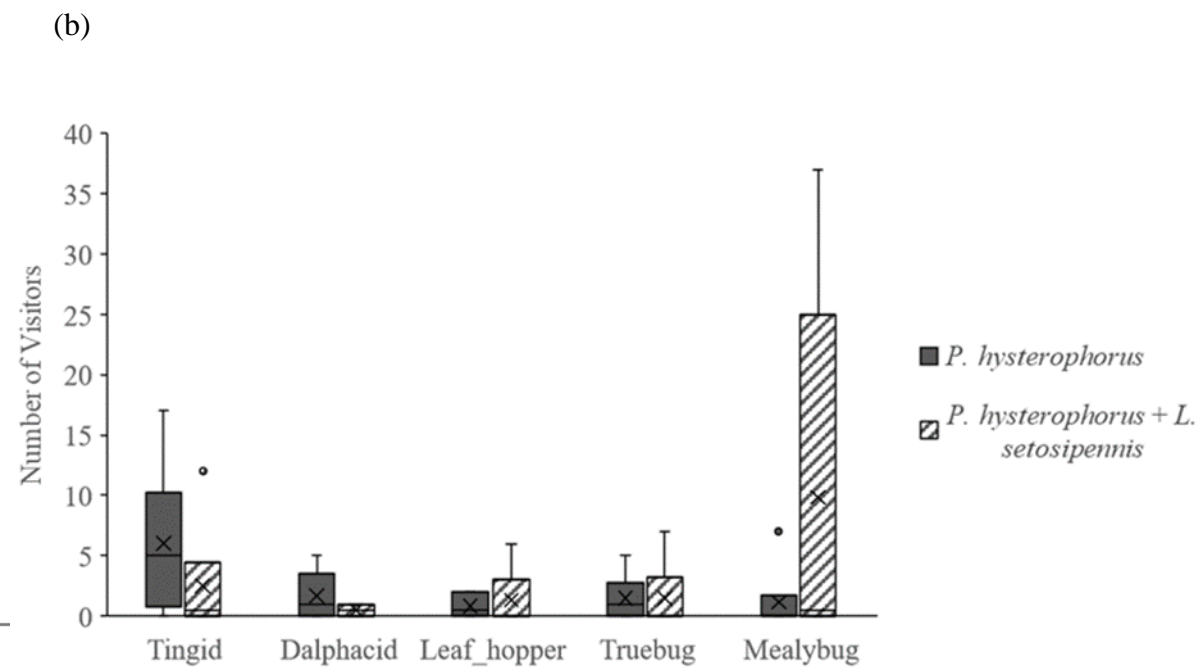
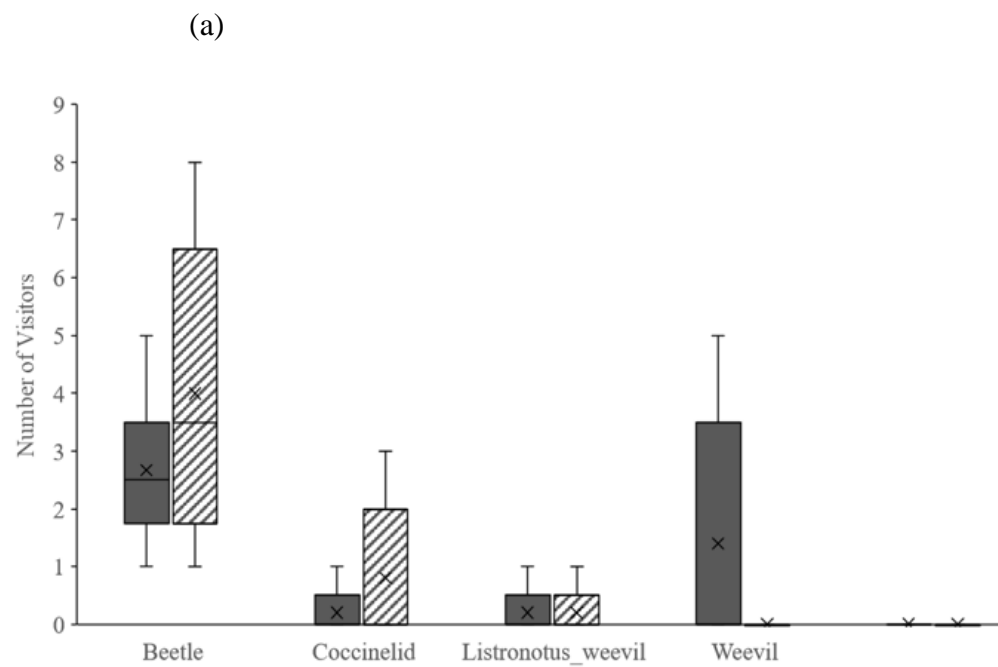


Figure 3.2: Number of arthropods per order as a proportion of the total number of arthropods that visited *P. hysterothorus* with and without *L. setosipennis* at six study sites in KwaZulu-Natal and Mpumalanga provinces during monthly sampling from December 2020 to March 2021.

The order Hymenoptera accounted for approximately 60% of the total number of arthropod visitors observed on *P. hysterothorus*, both with and without *L. setosipennis* (Fig. 3.2). The total number of Hymenopterans (wasps and ants), Coleoptera (beetles), and Hemiptera (true bugs, leafhoppers and mealybugs) to *P. hysterothorus* was not significantly different ($F = 1.543$, $p > 0.05$) between infestations with and without *L. setosipennis*, across all sites. Post-

hoc tests revealed that the number of beetles, coccinellids and mealybugs was higher on *P. hysterophorus* with *L. setosipennis* than on those plants without *L. setosipennis* (Figs 3.3a and 3.3b). Ants were the most common arthropod visitors to *P. hysterophorus* plants, both with and without *L. setosipennis* (Fig. 3.3c). Post-hoc comparisons revealed that there was no significant difference ($F = 4.294$, $p > 0.05$) in the total number of Coleopteran visitors or the total number of Hemipteran visitors ($F = 2.497$, $p > 0.05$) to *P. hysterophorus* with or without *L. setosipennis* (Figs 3.3a and 3.3b, respectively). However, there was a significant difference in the number of Hymenopteran visitors between *P. hysterophorus* with and without *L. setosipennis* ($F = 14.040$, $p < 0.05$) (Fig. 3.3c, respectively).



(c)

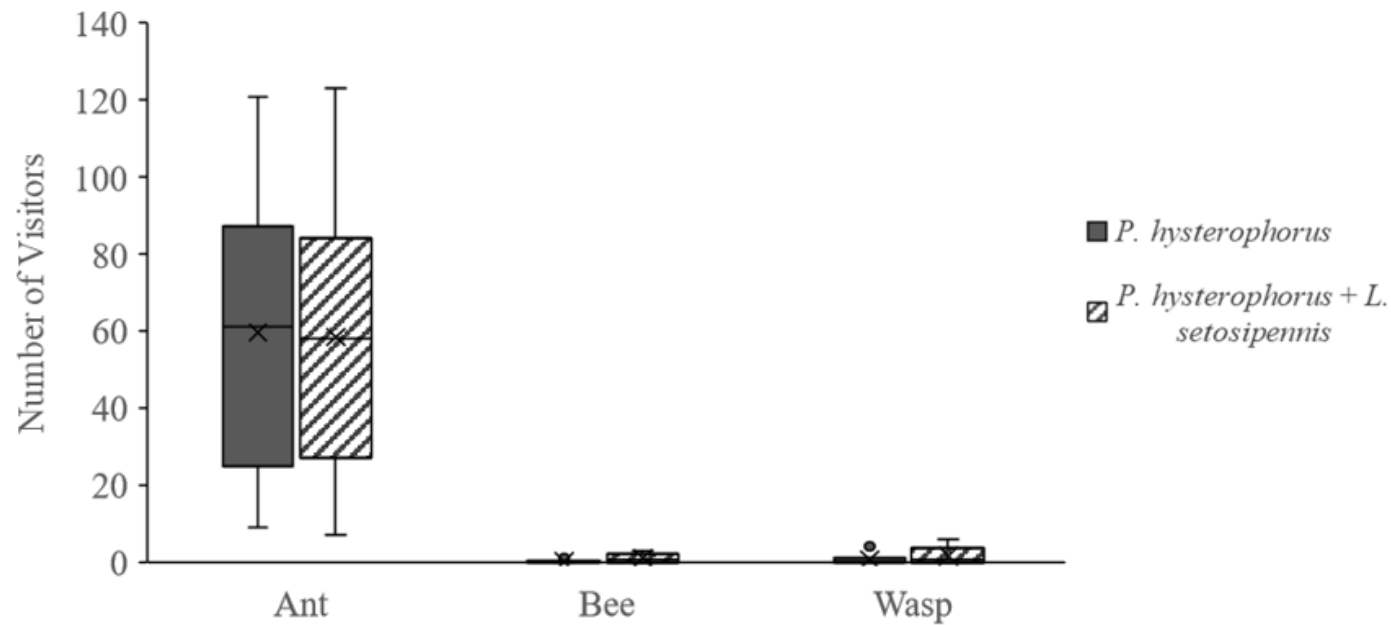


Figure 3.3: Total number of visitors, per order, to *Parthenium hysterophorus* plants with and without *Listrionotus setosipennis* for (a) Coleopterans ($F = 4.294$, $p > 0.05$); (b) Hemipterans ($F = 2.497$, $p > 0.05$), and (c) Hymenopterans ($F = 14.040$, $p < 0.05$). The arthropod visitors were analyzed per group within each order and compared using Tukey HSD test. Crosses and small circles the means ($p < 0.05$)

Across the study, the number of arthropods on *P. hysterophorus* flowers increased significantly as the number of *L. setosipennis* eggs on plants increased ($R^2 = 0.648$, $p < 0.05$) (Fig. 3.4).

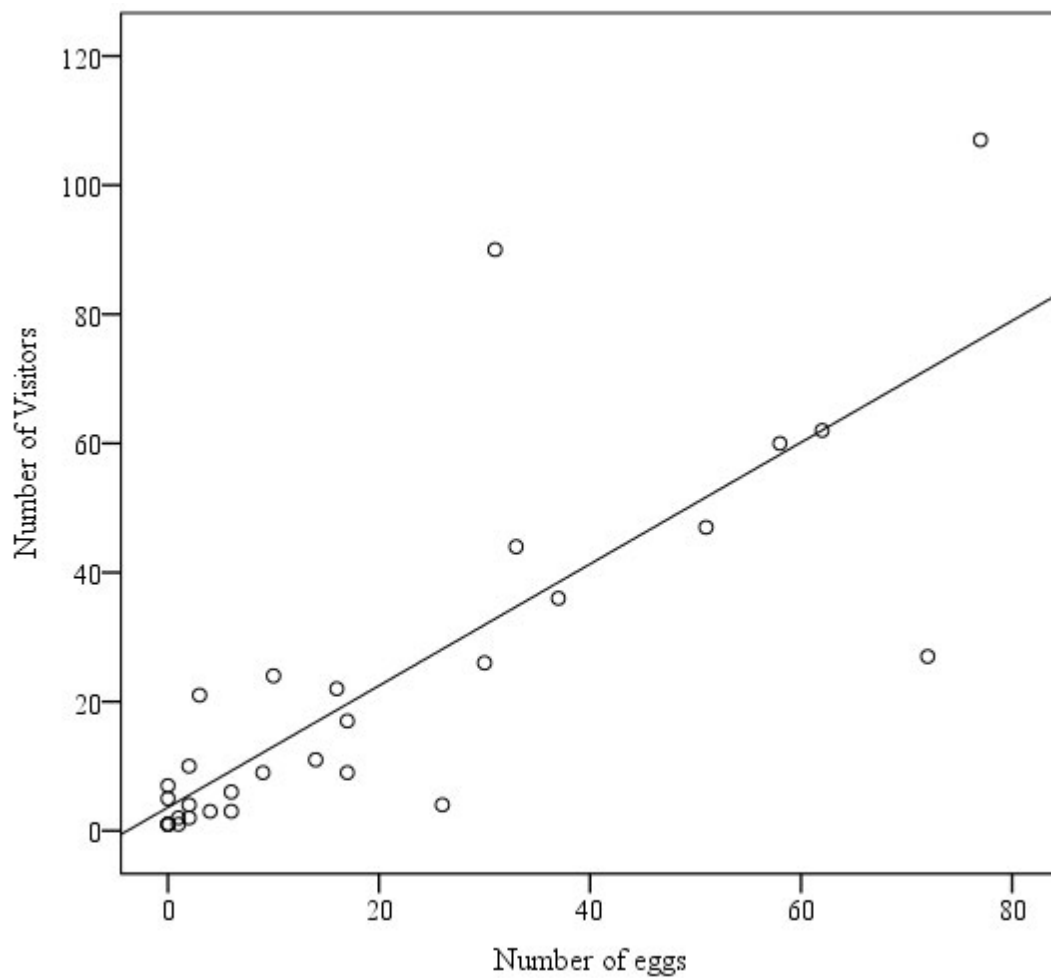


Figure 3.4: Linear regression indicating the number of arthropod visitors (per flower, per plant, per plot) in relation to the number of *Listronotus setosipennis* eggs (per flower, per plant, per plot); $R^2 = 0.648$, $p < 0.05$.

There was no significant difference (Chi-square = 41.855, df = 1, $p > 0.05$) in the visitation frequency of all arthropods across all sites to flowers with or without *L. setosipennis* eggs (Fig. 3.5).

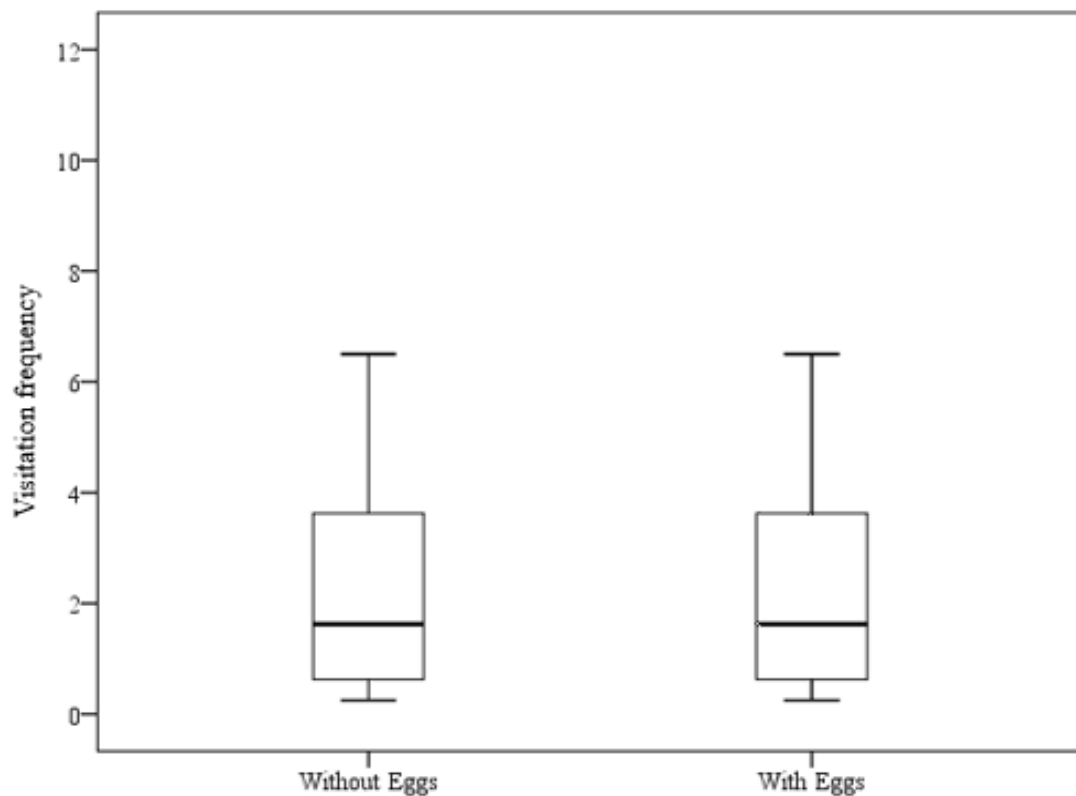


Figure 3.5: Visitation frequency of arthropods to *Parthenium hysterophorus* flowers with and without *Listronotus setosipennis* eggs (Chi-square = 41.855, df= 1, $p > 0.05$).

The presence or absence of *L. setosipennis* eggs had no significant impact on the type of activities (resting, exploratory or foraging) displayed by the arthropod visitors ($F = 0.001$, $p > 0.05$). However, a high proportion of arthropod visitors were observed in exploratory and foraging activities and resting on *P. hysterophorus*, both plants with and without *L. setosipennis* (Fig. 3.6).

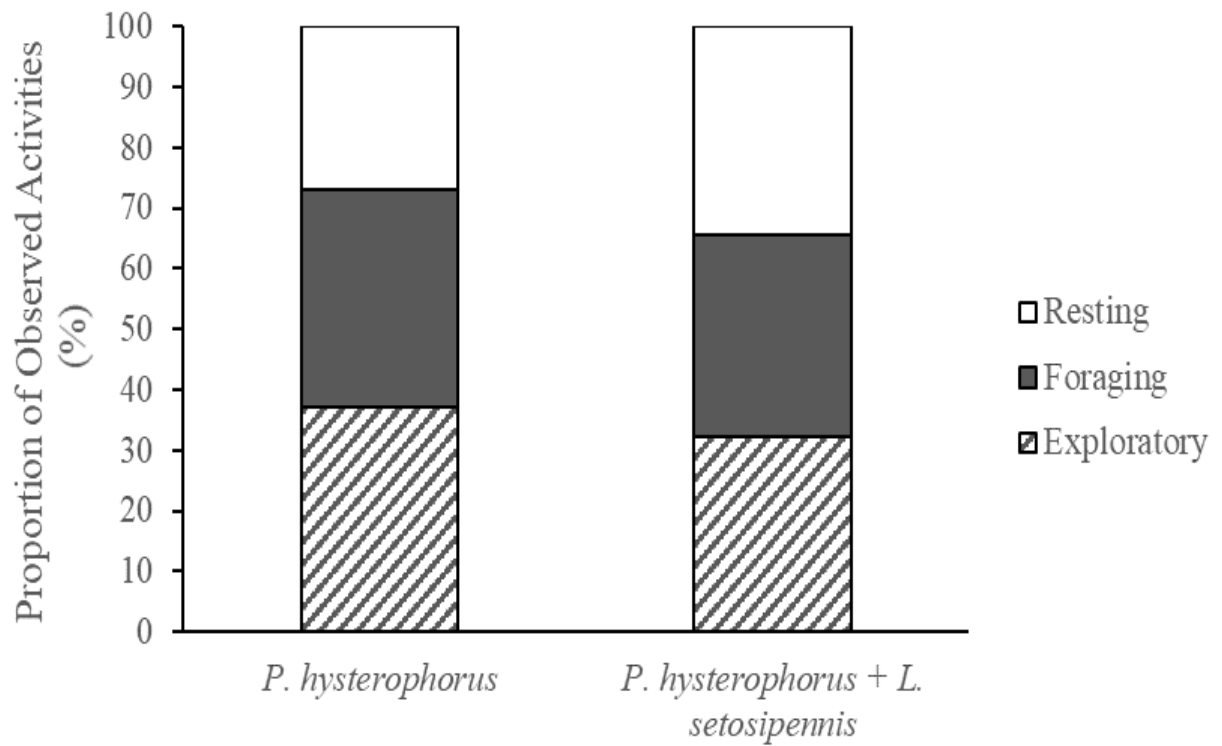


Figure 3.6: Proportion (%) of resting, foraging and exploratory activities by arthropod visitors on *Parthenium hysterophorus* plants with and without *Listronotus setosipennis* eggs.

Discussion

Parthenium hysterophorus invades various environments such as roadsides, overgrazed pastures, and cultivated lands, in many parts of South Africa as well as other countries, and it is frequently abundant where it has invaded (Cowie et al., 2022). A generalised statement of the impact of invasive alien plants on arthropods cannot be made as plant invasion has been shown to correspond with both increases (Marshall and Buckley, 2009) and decreases (Simao et al., 2010) in arthropod community abundance in different systems. In the present study, there were no significant differences in arthropod activity between *P. hysterophorus* plants with and without the biological control agent *L. setosipennis* when all arthropods were considered, as measured by the number of individuals or orders. The current study found that some groups of arthropods, such as Hemiptera and Thysanoptera (included in the category "Other"), were less common on *P. hysterophorus* both with and without *L. setosipennis*. This concurs with the study by Litt et al. (2014) which reported that Hemipterans were less abundant or absent when an invasive species dominated the vegetative community, resulting in changes in composition. Some studies (e.g. Samways et al., 1996; Lindsay and French 2006; Marshall and Buckley 2009; Litt and Steidl, 2010) reported that increased abundance of exotic plants might also have a significant impact on many species of Hemiptera (true bugs) and Lepidoptera, as well as a few Thysanoptera (thrips) and Coleoptera (beetles).

Thysanoptera may also be less abundant or absent in places dominated by exotic species, despite little research concentrating on non-agricultural environments (Cord, 2011; Litt et al., 2014). Many Hemiptera and Lepidoptera species, as well as a few Thysanoptera and Coleoptera species, are host specific during some or all life stages. Therefore, an increased abundance of foreign plants may significantly impact these species (Litt et al., 2014). Orthoptera populations have been reported to decline in areas dominated by exotic plants (Skórka et al., 2010). Different life forms of invasive plants and the degree of nutritional specialization may cause such variable reactions (Litt et al., 2014). Studies have reported a decrease in the abundance and richness of predatory arthropods such as Araneae (spiders) (Gerber et al., 2008), Odonata (dragonflies) (Samways and Sharrat, 2010), and a few Diptera (flies) (Topp et al., 2008), in response to plant invasions. In contrast, Wolkovich (2010) and Kappes et al. (2007) reported an increase in the abundance and richness of predatory arthropods in response to non-native grasses such as *Brachypodium distachyon* (L.) P. Beauv. (Poaceae), and knotweed *Reynoutria* spp. such as *Fallopia japonica* var. *japonica* (Houtt.) Ronse Decraene (Polygonaceae) invasions. Furthermore some studies have recorded no change in the abundance and richness

of predatory arthropods in response to plant invasions (Basset et al, 2011; Tang et al, 2012; Schreck et al, 2013). Although these arthropod predators do not rely on plants for food, plant invasions may indirectly impact them due to prey availability or vegetation structure (Litt et al., 2014). Beetles, epigaeic invertebrates, salt marsh arthropods, and spiders have shown a decrease in abundance, richness, or diversity in areas invaded by non-native plant species, even though none of these studies experimentally manipulated the presence of the invader, preventing the assignment of causality (Simao et al., 2010).

The current study observed much higher numbers of Hymenoptera individuals, comprised primarily of ants, than any other arthropod orders. These findings contrast those by Roets and Pryke (2012), who reported a decrease in Hymenopterans in increased plantations of 5 m tall *Eucalyptus camaldulensis* Dehnh. (Myrtaceae) trees abundance. Van der Colff et al. (2015) also reported a decrease in hymenopterans with an increased abundance of *Acacia mearnsii* De Wild. (Fabaceae) within the Garden Route National Park, with vegetation dominated by evergreen trees, with multi-layered vegetation underneath.

Non-native invasive plants tend to create monospecific stands over time, attracting many native insect pollinators, resulting in increased seed production and contributing to their successful spread and establishment (Sunny et al., 2015). Furthermore, invasive plants that produce food for ants, such as nutritious seeds or plants that increase aphid populations, may favour native ant populations. In this study, many visiting arthropods, particularly ants, were observed exploring and foraging on *P. hysterothorus*, both with and without *L. setosipennis* present. Ants were often observed visiting the ray florets situated along the outer rim of flowers, seemingly feeding on nectar for extended periods.

Although arthropod visitors increased with an increase in *L. setosipennis* eggs, the lack of a significant difference in the visitation frequency of arthropods to flowers with or without *L. setosipennis* eggs, indicates that *L. setosipennis* was not the reason for floral visits by arthropods. Of relevance to the biological control programme, no incidences of predation of *L. setosipennis* eggs, by ants or any other arthropods, were observed on any occasion during this study, despite numerous visitors to flowers containing *L. setosipennis* eggs. The effects of invasive plants on ants have been studied in some systems globally, and shown that their responses vary according to their different ecological roles as herbivores, predators, and detritivores (Lenda et al., 2013; Litt et al., 2014).

This study showed that the presence or absence of *L. setosipennis* did not significantly affect the exploratory, foraging, and resting activities of arthropods that visited *P. hysterothorus*. Many factors have been linked to the impact of insect variety and abundance on plants, which may have explained these results. It is possible that the presence of *L. setosipennis* had no significant impact on arthropod visitation as the weevil was not exceptionally abundant during the study. However, it is also possible that the presence of *L. setosipennis* does not influence arthropods visiting *P. hysterothorus*, on the whole, as this study seems to indicate. Regardless, a large proportion of the activities by arthropods that visited *P. hysterothorus* involved exploration and feeding. It therefore seems that *P. hysterothorus* is acting as a resource for native arthropods.

Invasive plants may provide a better feed, for example, increased nectar and pollen production, for native insects and arthropods, causing them to be drawn to them (Bezemer et al., 2014). Native insects may prefer invasive plants for feeding and oviposition, but they may struggle to survive and develop their larvae on the invasive plants (Sunny et al., 2015, Tallamy et al., 2021). Because the natural enemies of native insects may not link with invasive plants, host shift by native insects provides them with an added advantage to thrive on invading plants (Harvey and Fortuna, 2012). However, although invasive plants may initially provide a suitable niche, native insect fitness may deteriorate with time, resulting in a decline in native insect populations (Keeler and Chew, 2008). Sunny et al. (2015) reported that insect visits to non-native plants were remarkably similar to visits to native plants prior to non-native plant colonization and naturalization. However, as non-native plants become more naturalized, the frequency and diversity of native insect visits to non-native plants increases. For example, the inflorescences of *Acacia saligna* consist of multiple flowers that attract native insects due to easy access to nectar. Increased native insect visitation in *A. saligna* in invaded plots of the Cape Floristic Region has made the plant more invasive due to increased pollination (Gibson et al., 2013). Non-native plant introductions can introduce other non-native organisms, such as their biological control agents, changing the composition of native plant communities and, as a result, the insects that rely on them.

The present study could only investigate the effects of *P. hysterothorus* and *L. setosipennis* found in these study locations. Thus, generalizations about the effects of *P. hysterothorus* and *L. setosipennis* in other environments are not possible. It is possible that the methods of this study overlooked spatially or temporally uncommon interactions due to infrequent sampling. Nonetheless, any bias would have had an equivalent impact on all study sites, so it was not

likely to have influenced these findings. Seasonal changes that could affect the presence and activity of arthropods were not fully taken into account in this study. However, the sites were sampled at monthly intervals during the summer season when there is active growth of *P. hysterothorus* and the breeding activity of *L. setosipennis*.

An invader can potentially degrade an ecosystem by homogenizing the vegetation due to its dominance. *Parthenium hysterophorus* simplified the structure of study sites, replacing it with homogenized flora. Plant homogeneity and its impact on arthropods, in particular, are expected to have far-reaching detrimental consequences for ecosystem stability (Litt et al., 2014). Environmental factors, for example, soil properties, temperature, and salinity, influencing arthropod activity in *P. hysterothorus*-infested areas, need to be considered in future research. Furthermore, the influence of climate change should also be addressed. A change in climatic patterns could significantly impact the dispersal of alien plant species. As climates change, some exotic species that are currently non-invasive may become more invasive, leading to additional impacts on native flora and fauna. Additionally, climate change, through changed conditions, may also alter the current diversity and composition of native arthropods associated with invasive plants.

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Chapter 4: General Summary and Recommendations

4.1 Introduction

Invasive alien plants constitute a significant threat to native species and disrupt natural ecosystems worldwide. While alien plant invasions have been related to significant ecological changes in various environments, quantifying their impact on native arthropods has received limited attention. Arthropods are the most abundant group in terrestrial ecosystems, with insects accounting for the majority of all species (Dossey et al., 2010). Invasive plant species can cause irregular effects on arthropod structure, diversity, and abundance. Given that exotic plant invasions can significantly alter vegetation structure by displacing native herbs and plant seedlings it is not surprising that these invasions could also impact the diversity and abundance of arthropod populations (Tang et al., 2012; Spafford et al., 2013).

4.2 Revisiting the study aims and objectives

The current study aimed to investigate the impacts of *P. hysterophorus* and one of its biological control agents *L. setosipennis*, on ant diversity, assemblage composition, and arthropod activities. The results indicated that the occurrence of *P. hysterophorus* and *L. setosipennis* did not significantly impact on ant diversity and assemblage composition. The results of this study did not conform to the trends recorded by some studies showing increasing (Gerber et al., 2008) and decreasing (Simao et al., 2010; Cross et al., 2016) diversity and assemblage composition of arthropods in response to invasive alien plants. This study also explored vegetative drivers of ant diversity and assemblage composition. The results showed that *P. hysterophorus* and *L. setosipennis* population variables did not significantly influence ant assemblages. Furthermore, the observed variance in ant diversity was not explained by geographical variation in vegetation cover, or *P. hysterophorus* height and flowering.

Additionally, this study examined arthropod activity in relation to *P. hysterophorus* plants with and without the biological control agent *L. setosipennis*, to address whether the weed and its biological control agent influenced arthropods and their activities. Results revealed the array of arthropod groups that visited *P. hysterophorus*, with and without *L. setosipennis*. The presence of *L. setosipennis* was shown not to influence the activity of arthropods. However, this study demonstrated that some arthropods, particularly Hymenopterans and specifically ants, were more commonly associated with *P. hysterophorus* than other arthropod groups. This contrasts with research conducted by Litt et al. (2014) and Garcia and Clusella-Trullas (2017),

which demonstrated that when the abundance of invasive plants increased, the abundance of Hymenoptera (ants, wasps, and bees) decreased.

4.3 Contributions of the study

Few studies have examined the impacts of *P. hysterophorus* or the introduced natural enemies used to manage the weed, on native communities in the invaded range. Examining the efficacy of management or restoration treatments can reveal essential information on whether management techniques can reduce the effects of invasive plants on arthropods. The findings of this study have added to growing evidence demonstrating the impact of invasive plants on ant assemblages, which are often used as important indicators of local species richness.

4.4 Limitations and future recommendations

Since this study was limited to investigating the effects of *P. hysterophorus* in the study sites only, it is not feasible to draw broad conclusions about the impact of this invasive alien plant in other environments. With the restricted observation times, it is possible that methods used in the current study missed spatially or temporally rare interactions. The current study did not investigate seasonal changes that may have affected the presence and activity of arthropods, although seasonal fluctuations have been shown by some studies (Pearson and Derr, 1986; Wagner, 2001) to influence arthropods to some extent. Future research should investigate whether environmental effects play a role in modifying arthropod diversity and composition. Many weeds, including *P. hysterophorus* contain alkaloids that have been shown to be toxic to bees (Witt and Belgeri, 2018). So, given the escalating invasions despite biological interventions, research to investigate the allelopathic effects of *P. hysterophorus* on arthropod communities, including ants, is recommended.

4.5 Summary conclusions

This study has highlighted that ant diversity, as indicated by species richness and abundance, and assemblage composition were not significantly influenced by the presence of *P. hysterophorus* and *L. setosipennis*. It also highlighted that many arthropods, particularly Hymenoptera and specifically ants, use *P. hysterophorus* for resting, exploratory and foraging activities. However, their activities were not influenced by the presence of the biological control agent *L. setosipennis* in *P. hysterophorus* invaded sites, and the biocontrol agent did not appear to be at significant risk of egg predation under the study conditions. Ultimately, this information may be reassuring because even highly invaded sites may host diverse arthropod

communities and non-native species do not essentially have impoverished impacts on arthropod assemblages.

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Appendices

Appendix 1: Locality details for sites in *Parthenium hysterophorus* invaded areas in KwaZulu-Natal and Mpumalanga provinces, South Africa at which ant diversity and assemblage composition studies were undertaken during October 2019 to October 2020.

Site	Treatment	Coordinates	Description
KwaZulu-Natal province			
KwaMsane	<i>P. hysterophorus</i> + <i>L. setosipennis</i>	28°24'56"S 32°09'50.2"E	Along roadsides
	<i>P. hysterophorus</i>	28°24'45.0"S 32°09'55"E	Along a gravel road in a village
	No <i>P. hysterophorus</i>	28°24'53.5"S 32°09'49.2"E	Along a gravel road in a village
	<i>P. hysterophorus</i> + <i>L. setosipennis</i>	27°38'35.2"S 31°22'18.4"E	Near riparian habitats
KwaJobe	<i>P. hysterophorus</i>	27°38'37.4"S 32°22'36.4"E	Cultivated and abandoned cropland
	No <i>P. hysterophorus</i>	27°38'39.2"S 32°22'23.6"E	Near riparian habitats
Magudu Reserve	<i>P. hysterophorus</i> + <i>L. setosipennis</i>	27°28'51"S 31°41'26.5"E	Within a game reserve, near a dam
	<i>P. hysterophorus</i>	27°28'52.8"S 31°41'25.7"E	Within a game reserve
	No <i>P. hysterophorus</i>	27°28'53.1"S 31°41'27.9"E	Within a game reserve
	<i>P. hysterophorus</i> + <i>L. setosipennis</i>	27°29'32.6"S 31°44'19.2"E	Within a game reserve, on dam embankment
Ntibane Ranch	<i>P. hysterophorus</i>	27°29'30"S 31°44'24.3"E	Within a game reserve, on dam embankment

	No <i>P. hystrophorus</i>	27°29'29.8"S 31°44'25.7"E	Within a game reserve, near dam
Mpumalanga province			
Mananga	<i>P. hystrophorus</i> + <i>L. setosipennis</i>	25°54'48.6"S 31°46'49.5"E	Along roadside, near border to Eswatini
	<i>P. hystrophorus</i>	25°55'04.6"S 31°46'30.6"E	Along roadside, near border to Eswatini
	No <i>P. hystrophorus</i>	25°54'48.6"S 31°46'49.5"E	Along roadside
Mangweni	<i>P. hystrophorus</i> + <i>L. setosipennis</i>	25°54'48.6"S 31°46'49.5"E	Along a gravel road in a village, adjacent to open veld
	<i>P. hystrophorus</i>	25°43'07.2"S 31°50'41.3"E	Along a gravel road in a village, adjacent to open veld
	No <i>P. hystrophorus</i>	25°43'21.3"S 31°49'53.5"E	Along a gravel road in a village, in open veld
Malelane Municipal Area	<i>P. hystrophorus</i> + <i>L. setosipennis</i>	25°29'23.1"S 31°31'45.7"E	Abandoned cropland (sugarcane)
	<i>P. hystrophorus</i>	25°29'20.4"S 31°31'43.6"E	Abandoned cropland (sugarcane)
	No <i>P. hystrophorus</i>	25°29'27.2"S 31°31'36.5"E	Veld adjacent to abandoned cropland
Malelane Power Station	<i>P. hystrophorus</i> + <i>L. setosipennis</i>	25°30'11.1"S 31°31'42"E	Adjacent to cultivated cropland (sugarcane)
	<i>P. hystrophorus</i>	25°30'11.1"S 31°31'35.7"E	Fallow cropland (sugarcane)
	No <i>P. hystrophorus</i>	25°30'04.1"S 31°31'35.2"E	Veld near cultivated and fallow cropland (sugarcane)

Kaalrug	<i>P. hysterothorus</i> + <i>L.</i>	25°37'20.6"S	Woodland	near
	<i>setosipennis</i>	31°29'23.2"E	roadside	
	<i>P. hysterothorus</i>	25°37'006"S	Fallow	subsistence
		31°29'03.4"E	crop field	
	No <i>P. hysterothorus</i>	25°37'23.9"S	Along a gravel road	
		31°29'23.5"E		

Appendix 2: Monthly mean minimum and maximum temperatures recorded in canopy about 1m height during the study period (December 2019 to March 2020 and October 2020), using iButtons® (Maxim Integrated San Jose, CA, USA).

Site	December 2019		January 2020		February 2020		March 2020		October 2020	
	Min (°C)	Max (°C)	Min (°C)	Max (°C)	Min (°C)	Max (°C)	Min (°C)	Max (°C)	Min (°C)	Max (°C)
KwaZulu-Natal										
Magudu Game Reserve	15.2	23.6	15.2	25.7	14.6	25.7	13.1	25.1	12.8	24.5
Ntibane Game Ranch	18.7	28.3	19.6	28.5	19.6	28.7	18.7	28.1	15.3	26.1
KwaJobe	22.2	25.6	23.5	26.9	23.7	27.2	23.3	26.9	19.9	23.4
KwaMsane	20.9	28	21.7	28.5	21.9	28.7	21.3	28.1	17.9	25.3
Mpumalanga										
Malelane	19.3	29.1	20	28.9	20.1	29.2	19.1	28.6	16.1	27.9
Mangweni	17	29	18	29	17	28	16	27	16	28
Mananga	21.5	30.5	27.1	30.2	21.6	30.7	21	30.3	19.4	28.3
Kaalrug	16.2	25	17.3	25.7	17.3	26.2	16.6	25.7	13.7	23.7

Appendix 3a : Ant specimens collected in pitfall traps at four study sites in KwaZulu-Natal province, grouped by treatments in habitats uninvasion and invasion by *Parthenium hysterophorus*, with and without *Listronotus setosipennis*, collected during monthly sampling during October 2019 to March 2020 and October 2020.

Species	No	<i>P. hysterophorus</i>	<i>P. hysterophorus</i>	<i>P. hysterophorus</i> + <i>L. setosipennis</i>
<i>Anoplolepis custodiens</i>	88		68	658
<i>Bothroponera kruegeri</i>	4		3	3
<i>Bothroponera soror</i>	0		0	1
<i>Camponotus</i> UKZN_02 (<i>cinctellus</i> gp.)	27		26	31
<i>Camponotus</i> UKZN_05 (<i>maculatus</i> gp.)	1		11	1
<i>Camponotus</i> UKZN_06 (<i>niveosetosus</i> gp.)	0		0	2
<i>Camponotus</i> UKZN_07 (<i>cinctellus</i> gp.)	1		4	3
<i>Cardiocondyla</i> UKZN_02 (<i>elegans</i> gp.)	0		14	4
<i>Cardiocondyla</i> UKZN_05	0		1	0
<i>Carebara vidua</i>	0		0	6
<i>Crematogaster</i> nr. <i>rufigena</i>	0		13	0
<i>Crematogaster</i> UKZN_01 (<i>catsanea</i> complex gp.)	0		1	0
<i>Dorylus helvolus</i>	3		0	39
<i>Lepisiota capensis</i>	1		1	2
<i>Lepisiota crinite</i>	1		0	0
<i>Lepisiota incisa</i>	5		1	2
<i>Lepisiota</i> UKZN_04 (<i>spinosior</i> gp.)	1		2	3
<i>Leptogenys intermedia</i>	0		1	0
<i>Leptogenys schwabi</i>	2		11	1
<i>Megaponera analis</i>	3		0	1
<i>Meranoplus magretti</i>	2		0	0

<i>Mesoponera caffraria</i>	0	2	0
<i>Messor UKZN_01</i>	3	6	0
<i>Monomorium damarensense</i>	28	26	0
<i>Monomorium junodi</i>	366	124	183
<i>Monomorium UKZN_02</i> (<i>salamonis</i> gp.)	29	27	105
<i>Monomorium UKZN_06</i> (<i>emeryi</i> gp.)	166	2	4
<i>Monomorium UKZN_07</i> (<i>notulum</i> gp.)	13	0	0
<i>monomorium UKZN_08</i> (<i>drapenum</i> gp.)	45	20	19
<i>Nylanderia boltoni</i>	0	1	0
<i>Ocymyrmex flaviventris</i>	7	0	0
<i>Ocymyrmex fortiori</i>	139	42	30
<i>Ophthalmopone UKZN_01</i>	6	1	8
<i>Parasyscia UKZN_03</i>	0	0	1
<i>Pheidole UKZN_01</i> (<i>megacaphala</i> gp.)	137	72	340
<i>Pheidole UKZN_02</i> (<i>megacephala</i> gp.)	857	1275	2474
<i>Pheidole UKZN_03</i>	135	21	19
<i>Pheidole UKZN_04</i> (<i>crassinoda</i> gp.)	9	33	12
<i>Plagiolepis UKZN_01</i>	0	1	0
<i>Plectroctena mandibularis</i>	0	1	1
<i>Polyrhachis UKZN_02</i> (<i>schistacea</i> gp.)	0	2	1
<i>Tapinolepis UKZN_01</i>	82	22	8
<i>Tapinolepis UKZN_02</i>	2	0	1
<i>Tapinolepsis UKZN_05</i>	2	0	0
<i>Tetramorium setuliferum</i>	14	9	1
<i>Tetramorium UKZN_04</i> (<i>setigerum</i> gp.)	24	40	91

<i>Tetramorium UKZN_10</i> (<i>similimum</i> gp.)	6	21	3
<i>Tetramorium UKZN_11</i> (<i>similimum</i> gp.)	2	5	7
<i>Tetramorium UKZN_17</i> (<i>sericeiventris</i> gp.)	87	15	105
<i>Tetramorium UKZN_21</i> (<i>squaminode</i> gp.)	1	3	14
<i>Tetramorium UKZN_24</i> (<i>gabonense</i> gp.)	0	0	7
<i>Tetramorium UKZN_31</i> (<i>weitzackeri</i> gp.)	8	16	196
<i>Tetramorium UKZN_40</i> (<i>squaminode</i> gp.)	0	0	1
<i>Tetramorium UKZN_41</i> (<i>baufra</i> gp.)	1	0	0
<i>Tetramorium UKZN_42</i>	0	1	0
Grand Total	2308	1945	4388

Appendix 3b: Ant specimens collected in pitfall traps at five study sites in Mpumalanga province, grouped by treatments in habitats uninhabited and invaded by *Parthenium hysterophorus*, with and without *Listronotus setosipennis*, collected during monthly sampling during October 2019 to March 2020 and October 2020.

Species	No	<i>P. hysterophorus</i>	<i>P. hysterophorus</i> + <i>P. hysterophorus</i> <i>L. setosipennis</i>
<i>Anoplopepis custodiens</i>	1387	8	117
<i>Bothroponera kruegeri</i>	1	7	12
<i>Bothroponera soror</i>	2	0	0
<i>Brachyponera UKZN_01</i>	35	17	30
<i>Camponotus UKZN_02 (cinctellus</i> gp.)	55	26	57
<i>Camponotus UKZN_05 (maculatus</i> gp.)	3	10	15
<i>Cardiocondyla UKZN_01</i>	12	12	20
<i>Cardiocondyla UKZN_02</i>	0	8	3
<i>Crematogaster nr. rufigena</i>	0	0	2
<i>Hypoconerops UKZN_01</i>	1	0	0
<i>Lepisiota capensis</i>	15	0	5
<i>Lepisiota crinata</i>	0	0	1
<i>Lepisiota incisa</i>	1	1	3
<i>Leptogenys schwabi</i>	0	1	0
<i>Mesoponera caffraria</i>	10	52	39
<i>Mesoponera UKZN_01</i>	1	1	0
<i>Monomorium junodi</i>	234	307	200
<i>Monomorium UKZN_02 (salamonis</i> gp.)	8	6	0
<i>Monomorium UKZN_04 (salamonis</i> gp.)	77	29	47
<i>Monomorium UKZN_08 (drapenum</i> gp.)	14	1	12
<i>Myrmecaria natalensis</i>	104	12	198
<i>Ocymyrmex fortiori</i>	163	32	42

<i>Odontomachus troglodytes</i>	1	0	11
<i>Ophthalmopone UKZN_01</i>	20	16	27
<i>Pheidole UKZN_01 (megacephala gp.)</i>	0	23	5
<i>Pheidole UKZN_02 (megacephala gp.)</i>	215	1657	1446
<i>Pheidole UKZN_03</i>	106	91	40
<i>Pheidole UKZN_04 (crassinoda gp.)</i>	76	118	144
<i>Plectroctena mandibularis</i>	0	2	0
<i>Polyrhachis schistacea</i>	1	2	4
<i>Tapinolepsis UKZN_01</i>	0	3	1
<i>Tapinolepsis UKZN_02</i>	14	3	4
<i>Tetramorium UKZN_04 (setigerum gp.)</i>	7	29	20
<i>Tetramorium UKZN_08 (setigerum gp.)</i>	0	1	0
<i>Tetramorium UKZN_10 (similimum gp.)</i>	0	0	2
<i>Tetramorium UKZN_11 (similimum gp.)</i>	0	0	1
<i>Tetramorium UKZN_15 (sericeiventris gp.)</i>	167	50	31
<i>Tetramorium UKZN_21 (squaminode gp.)</i>	0	0	1
<i>Tetramorium UKZN_31 (weitzackeri gp.)</i>	14	7	4
<i>Meranoplus glaber</i>	1	0	0
<i>Technomyrmex UKZN_01</i>	0	0	1
Grand Total	2745	2532	2545

Appendix 4a: Species names used in the CCA triplot for ant specimens collected in pitfall traps at four study sites in KwaZulu-Natal province, in habitats uninvaded and invaded by *Parthenium hysterophorus*, with and without *Listronotus setosipennis*

Abbreviation	<i>Species name</i>
Anocus	<i>Anoplolepis custodiens</i>
Botkrue	<i>Bothroponera kruegeri</i>
Botsor	<i>Bothroponera soror</i>
Campcin	<i>Camponotus UKZN_02 (cinctellus gp.)</i>
Campmac	<i>Camponotus UKZN_05 (maculatus gp.)</i>
Campniv	<i>Camponotus UKZN_06 (niveosetosus gp.)</i>
Campcin7	<i>Camponotus UKZN_07 (cinctellus gp.)</i>
Cardioele	<i>Cardiocondyla UKZN_02 (elegans gp.)</i>
Cardio5	<i>Cardiocondyla UKZN_05</i>
Carevid	<i>Carebara vidua</i>
Crem.ruf	<i>Crematogaster nr. rufigena</i>
Cremcat	<i>Crematogaster UKZN_01 (catsanea complex gp.)</i>
Doryhel	<i>Dorylus helvolus</i>
Lepicap	<i>Lepisiota capensis</i>
Lepicri	<i>Lepisiota crinita</i>
Lepiinc	<i>Lepisiota incisa</i>
Lepispi	<i>Lepisiota UKZN_04 (spinosior gp.)</i>
Leptoint	<i>Leptogenys intermedia</i>
Leptosch	<i>Leptogenys schwabi</i>
Megaana	<i>Megaponera analis</i>
Meramag	<i>Meranoplus magrettii</i>
Mesocaf	<i>Mesoponera caffraria</i>
Messor	<i>Messor UKZN_01</i>
Monodam	<i>Monomorium damarense</i>
Monojun	<i>Monomorium junodi</i>
Monosal	<i>Monomorium UKZN_02 (salamonis gp.)</i>
Monoeme	<i>Monomorium UKZN_06 (emeryi gp.)</i>
Mononot	<i>Monomorium UKZN_07 (notulum gp.)</i>

monodrap	<i>monomorium UKZN_08 (drapenum gp.)</i>
Nylabol	<i>Nylanderia boltoni</i>
Ocyflav	<i>Ocymyrmex flaviventris</i>
Ocyfor	<i>Ocymyrmex fortior</i>
Ophtha	<i>Ophthalmopone UKZN_01</i>
Para	<i>Parasyscia UKZN_03</i>
Pheimeg1	<i>Pheidole UKZN_01 (megacaphala gp.)</i>
Pheimeg3	<i>Pheidole UKZN_02 (megacephala gp.)</i>
Phei3	<i>Pheidole UKZN_03</i>
Pheicras	<i>Pheidole UKZN_04 (crassinoda gp.)</i>
Plagio	<i>Plagiolepis UKZN_01</i>
Plectmand	<i>Plectroctena mandibularis</i>
Polysch	<i>Polyrhachis UKZN_02 (schistacea gp.)</i>
Tapi1	<i>Tapinolepis UKZN_01</i>
Tapi2	<i>Tapinolepis UKZN_02</i>
Tapi5	<i>Tapinolepsis UKZN_05</i>
Tetraser	<i>Tetramorium setuliferum</i>
Tetrasetig	<i>Tetramorium UKZN_04 (setigerum gp.)</i>
Tetrasim10	<i>Tetramorium UKZN_10 (similimum gp.)</i>
Tetrasim11	<i>Tetramorium UKZN_11 (similimum gp.)</i>
Tetraser	<i>Tetramorium UKZN_17 (sericeiventre gp.)</i>
Tetrasqua	<i>Tetramorium UKZN_21 (squaminode gp.)</i>
Tetragab	<i>Tetramorium UKZN_24 (gabonense gp.)</i>
Tetrawei	<i>Tetramorium UKZN_31 (weitzeckeri gp.)</i>
Tetrasqua40	<i>Tetramorium UKZN_40 (squaminode gp.)</i>
Tetrabau	<i>Tetramorium UKZN_41 (baufra gp.)</i>
Tetra42	<i>Tetramorium UKZN_42</i>

Appendix 4b: Species names used in the CCA triplot for ant specimens collected in pitfall traps at five study sites in Mpumalanga province, in habitats uninhabited and invaded by *Parthenium hysterophorus*, with and without *Listronotus setosipennis*

Abbreviation	<i>Species name</i>
Anocus	<i>Anoplopepis custodiens</i>
Botkrue	<i>Bothroponera kruegeri</i>
Botsor	<i>Bothroponera soror</i>
Brachy01	<i>Brachyponera</i> UKZN_01
Campcin	<i>Camponotus</i> UKZN_02 (<i>cinctellus</i> gp.)
Campmac	<i>Camponotus</i> UKZN_05 (<i>maculatus</i> gp.)
Cardio01	<i>Cardiocondyla</i> UKZN_01
Cardio02	<i>Cardiocondyla</i> UKZN_02
Cremruf	<i>Crematogaster</i> nr. <i>rufigena</i>
Hypo01	<i>Hypoconera</i> UKZN_01
Lepcap	<i>Lepisiota capensis</i>
Lepcri	<i>Lepisiota crinata</i>
Lepinc	<i>Lepisiota incisa</i>
Leptsch	<i>Leptogenys schwabi</i>
Mescap	<i>Mesoponera caffraria</i>
Meso01	<i>Mesoponera</i> UKZN_01
Monjun	<i>Monomorium junodi</i>
Monsal	<i>Monomorium</i> UKZN_02 (<i>salamonis</i> gp.)
Mon04	<i>Monomorium</i> UKZN_04 (<i>salamonis</i> gp.)
Mondrap	<i>Monomorium</i> UKZN_08 (<i>drapenum</i> gp.)
Myrnat	<i>Myrmecaria natalensis</i>
Ocyfor	<i>Ocymyrmex fortior</i>
Odontro	<i>Odontomachus troglodytes</i>
Ophth01	<i>Ophthalmopone</i> UKZN_01

Phemeg1	<i>Pheidole</i> UKZN_01 (<i>megacephala</i> gp.)
Phemeg2	<i>Pheidole</i> UKZN_02 (<i>megacephala</i> gp.)
Phei03	<i>Pheidole</i> UKZN_03
Pheicra	<i>Pheidole</i> UKZN_04 (<i>crassinoda</i> gp.)
Plecman	<i>Plectroctena mandibularis</i>
Polysch	<i>Polyrhachis schistacea</i>
Tapi01	<i>Tapinolepsis</i> UKZN_01
Tapi02	<i>Tapinolepsis</i> UKZN_02
Tetrset	<i>Tetramorium</i> UKZN_04 (<i>setigerum</i> gp.)
Tetret8	<i>Tetramorium</i> UKZN_08 (<i>setigerum</i> gp.)
Tetsim10	<i>Tetramorium</i> UKZN_10 (<i>similimum</i> gp.)
Tetsim11	<i>Tetramorium</i> UKZN_11 (<i>similimum</i> gp.)
Tetser	<i>Tetramorium</i> UKZN_15 (<i>sericeiventris</i> gp.)
Tetsqua	<i>Tetramorium</i> UKZN_21 (<i>squaminode</i> gp.)
Tetwei	<i>Tetramorium</i> UKZN_31 (<i>weitzackeri</i> gp.)
Mergla	<i>Meranoplus glaber</i>
Techno	<i>Technomyrmex</i> UKZN_01

Appendix 5: Site details for the study of arthropod visitations to *Parthenium hysterophorus* invaded habitats with and without *Listronotus setosipennis*, in KwaZulu-Natal and Mpumalanga provinces

Province	Site		Coordinates	Description
KwaZulu-Natal	KwaJobe		27°33'0" S 32°17'0" E	Near cultivated and fallow cropland next to a wetland
	Makhatini Factory	Cotton	27° 25' 3.1" S 32° 9' 28.4" E	Along roadside and in fallow cotton field/veld adjacent to cotton factory
	Magudu Reserve	Game	27°23'47.1" S 31°39'25.3" E	Within a game reserve, on the banks of a dam
Mpumalanga	Umbhaba Farm	Banana	25°25'34.8" S 31°48'11.1" E	Veld adjacent to cultivated cropland
	Mangweni		25° 44' 0" S 31° 49' 0" E	Along a gravel road in a village
	Mananga		25° 55' 59.16" S 31° 45' 40.68" E	Along roadside (near the Eswatini border)