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2019

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The effect of habitat management on the impact of *Liothrips tractabilis* Mound and Pereyra (Thysanoptera: Phlaeothripidae), on Pompom weed in South Africa

Phuluso Mudau

2019



School of Animal, Plant and Environmental Sciences

A Dissertation submitted to the Faculty of Science, University of the Witwatersrand, in partial fulfilment of the requirements for the degree of Master of Science, Johannesburg, South Africa.

May 2019

Declaration

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



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May 2019

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Dedication

This Dissertation is dedicated to my dad (Takalani Mudau),

mom (Gladys Mudau), and my one and only brother Tumelo Mudau.

Thank you for your support.

Acknowledgements

I would like to thank my supervisors Prof. Marcus J. Byrne, Ms. Liame van der Westhuizen and Prof. Ed T.F. Witkowski for their supervision, guidance and constructive comments towards completion of this project. I am also grateful to Phillimon Mpedi for his advice and guidance on propagation and culturing of pompom weed and the thrips biocontrol agent.

I would also like to thank the Waterkloof Airforce Base staff members, in particular Major Mariska Vogel, Thato Chauke and Coert Theron for always assisting me in gaining access to the base. Macphee Madzivhe, Sanele Mtetwa and Joe Venturi thank you for assisting me with field work and driving me to the base and Bonginkosi Hlalukane for always being there for me when I needed field equipment.

To the Biocontrol lab; Sipho Mbonani, Lerato Molekoa, Macphee Madzivhe, Lyriche Drude, Sanele Mtetwa, Kuda Musengi, Prisca Thobejane, Bongkuhle Mabuya, Lusungu Nkoma, Abubakar Bello, Zanele Machimane, Tumi Mathige, Guelor Mayonde, Amy Burness, Peter Kgampe, Tshuxekani Maluleke, Jeanne Mukarugwiro, Naweji Katembo, Archie Sassa, Danica Marlin, Solomon Newete, Joe Venturi and Blair Cowie thank you for your support and company.

I would like to thank the NRF for funding my project. I am also grateful to FEENIX crowd funding for clearing my historical debt that threatened my stay at the University of the Witwatersrand. Would also like to thank South African Weather Service (SAWS) for the temperature data.

To my mom and dad, thank you for the support and for always encouraging me to work hard.

Abstract

Campuloclinium macrocephalum (Less.) DC. (Asteraceae), is an invasive perennial herb, mainly in the grassland biome of South Africa, commonly known as pompom weed. It is native to South and Central America. Since the 1980s it has invaded wetlands, roadsides and grasslands and poses a significant environmental threat, such as displacement of indigenous vegetation, reduction in grazing capacity of farms and game reserves because of its unpalatability to wildlife and livestock. Given the shortcomings of chemical and mechanical control methods, biological control is considered the most sustainable and environmentally friendly method of controlling some invasive plants such as pompom weed.

Liothrips tractabilis Mound and Pereyra (Thysanoptera: Phlaeothripinae) is a stem and leaf deforming biological control agent released against *C. macrocephalum* in 2013. During laboratory impact studies *L. tractabilis* was found to significantly reduce the growth of *C. macrocephalum*. Young and regrowth plants that were inoculated with thrips demonstrated a significant reduction in leaf production, biomass and height compared to the control plants, with a reduced floral production.

Light intensity is one of the factors that has been found to affect plant growth and population structure and consequently that of other living organisms, including insects. Exposure to light alters leaf quality, such as phenolic and nitrogen contents, water content and structural defences. Plants that are exposed to high light are often less palatable, making herbivores more likely to colonise plants in the shade. Many studies have observed increased rates of herbivory on plants in shaded environments, while conversely other studies found increased herbivory on plants in full sun. The first aim of this study was to compare the impact of *L. tractabilis* on the growth of *C. macrocephalum* in sunny (areas with short, mown grass) versus shaded environments (areas with long unmown grass).

In this study, the number of adults, nymphs and eggs of *L. tractabilis* were found to be significantly higher in the sunny environment, which resulted in 78% of *C. macrocephalum* plants with deformed growth (plants with altered apical shoot tips), as well as a 64% reduction in plant height and a 74% reduction in the proportion of plants with flowers. Therefore, an early season mowing of *C. macrocephalum* in invaded veld, has the potential to enhance the performance of the biocontrol agent, *L. tractabilis* in reducing the vigour and reproduction of pompom weed.

The second aim of this study was to investigate the life stages of *L. tractabilis* that overwinter and assess their impact on the fleshy rootstocks of *C. macrocephalum*. In the southern hemisphere, perennial plants such as *C. macrocephalum* respond to seasonal reductions in rainfall with dieback to below-ground plant tissue during the dry and cold winter from May onwards followed by regrowth from the fleshy rootstocks in spring (October) at the commencement of the new growing (rainy) season. *Liothrips tractabilis* was found to display a similar pattern by moving to the underground plant parts of *C. macrocephalum* in winter, only to reappear in spring on the above-ground shoot regrowth. Adults of *L. tractabilis* were found to be the main life stage that overwinter on the plant roots, with only 4% of *L. tractabilis* individuals being nymphs. *Liothrips tractabilis* had no significant effect on the number, mass, thickness or length of the weed's roots during the dry season. Therefore, *L. tractabilis* is only effective in controlling *C. macrocephalum* above-ground growth during the growing season. The effect of habitat management such as mowing enhanced the impact of *L. tractabilis* on *C. macrocephalum.* Overwintering *L. tractabilis* adults were most frequently found at the 4-6 cm depth from the soil surface (52%), which suggests that they could survive the typical Highveld winter fires. Thus, integrated control of *C. macrocephalum* using both fire and the biocontrol agent *Liothrips tractabilis* is feasible and should be trialed.

Keywords: Alien invasive plants, impact, insect development, light intensity, mowing, overwintering, plant damage

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Glossary of abbreviations

ANOVA -Analysis of Variance

DEA -Department of Environmental Affairs

DPM –Disk Pasture Meter

IAS -Invasive Alien Species

NRMP -National Research Management Programmes

SAWS -South African Weather Service

CHAPTER 1:

General introduction

1.1 Invasive plants

Invasive Alien Species (IAS) are species introduced to a non-native ecosystem (Zimmermann *et al.* 2004). An alien plant becomes invasive when it establishes in a new non-native region in the absence of natural enemies to suppress their populations and spread without human assistance (Zimmermann *et al.*, 2004). Once established, many invasive plants can have a negative impact on the environment. They disrupt ecosystem processes (Witkowski, 1991a; Holmes *et al.*, 2005), displace indigenous vegetation (Witkowski, 1991b; Culliney, 2005; Zimmermann *et al.*, 2004; Goodall *et al*. 2012; Holmes *et al.*, 2008), alter fire regimes and impact on hydrological cycles (Culliney, 2005; Witkowski and Wilson, 2001; Mugwedi *et al.*, 2015). Invasive alien species are considered to be the second biggest threat to biodiversity after habitat loss (Kumar, 2010; Simberloff *et al.*, 2013). Human disturbances such as habitat transformation, habitat fragmentation and commercial practices have escalated the invasion rate by creating niches for plant invasions worldwide (Culliney, 2005; Dehnen-Schmutz *et al.*, 2007; Faulkner *et al.*, 2016).

Since the 1600s, hundreds of alien invasive plants have been brought into South Africa for various reasons (Zimmermann *et al.*, 2004). Most of the alien plants that establish in South Africa are from Australia as well as South and Central America (Zimmermann *et al.*, 2004). Some of the ecosystems most vulnerable to invasion by IAS include riparian habitats, water ways, grassland habitats and disturbed areas (Culliney, 2005; Daehler, 2003; Beater *et al.*, 2008; Morris *et al.*, 2008; Esler *et al.*, 2008; Witkowski and Garner, 2008; Mugwedi *et al.*, 2015: Marlin *et al.*, 2017; Venter *et al.*, 2017). As of 2016, the Southern African Plant Invaders Atlas (SAPIA) project had records for 773 alien plant taxa, which was an increase of 172 from 2006 (Henderson and Wilson, 2017). Invasive plants that showed the greatest increase in range include *Cylindropuntia fulgida* var. *mamillata* Backeb (Cactaceae), *Argemone ochroleuca* subsp. *ochroleuca* (Papaveraceae), *Parthenium hysterophorus* L. (Asteraceae), *Opuntia engelmannii* (Cactaceae), and *Campuloclinium macrocephalum* (Less.) DC. (Asteraceae) (Henderson and Wilson, 2017). Introductions of alien plants are mainly as a result of increased trade, emigration, international air travel, human population increase (Faulkner *et al.*, 2016) while many others are not accidental, but the intentional movement of plant species from their native range. Some invasive plants were deliberately introduced into

new ranges as ornamental plants, crops, hedge plants, agroforestry species and as fodder (Mgidi *et al*., 2007).

1.2 Management and Biological control

Methods used to control and manage alien invasive plants include, chemical, mechanical, fire and biological control, and combining a number of these methods is known as integrated control (Goodall *et al*., 2011; DiTomaso *et al*., 2017; Hajek and Eilenberg, 2018). Mechanical control includes mowing, grubbing, chaining, harvesting and bulldozing, basal stem cutting of trees and shrubs (with and without the application of herbicides to the cut stump) (Witkowski and Garner, 2008; DiTomaso *et al*., 2017). These practices can result in the displacement of wildlife, habitat disruptions as well as soil compaction and erosion (DiTomaso, 2000; Zimmermann *et al*., 2004). Fire is also a management tool that is used to control invasive plants (e.g. Goodall and Erasmus, 1996; Witkowski and Wilson 2001). Chemical control on the other hand includes the use of herbicides that inhibit respiration, cell division, photosynthesis and lipid biosynthesis in plants (Goodall *et al*., 2011; Capinera, 2017). The misuse of herbicides can, however, result in the contamination of soil and fresh water systems and cause non-target effects (Culliney, 2005; Capinera, 2017; DiTomaso *et al*., 2017).

In aquatic systems, it was shown that fish were vulnerable to some herbicides, such as nitrile, and phenoxy groups, that damage their reproductive and respiratory organs (Pemberton *et al.*, 2002; Fabricius, 2005; Capinera, 2017). Even under normal field application procedures, some evidence suggested that herbicides pose a significant risk to arthropods (Norris and Kogan, 2000), and that aerial applications can cause direct and indirect effects on the diversity and abundance of wildlife, plants and soil biota (Pemberton *et al.*, 2002; Relyea, 2005; Heyes *et al*., 2007; DiTomaso *et al*., 2017). Integrated control involves the use of more than one control method to manage invasive weeds, which can include biological control (Goodall and Erasmus, 1996).

Given the shortcomings of chemical and mechanical control methods, biological control is considered the most sustainable and environmentally friendly method of weed control (Liebenberg, 2007; Turpie *et al.*, 2007; Bean *et al.*, 2012). Biological control is the use of host-specific, exotic natural enemies such as parasitoids, predators and pathogens (Goldson *et al.*, 2014; Cowie *et al.* 2018). Although insects are most frequently used as biological control agents because of their characteristic host-specific nature; other agents used include mites

(Mukwevho *et al.*, 2018), fungi (Klein, 2011; Buccellato *et al.*, 2012), nematodes and bacteria (Hajek *et al.*, 2007; Goldson *et al.*, 2014).

Before biocontrol agents are released they are tested for host specificity to determine their impact on economically important non-target plants and native plants that are closely related to the target plant (Zimmermann *et al*. 2004; McConnachie *et al*. 2011; Ramanand *et al.* 2016). Agents are only considered safe for release, once they have been proven to have an acceptably narrow host range (McFadyen, 1998; Muniappan *et al.*, 2009). Under favourable conditions, successful agents have the ability to spread and establish in their introduced range, reducing the weed population by decreasing the reproductive ability or vigour of the invasive plant target (McFadyen, 1998; Byrne et al. 2011; Cowie et al. 2017). Once established the agent population becomes self-sustaining (Muniappan *et al.*, 2009) and no further releases are required (Muller-Scharer and Schaffner, 2008; Barratt *et al*. 2010).

In South Africa, the first biological control agent was released in 1913 against drooping prickly pear (*Opuntia monacantha)* (Zimmerman *et al.*, 2004; Paterson *et al.*, 2011). Biological control in South Africa started using research that was originally done in the United States of America and Australia (Moran *et al*. 2013). South Africa has since advanced and is considered among the most active and leading countries in biological weed control research alongside the U.S.A. and Australia (Zimmermann and Klein, 2000; Zachariades, *et al.*, 2017).

1.3 Factors affecting establishment of biological control agents

Climate incompatibility between the area of introduction and origin is one of the reasons why biological control agents fail to establish and spread (Byrne *et al.*, 2002; Cowie *et al.*, 2018). Climate may also have a negative impact on the vigour of agent populations (Byrne et al., 2002; McConnachie and McKay, 2015; Cowie *et al.*, 2018). Factors such as frost, flooding and drought were found to drastically affect populations of *Neochetina eichhorniae* Warner (Coleoptera: Curculionidae) weevils on water hyacinth, *Eichhornia crassipes*, Martius (Pontederiaceae) (Bownes *et al.*, 2013). Climate matching is therefore used to identify climatically suitable regions for biological control agents on invasive plants (Byrne *et al.*, 2002; Senaratne *et al.*, 2006). It can also be used during exploration to identify potential biological control agents that are climatically adapted to release sites, from the native range of the alien invasive plant (Senaratne *et al.*, 2006 Cowie *et al.*, 2018). Altitude can also affect the establishment of biological control agents (Byrne *et al.*, 2002). A leaf-feeding beetle

Gratiana spadicea (Klug) was released against *Solanum sisymbriifolium* (Lamarck) and failed to establish on some of the release areas because of elevation (Senaratne *et al.*, 2006). Other factors that may affect the establishment and reproduction of agents include plant quality, disease, application of herbicides (Katembo *et al.*, 2013) and predators (Newman *et al.*, 1998; Goodall *et al.* 2011; Goodall and Witkowski, 2014).

1.4 *Campuloclinium macrocephalum* and its native and invasive distributions

Campuloclinium macrocephalum (Less.) DC. (Asteraceae), is an invasive perennial plant in the South African grassland biome, commonly known as pompom weed (Henderson *et al*., 2006). Native to South and Central America (Argentina, Bolivia, Brazil and Paraguay) (Figure 1.1), the earliest herbarium record of *C. macrocephalum* South Africa is lodged at the Pretoria National Herbarium of a specimen, collected in Johannesburg in 1962 (Henderson *et al*., 2006). Naturalised populations were first recorded in Westville (Durban) and Fountains Valley (Pretoria) in 1972 and 1974 respectively (Henderson *et al*., 2006).



Figure 1.1: The native distribution of *Campuloclinium macrocephalum* in South and Central America (Source: McConnachie *et al.*, 2011).

Campuloclinium macrocephalum was in some ways a sleeper weed, displaying a lag phase of more than 30 years after its first introduction before notably becoming invasive (Grice and

Ainsworth, 2003; Goodall *et al.*, 2010, 2011; Gitonga *et al.*, 2015). Since the 1980s it has invaded wetlands, roadsides and grasslands in provinces such as Gauteng (G), Mpumalanga (M), Free State (FS), Limpopo (L), North West (NW) and KwaZulu-Natal (KZN) (Figure 1.2) (Goodall, 2016). The presence of *C. macrocephalum* poses a significant environmental threat by displacing indigenous vegetation, resulting in a reduction in species richness and biodiversity in areas that have been invaded (Aileen, 2005). It also reduces the grazing capacity of farms and game reserves because of its unpalatability to wildlife and livestock (Goodall *et al.*, 2011; McConnachie and McKay, 2015). Allelopathy does not play a role in the invasion biology of *C. macrocephalum* (Goodall *et al.*, 2010).

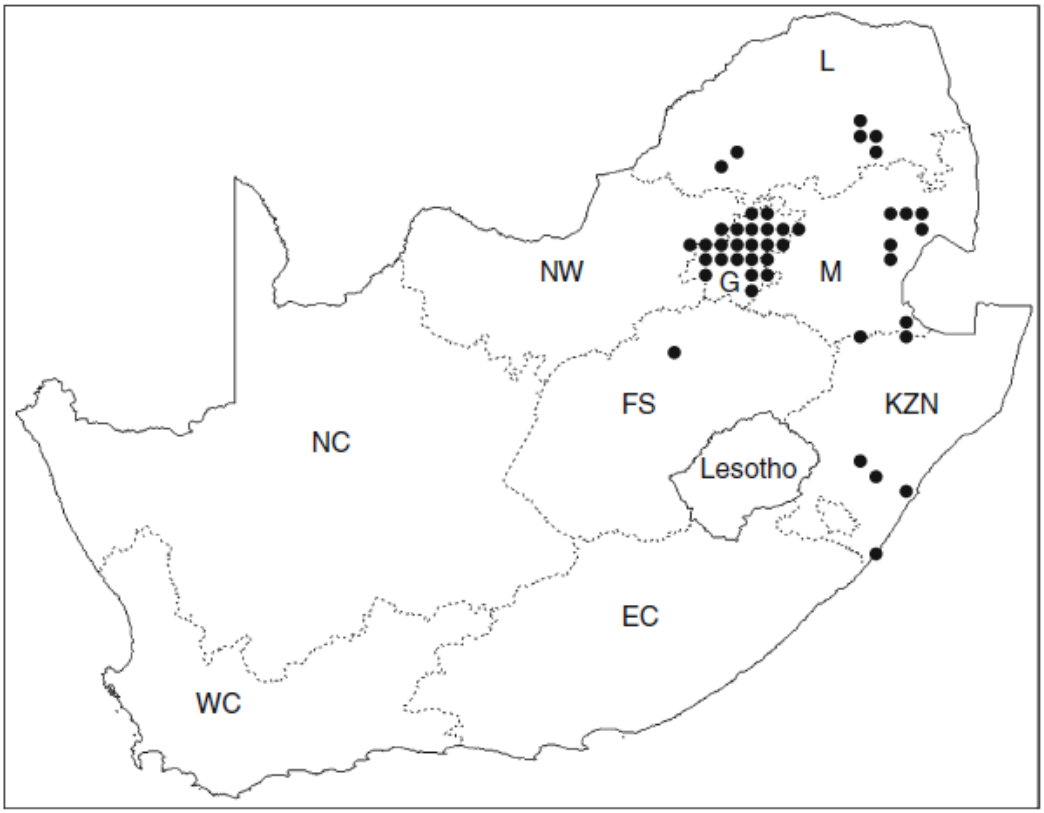


Figure 1.2: Distribution (black dots) of *Campuloclinium macrocephalum* in South Africa. The black circle represents weed presence in quarter degree squares (ca. 700 km2) (Source: Goodall, 2016).

Campuloclinium macrocephalum grows up to 1.5 m in height and has leaves and stems that are covered with rough, bristly hairs (Figure 3) (McConnachie *et al*., 2011). It is able to spread through prolific seed production (McConnachie *et al*., 2011). In late autumn (May) the stems senesce and the plant dies back to perennial rootstocks in winter which regrow in spring (October) (Goodall and Witkowski, 2014). Consequently, in winter pompom weed is hardly affected by frost, fire or the insignificant amount of rainfall during the highveld dry season (Goodall *et al.*, 2012). It grows rapidly and can reach an average height of 1.3 m by

December (Goodall and Witkowski, 2014). Stems that arise from the perennial rootstocks in spring are called primary stems and any physical damage to these stems results in copious regrowth (Goodall *et al.*, 2012). It produces dense clusters of pink flowers on aerial stems and each flower head has hundreds of tiny florets that are enclosed by purple bracts (Figure 1.3) (Henderson 2001; McConnachie *et al*., 2011). When the florets are mature each produces a dry fruit that is single seeded with a cluster of hairs that promote wind dispersal (McConnachie *et al*., 2011)

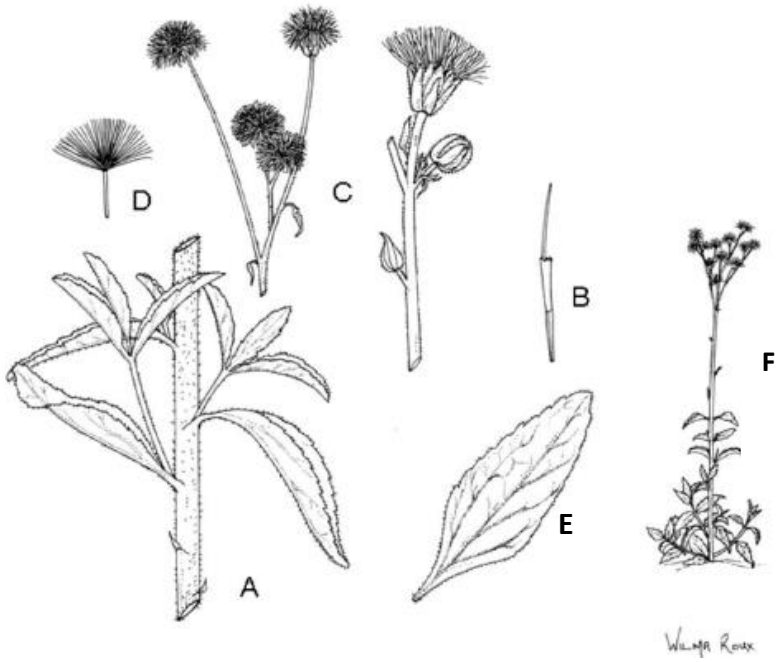


Figure1.3: Stem and leaves (A), inflorescence (B), florets (C), achenes (D), leaf (E) and mature plant (F) of *Campuloclinium macrocephalum* (Source: McConnachie *et al.*, 2011).

1.5 Management of *Campuloclinium macrocephalum*

The management of this weed has relied mainly on mechanical and chemical methods before the introduction of biological control agents (McConnachie *et al*., 2011; Ramanand *et al*., 2016). Mechanical control methods such as hoeing and physical removal of the weed were found to be ineffective (McConnachie *et al*., 2011), and the disturbance made the problem worse because damage to the rootstocks stimulates and promotes further vegetative growth which increases the density of the weed (McConnachie *et al*., 2011). Furthermore, because of the difficulties in removing the roots of the weed in rocky areas, the flowering stems were cut off to reduce seed production, and hence this was an attempt to reduce the rate of spread (E. Witkowski, pers. comm.). The use of chemicals such as registered herbicides is effective (Figure 1.4), however annual application is required to eliminate the plant from the area (Goodall, 2016). Herbicide application is also not ideal for environments that are sensitive,

such as areas that are next to dams, wetlands and rivers (Capinera, 2017; DiTomaso *et al*., 2017. Despite the application of herbicides, the range of *C. macrocephalum* continues to expand in South Africa (McConnachie *et al*., 2011; Henderson and Wilson, 2017). High fire frequency was also found to increase the density of *C. macrocephalum*, as it stimulated the emergence of shoots before the native grasses emerged (Goodall *et al.*, 2011). The use of fire during the dry season is also being considered as a control method to reduce the seedbank. Overall, mechanical and chemical control methods are expensive, unsustainable and possibly harmful to the ecosystem.



Figure 1.4*:* *Campuloclinium macrocephalum* at Voortrekker monument, Pretoria South Africa, before application of herbicides (**A**, February 2011) and after application (**B**, January 2012) by the then Department of Water Affairs, Working for Water programme.

1.6 Biological control of *Campuloclinium macrocephalum*

The spread of the weed and its negative impacts resulted in the initiation of a biological control programme in 2003, funded by the then Working for Water Programme of the Department of Water Affairs (now under the Department of Environmental Affairs, National Research Management Programmes (DEA NRMP)) (Olckers, 2004; Goodall *et al.*, 2011). According to McConnachie *et al*. (2011) the highest diversity of natural enemies of *C. macrocephalum* in South America was found in the northern parts of Argentina. A total of eight biocontrol candidate agents were brought back to South Africa (Table 1).

Based on their distribution and impact on the plant, four species were considered suitable potential biological control agents (McConnachie *et al*., 2011). Of the four species there were three insect species, namely *Liothrips tractabilis* Mound and Pereyra (Thysanoptera: Phlaeothripinae), *Zeale* (=*Adesmus*) *nigromaculatus* (Cerambycidae), and *Cochylis campuloclinium* (Lepidoptera: Tortricidae) and one fungal pathogen, *Puccinia eupatorii* (Pucciniales: Pucciniaceae) (Table 1) (McConnachie *et al*., 2011). In 2006, a rust fungus was

discovered on *C. macrocephalum* in Pretoria and it was confirmed to be *P. eupatorii* (McConnachie *et al.* 2011; Goodall et al. 2012; Wood and Besaans, 2013). Only the stem-deforming thrips and the leaf rust have been released in South Africa to date, with the leaf rust being an adventive release (Ramanand *et al*., 2016).

Puccinia eupatorii is an autoecious macrocyclic leaf rust that has several spore stages on its host (Cummins, 1978). Physical symptoms of urediniospore germination and infection appear after 11-14 days, with black spots appearing on both leaf surfaces (Goodall *et. al.*, 2012). The presence of *P. eupatorii* on the leaves causes necrosis and abscission which results in the premature death of stems. After detection in the field in 2006, *P. eupatorii* has established in most of the introduced range of *C. macrocephalum* in South Africa (Goodall *et. al.*, 2012). The application of herbicides may not be effective during the peak pathogen infection stages in February and March because leaf necrosis reduces absorption and translocation of active ingredient (Goodall *et al.* 2011).

Table 1.1: Potential biological control candidates of *Campuloclinium macrocephalum* imported from South America to South Africa in 2003 (Source: McConnachie *et al*., 2011).

Potential agent	**Country of origin**	**Guild**
Zeale (=Adesmus) nigromaculatus Klug (Cerambycidae)	Argentina and Brazil	Stem borer
Unidentified pintail beetle (Mordellidae)	Argentina	Stem borer
Unidentified *Carmenta* sp. Eichlin (Sesiidae)	Argentina	Stem borer
Liothrips tractabilis Mound and Pereyra (Thripidae)	Argentina	Stem deformer
Unidentified fly (*Trupanea* sp.) (Tephritidae)	Argentina and Brazil	Flower feeder
Adaina sp. prob. *simplicius* Brown (Pterophoridae)	Argentina	Flower feeder
Cochylis campuloclinium Brown (Tortricidae)	Argentina and Brazil	Flower feeder
Puccinia eupatorii Dietel (Pucciniaceae)	Argentina	Leaf rust

1.7 *Liothrips tractabilis*

The genus *Liothrips* Uzel consists of about 282 species worldwide (Mirab-Balou, 2016), 74 species recorded in Indonesia, 25 species in China, with 35 species listed from the south of Mexico, 17 from Brazil and five from Argentina (Zamar *et al.* 2013). The five species from Argentina include *L. tandiliensis* (Minaei and Mound, 2014), *L. vernoniae*, *L. atricolor*, *L. ludwigi* (Zamar *et al.*, 2013) and *L. tractabilis* Mound and Pereyra (2008). *Liothrips tractabilis* was first recorded in Argentina in 2004 on *C. macrocephalum* (McConnachie and McKay, 2015) and was described as a new species by Mound and Pereyra (2008). Most of the host plant species that *Liothrips* spp. depend on, are not known and poorly understood,

which makes the identity of most *Liothrips* spp. doubtful (Mound *et al.*, 2016). Three species within the genus *Liothrips* were considered and used as biological control agents (Mound *et al.*, 2016). *Liothrips mikaniae* was introduced into South-East Asia against *Mikania micrantha* (Asteraceae) (Cock, 1982) and *L. urichi* was used against *Clidemia hirta* in Hawaii, Fiji and Samoa (Melastomataceae) (Simmonds, 1933).

Permission to release *L. tractabilis* in South Africa was approved in 2013 after quarantine evaluations showed that the agent is host specific and causes significant damage to the target plant (McConnachie and McKay, 2015). It deforms the stems of *C. macrocephalum* by altering the apical shoot tips of stems; instead of vertical growth the stems bend over downwards (Figure 1.5B). The growth of *C. macrocephalum* was significantly reduced by the thrips even at low inoculation densities (two thrips per test plant) in lab trials (McConnachie and McKay, 2015). Young and regrowth plants inoculated with thrips demonstrated a significant reduction in leaf and floral production, as well as biomass and height compared to control plants (McConnachie and McKay, 2015).

Liothrips tractabilis adults are black (Figure 1.5A) and females lay their eggs either singly or in batches on the leaves, sepals and stems of the weed (McConnachie and McKay, 2015). Eggs of the thrips are 0.19 ± 0.01 mm wide, 0.45±0.02 mm long, yellow to orange and oval. They also have bumps that are evenly distributed on the surface of the egg (McConnachie *et al*., 2011). Eggs hatch within 10 days at 25 °C and three nymphal stages and two pre-adult stages can be distinguished. Both nymphal and pre-adult stages are red in colour and developmental times are eleven and seven days respectively. Total developmental time from egg to adult is 28 days (Ramanand *et al*., 2017).



Figure 1.5: Adults (black) and nymphs (red) of *Liothrips tractabilis* **(A)**. The deformation of *Campuloclinium macrocephalum* stems **(B)** (Source: Besaans, 2014).

Thrips in general, including *Thrips obscuratus* (Crawford) (Thysanoptera: Thripidae), a New Zealand flower thrips, are amongst the weakest flying insects, yet they are commonly found at a height of 600m from the ground traveling over long distances (Lewis, 1973). Before thrips take off they stand in an elevated position with their wings held backwards at a steep angle with their abdomen curved upwards to assist in spreading their wings, they launch with a kick from their legs (Lewis, 1973). Once launched thrips have minimal directional control and depend greatly on wind for their dispersal (Lewis, 1973).

1.8 Developmental rates of insects and overwintering

Climatic conditions such as temperature, rainfall, humidity, light and wind significantly affect the population parameters (distribution and abundance) of insects, plants and other living organism (Byrne *et al*., 2003; May and Coetzee, 2013; Ramanand *et al*., 2017). As a result of seasonal changes in climatic conditions, insects, plants and other organisms experience both favourable (wet season) and unfavourable (dry season) growth periods (Surthest, 2003). Most plants, including invasive weeds, lose their leaves during the dry season in response to reduced rainfall and decreased temperatures. However, such plants usually regrow from buds, at or below the soil surface (where they are protected from dry season fires) as the temperatures and rainfall increase again (wet season). Because of the loss of leaves and the often-freezing conditions, insects associated with such plants (e.g. *L. tractabilis* and *C. macrocephalum*) enter a period of dormancy (diapause) and/or move underground to overwinter (Bean, *et al*., 2012).

The rate of development of insects is largely governed by temperature because biological activities of poikilotermic organisms depend on chemical reactions which are proportional to temperature (Campbell *et al*., 1974). This reaction is limited by upper and lower temperature thresholds (Campbell *et al*., 1974; Sutherst *et al*., 1985). High light environments are exposed to direct solar radiation resulting in higher temperatures (Muth *et al*., 2008; Ruban, 2009; Patrick and Olckers, 2014). As opposed to full sun environments, shaded habitats experience reduced temperatures (Ruban, 2009). In both shade and sunny environments, plants defence against herbivory may be either be structural, chemical and/or physiological (Dungan *et al*., 2007). *Liothrips tractabilis* causes significant damage to *C. macrocephalum* (McConnachie and McKay, 2015), however ways to optimize the impact of *L. tractabilis* should be explored.

In winter the host plant of *L. tractabilis* dies back to rootstocks, which causes the agent to also move below ground in order to overwinter. During dry cold conditions insects cope

through various physiological and behavioural adaptations (Tauber *et al.* 1986). Heavy rainfall during the rainy season negatively affects the population of some species of thrips and may suppress dispersal (Lewis, 1963). In contrast, rainfall can also positively impact the population of thrips growth and dispersal by delaying the senesce of the host plant to allow thrips to thrive for an extended period.

Liothrips vaneeckei (Priesner) was found to be able to persist through winter as adults in the laboratory in the United Kingdom on species of *Lilium* (Morison, 1957). Other insects that also overwinter include, the Colorado potato beetles (*Leptinotarsa decemlineata*) (Milner *et al.*,1992), and *Diorhabda elongata deserticola* (Lewis *et al.*, 2003). Although *L. tractabilis* was able to overwinter under favourable laboratory conditions (18- 30 °C) (Ramanand *et al*., 2017), it is not clear whether they overwinter as adults or nymphs in the field either in South Africa or South America and if they feed on and cause any damage to the roots (McConnachie *et al*., 2011). In Argentina, *L. tractabilis* was observed underground in winter (McConnachie and McKay, 2015), however, this has not been verified or quantified in South Africa (Ramanand *et al*., 2017).

1.9 Aims and objectives

The aim of this study was to assess the impact of *L. tractabilis* on *C. macrocephalum* during the growing season (summer) and dry season (winter). The research objectives were as follows:

Objective 1. Compare the impact of *L. tractabilis* on the above ground-growth of *C. macrocephalum* under full-sun and partial shade conditions over the growing season (Chapter 2).

Objective 2. To investigate the impact of *L. tractabilis* underground on the rootstock of *C. macrocephalum* during winter or the dormant season (Chapter 3).

CHAPTER 2:

Does early season mowing increase the impact of *Liothrips tractabilis* on *Campuloclinium macrocephalum* during the growing season?

Abstract

Temperature is one of the factors that affects the distribution and abundance of animals and plants, and the rate of insect development. Developmental rates of insects increase from a low temperature threshold up to a maximum at an optimal temperature. Higher temperatures result in increased population size because of increased developmental rates. However, some studies have observed increased rates of herbivory for plants occurring in shaded environments, while other studies have observed increased herbivory on plants in full sun environments. The aim of this study was to compare the effect of the biological control agent, *L. tractabilis* on the growth of *C. macrocephalum* under sunny (areas with mown short grass) versus more shaded (areas with unmown long grass) environments. One plot was mown when *C. macrocephalum* was dormant in early October, the other plot was left unmown. The mown plot experienced increased temperatures compared to the unmown plot. The number of *L. tractabilis* adults was significantly higher by 76% in the sun compared to the shade with 36%. The impact of *L. tractabilis* on the plants was also significantly greater by 78% in the sun compared to the shade, whilst plant height was reduced by 64% and the proportion of plants flowering reduced by 74%. Mowing may have also influenced the nutritional quality of *C. macrocephalum* which in turn increased the impact of *L. tractabilis*. Therefore, within an integrated management system, an early season mowing of pompom invaded veld, has the potential to enhance the performance of the biocontrol agent, *L. tractabilis* in controlling *C. macrocephalum.*

Keywords: Biological control, light intensity, mowing, shade, sun, temperature

2.1 Introduction

Temperature is one of the most important climatic conditions that affects insect development and distribution (Campbell *et al*., 1974; van Lenteren *et al*., 2006; Ramanand *et al*., 2017). Biological activities of poikilothermic organisms are dependent on the energy from chemical reactions that depend on the organisms lower and upper temperature thresholds (Ramanand *et al*., 2017). Developmental rates of insects increase from zero at a low temperature threshold up to a maximum at an optimal temperature, and then progressively decline again to zero beyond the species upper threshold temperature (Wagner *et. al.*, 1991). Based on Logan *et. al.* (1976), increased temperature also positively influences the population size of insects because it increases their developmental rates.

The lower temperature threshold at which *L. tractabilis* completes development is 17 °C, while the optimal temperature threshold is 30 °C (Ramanand *et al*., 2017). During laboratory studies development of *L. tractabilis* was not supported at temperatures lower than 15 °C and temperatures higher than 32 °C (Ramanand *et al*., 2017). Environments that are exposed to high light intensity experience higher temperatures because of direct solar radiation, compared to low intensity light (shady) environments which maintain cooler microhabitat conditions that may be more favourable to some insects (Muth *et al.*, 2008; Ruban, 2009; Patrick and Olckers, 2014). For example, *Gargaphia decoris* (Hemiptera: Tingidae) had greater effects on *Solanum mauritianum* Scop. (Solanaceae) plants growing in full sun compared to plants in the shade (Cowie *et al.*, 2016).

Plant quality and growth is not only affected by nutrient availability for plants in shaded environments, light is generally a more limiting resource than nutrients (Kopper *et al.*, 2002; Barber and Marquis, 2011). Light promotes the growth of plants, however extreme exposure to high temperatures may result in plant damage due to overheating (Barber and Marquis, 2011). Generally, different light conditions have variable effects on plant physiology, insect herbivory and insect-plant interactions (Roberts and Paul, 2006; Venter *et al.*, 2013).

Plants that are exposed to high light generally have tough leaves with high water content, low nitrogen and high levels of phenolics compared to plants in the shade (Henriksson *et al.* 2003). As a result, plants that are exposed to high light are less palatable to herbivores, which may make herbivores more likely to colonise plants in the shade (Henriksson *et al.* 2003). The reduction in herbivore abundance results in reduced foraging intensity and lower plant

damage (Barber *et. al.* 2011). High phenolic content such as tannins negatively affect the growth of plants and abundance of herbivores (Kopper *et al.* 2002).

Campuloclinium macrocephalum is an invasive perrenial forb that invades grasslands, and it particularly dominantes many grasslands in Gauteng Province (McConnachie *et al*., 2011). *Campuloclinium macrocephalum* grows well in areas that have either long or short grass (Goodall *et al.*, 2012). *Liothrips tractabilis* was released against *C. macrocephalum* in 2013, and it causes significant damage to the weed (McConnachie *et al*., 2011; McConnachie and McKay, 2015).

There is anecdotal evidence that *L. tractabilis*'s impact and reproductive ability is greater on plants in full sunlight rather than those growing in partial shade cast by tall grasses. Areas with short grass receive more sun light (full-sun) on the soil surface compared to areas with long grass (shade). Light intensity can vary and has direct influence on plant-insect interactions and photosynthetic capabilities of the host plant (Ruban, 2009). Therefore, the aim of this chapter was to quantify and compare the impact of *L. tractabilis* on *C. macrocephalum* growing in short grass (grass was mown in early spring) and long grass (grass was left unmown).

Objective 1. Measure the effect of long (unmown) and short (mown) grass on the abundance of *L. tractabilis* (adults, nymphs and eggs) on *C. macrocephalum*.

Objective 2. Compare the impact of *L. tractabilis* on *C. macrocephalum* growing in long versus short grass.

2.2 Methods and Materials

Study site

This study was conducted at Waterkloof Airforce Base, Pretoria, South Africa (25°48'54.57"S, 28°13'27.68"E) during spring, summer and autumn, from October 2017 to May 2018. The vegetation of the site is dominated by grasses and is classified as Carletonville Dolomite Grassland (Mucina and Rutherford, 2006). Some of the dominant grass species include, *Eragrostis curvula*, *Hyparrhenia hirta* and *Cymbopogon caesius*. The region is characterised by severe frost in winter, high summer temperatures, summer rainfall with an overall mean annual precipitation of 593 mm. It is an open and flat area that currently has no grazers. Some of the invasive plants within the same area include *Solanum mauritianum* Scopoli (Solanaceae) and *Lantana camara* L. (Verbenaceae). In South Africa *C.*

macrocephalum is able to survive the devastating effects of *P. eupatorii* (Goodall *et al.*, 2012). *Puccinia eupatorii* was present at Waterkloof Airforce Base before *L. tractabilis* was introduced, with insignificant effects on *C. macrocephalum* at the site (L. Van Der Westhuizen, pers. comm.). *Puccinia eupatorii* causes dark brown spots on both leaf surfaces resulting in leaves turning brown and then leaf abscission (Goodall *et. al.*, 2012). *Liothrips tractabilis* was released in November 2014 and has become well established in the study site.

Experimental design and protocol

Two plots of 10 m x 10 m each, positioned 10 m apart, were laid out within the Waterkloof Airforce Base study site, where *L. tractabilis* has already established. In mid-October, before *C. macrocephalum,* plants had regrown from their rootstocks, the herbaceous layer, which was mostly composed of grass was mown in one plot (mown plot) while the other plot was left unmown (unmown plot). Grass on the mown plot was mowed to a height of at most 20 cm whilst the unmown plot had an average grass height of about 65 cm at the time. Mowing was done when *C. macrocephalum* was still dormant. As a result, *C. macrocephalum* growth in the mown plot received relatively more direct solar radiation compared to *C. macrocephalum* growing in the more shaded, unmown plot.

Field work

Each month from October 2017 to May 2018 inclusive, six 1 m x 1 m quadrats were randomly placed in each plot per month (using random number tables). A total of 96 quadrats were assessed over the study period (48 for each plot). Each month six new quadrats were sampled within the plots (no resampling of exactly the same quadrat was done). A record of sampled quadrat position was kept, ensuring that the same area was not sampled more than once. One facing north plant closest to the bottom left corner of each quadrat was selected and the following plant parameters were measured:

1) Height of *C. macrocephalum,* measured on the main stem from the ground to the tip of the inflorescence using a plastic measuring tape.
2) Number of leaves (only live leaves on stems were counted, with dead leaves being excluded).
3) Number of flowering plants. Note that flowers were not collected from the plants within the quadrats as this promotes lateral growth and further flowering.

4) The total number of deformed plants within the quadrat was also counted. Deformation of *C. macrocephalum* was defined as plants with altered apical shoot tips; showing inhibited vertical growth of the stems (Figure 1.5B).

Each plant that was randomly selected within a quadrat in the field was dug up as completely as possible, including the below-ground fleshy roots, labelled and placed in a separate container for further analysis in the laboratory. The above ground plant biomass (standing crop) of each plot was measured monthly for six months using a Disc Pasture Metre (DPM) for each quadrat where plant samples were collected. The biomass was then calculated using the equation of Trollope and Potgieter (1986). The temperature of the two plots was obtained by placing a single temperature iButton (1-Wire Thermochron, DS1921G) at the centre of each plot, 10 cm above the soil surface, and recorded every hour. The average maximum and minimum temperature of the two plots was calculated by obtaining the maximum and minimum temperatures of each day within the month from October 2017 to May 2018 inclusive. Data from the two ibuttons was extracted every three months using 1-Wire iButton Drivers for Windows and then replaced with new ibuttons for the next three months.

Laboratory work

The abundance of the biological control agent, *L. tractabilis* was counted on each of the plants that were dug up. Each plant sample from the two plots was carefully examined under a dissecting microscope (S6-BLED Stereo Zoom Dissecting Microscope) in the laboratory and the number of adults, nymphs and eggs of *L. tractabilis* were recorded. Each plant was examined for 40 minutes, a magnifying glass (Waltex pocket sliding magnifier 3x) was also used for these observations. Thereafter, above ground plant tissues were placed in brown paper bags and oven dried for three days at 55 °C and then the mass of stems (including flowers) and leaves were recorded separately once all plant tissues were completely dry.

Predicting the number of *L. tractabilis* generations

Degree day calculations were done to estimate and compare the number of generations on the two plots. Daily minimum and maximum temperature records were obtained from the iButtons (1-Wire Thermochron, DS1921G) from the two plots, with the temperature being offset using the temperature from SAWS as temperature values from the Ibutton was too high because of direct sunlight. The following equation was used:

$$K = \sum \left(\frac{T_{max} + T_{min}}{2} - t \right) (\text{if } T_{min} < t, t \text{ was used}).$$

Where k is rate of development, Tmax and Tmin represent the maximum and minimum temperatures, and t represents the lower developmental threshold for *L. tractabilis*. The lower temperature threshold (t) was 9.6 °C and the thermal constant at 546.9 °D, these values were obtained from (Ramanand *et al.*, 2017). Given the °D of *L. tractabilis*, the developmental rate was then calculated which enabled the calculation of the number of generations that could develop in the mown and unmown plots.

Data analysis

T-tests were used to examine the differences for each of the parameters measured between the mown and unmown plots for each month. One-way repeated measures ANOVA was used to compare the overall difference between the parameters from October 2017 to May 2018. All analyses were conducted using SPSS Statistics 22.0, and Microsoft Excel 2016.

2.3 Results

Early season mowing of the grass in the mown plot reduced the overall plant biomass compared to the unmown plot by a factor of 2.2 because there was still long grass from the previous season on the unmown plot (Figure 2.1; $P<0.0001$; Table 2.1). There was a strong seasonal increase in biomass for both plots from October to December because of the start of the summer rains that promoted grass growth, but thereafter it declined slightly from December to May ($P<0.0001$; Table 2.1). There was also a weak, although significant interaction, between the mown versus unmown treatments over time (months), based on higher relative differences in plant biomass between mown versus unmown plots, particularly in January and May ($P= 0.0486$; Figure 2.1; Table 2.1).

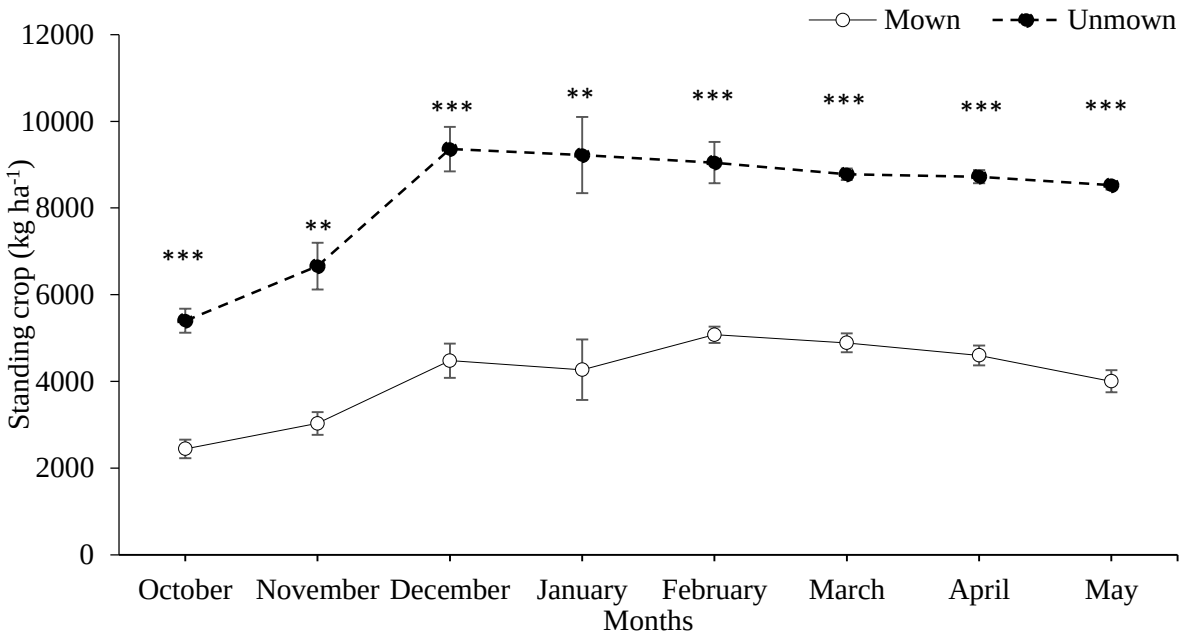


Figure 2.1: Comparison of standing biomass of mown and unmown plots from October 2017 to May 2018 (mean ± SE). For each data point n = 6 disk pasture meter (DPM) measures for each data point. Overall difference between plots assessed using one-way repeated measures ANOVA (Table 2.1). Asterisks (**= P < 0.01 and ***= P < 0.001) indicate significant differences between plots for corresponding months.

Table 2.1: Results of one-way repeated measures ANOVAs of the variables measured for the mown and unmown plots from October 2017 to May 2018. P-values in **bold** indicate significant differences.

Variable	**Treatment (mown versus**	**Time (month)**	**Treatment * Time**

	unmown plot)								
	F	**d.f**	**P**	**F**	**d.f**	**P**	**F**	**d.f**	**P**
Standing crop (Figure 2.1)	226.9528	1, 10	**3.354E-8**	17.9408	7, 70	**2.04E-13**	2.1576	7, 70	**0.0485**
Maximum temperature (Figure 2.2a)	8.969	1, 6	**0.005264**	6.208	7, 224	**0.0001**	0.685	7, 224	0.6844
Minimum temperature (Figure 2.2b)	14.464	1, 6	**0.000607**	31.877	7, 224	**0.0002**	1.824	7, 224	0.0837
Number of plants /quadrat (Figure 2.3)	0.1841	1, 10	0.677	6.0327	6, 60	**1.60E-05**	0.8696	6, 60	0.534
Deformed plants/ quadrat (Figure 2.4)	16.971	1, 10	**0.0021**	4.0851	6, 60	**0.0017**	0.8591	6, 60	0.5302
Height of plants (Figure 2.5)	183.974	1, 10	**0.0092**	73.358	6, 60	**0.0002**	61.5277	6, 60	**9.68E-04**
Number of flowering plants (Figure 2.6)	17.4227	1, 10	**0.0019**	4.2167	3, 30	**0.0028**	3.3399	3, 30	**0.0111**
Number of leaves/plant (Figure 2.7a)	2.331	1, 10	0.1578	11.4926	6, 60	**2.47E-06**	3.9302	6, 60	**0.0022**
Leaf mass/plant (Figure 2.7b)	3.3894	1, 10	0.0954	8.6004	6, 60	**9.38E-07**	1.2529	6, 60	0.2928
Stem mass /plant (Figure 2.7c)	56.0626	1, 10	**0.0021**	5.7086	6, 60	**0.0001**	2.1323	6, 60	0.0626
Number of *L. tractabilis* (adult)/ plant (Figure 2.8)	6.2665	1, 10	**0.0313**	3.0321	6, 60	**0.0118**	1.3704	6, 60	0.2412
Number of *L. tractabilis* nymphs/plant (Figure 2.9)	4.7124	1, 10	0.0551	2.9224	6, 60	**0.0144**	1.4279	6, 60	0.0654
Number of *L. tractabilis* eggs/ plant (Figure 2.10)	2.3617	1, 10	**2.48E-04**	4.2603	5, 50	**0.0026**	2.0179	5, 50	0.0921

Campuloclinium macrocephalum rosettes from the mown plot received more direct solar radiation compared to *C. macrocephalum* in the unmown plot, as indicated in the differences

in maximum and minimum temperatures between the mown and unmown plots (Figure 2.2). The maximum temperatures tended to be higher for the mown plot compared to the unmown plot by 1.4 ^{o}C on average, the seasonal change in maximum temperatures of the two plots was significant (P=0.0001; Table 2:1). The seasonal change of the minimum temperatures for the two plots was also significant (P=0.00021; Table 2:1).

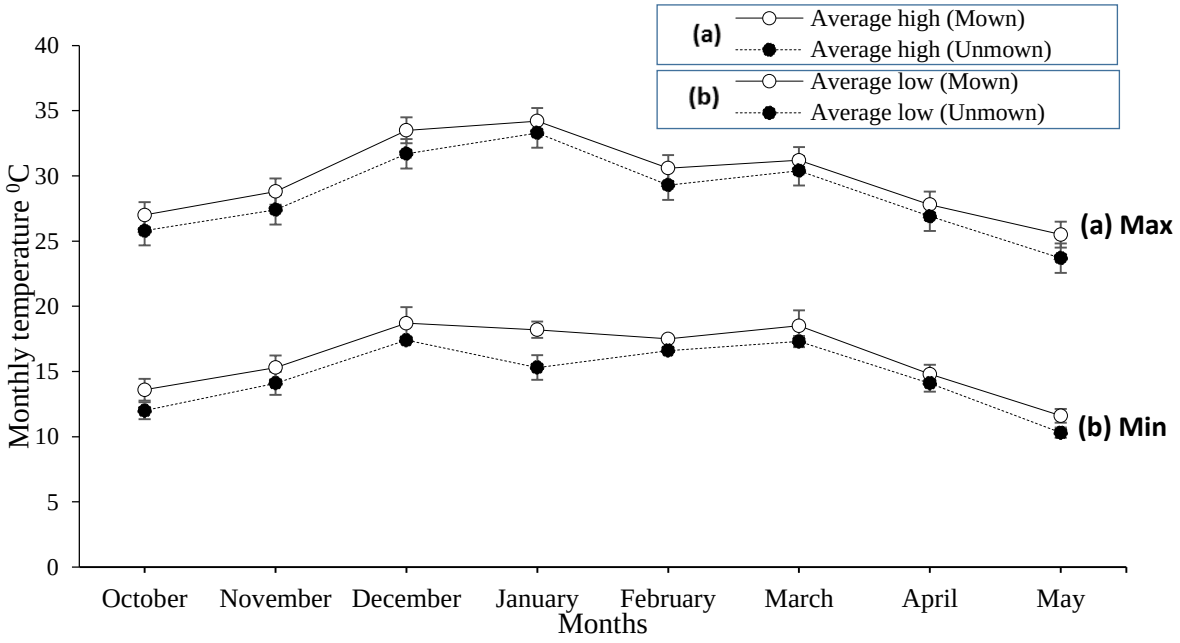


Figure 2.2: Maximum (a) and minimum (b) monthly temperatures (^{o}C) for mown and unmown plots from October 2017 to May 2018 (mean ± SE). Overall differences between plots was assessed using one-way repeated measures ANOVA in Table 2.1.

The mown plot had a greater overall number of individual *C. macrocephalum* plants compared to the unmown plot (Figure 2.3). There was no interaction between the number of *C. macrocephalum* plants between the mown and unmown plots over time (P= 0.5349; Table 2.1). There was an increase in the number of *C. macrocephalum* from October till December in both plots, followed by a decrease till none were recorded in May, as they had completely died back. By the end of May *C. macrocephalum* senesced to its underground tubers in both plots (Table 2.1).

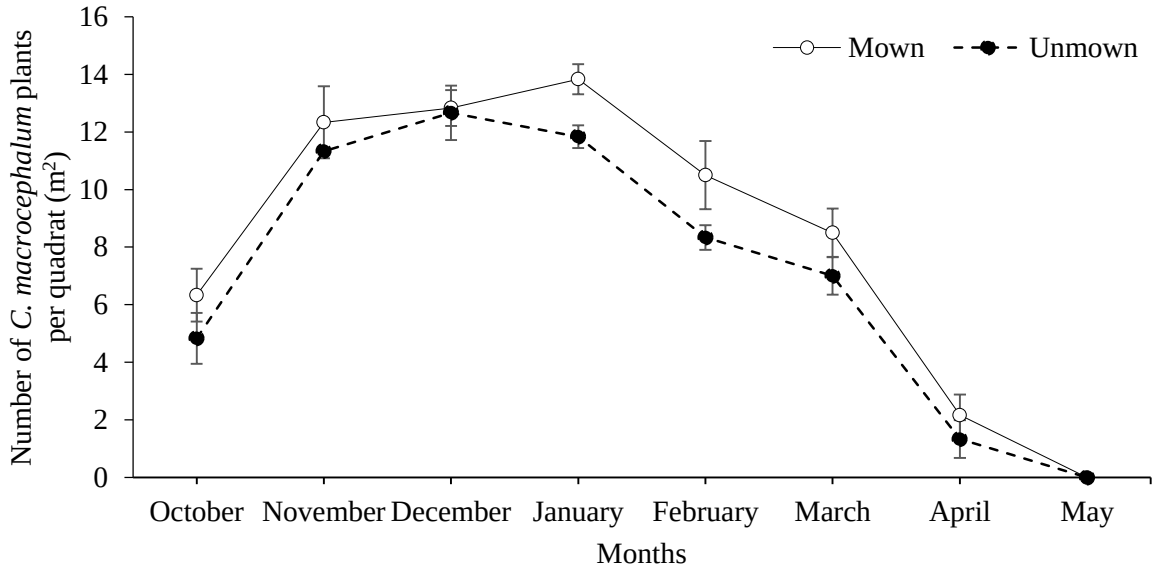


Figure 2.3: The effect of mowing on the number of *Campuloclinium macrocephalum* per quadrat (1 m x 1 m) for mown and unmown plots from October 2017 to May 2018 (mean ± SE). For each data point n = 6 quadrats for each data point. Overall differences between plots assessed using one-way repeated measures ANOVA (Table 2.1).

The greater number of *L. tractabilis* found on plants in the mown plot resulted in greater deformations of *C. macrocephalum* plants compared to those in the unmown plot (P= 0.0021; Table 2.1; Figure 2.4). There were no deformed plants in the unmown plot in October 2017, while 47% of *C. macrocephalum* plants were deformed in the mown plot by that date. In the mown plot the highest percentage of deformation (97%) was recorded in February, with 56% of the plants being deformed in the unmown plot for the same time period. There was a decrease in the deformation percentage from February to May 2018 in the mown plot, and to April 2018 in the unmown plot when all the *C. macrocephalum* plants had senesced (P=0.0304; Table 2.1). Hence complete senescence was a month earlier in the unmown compared with the mown plot. The seasonal difference in percentage plant deformation was significant (P= 0.0017; Table 2.1).

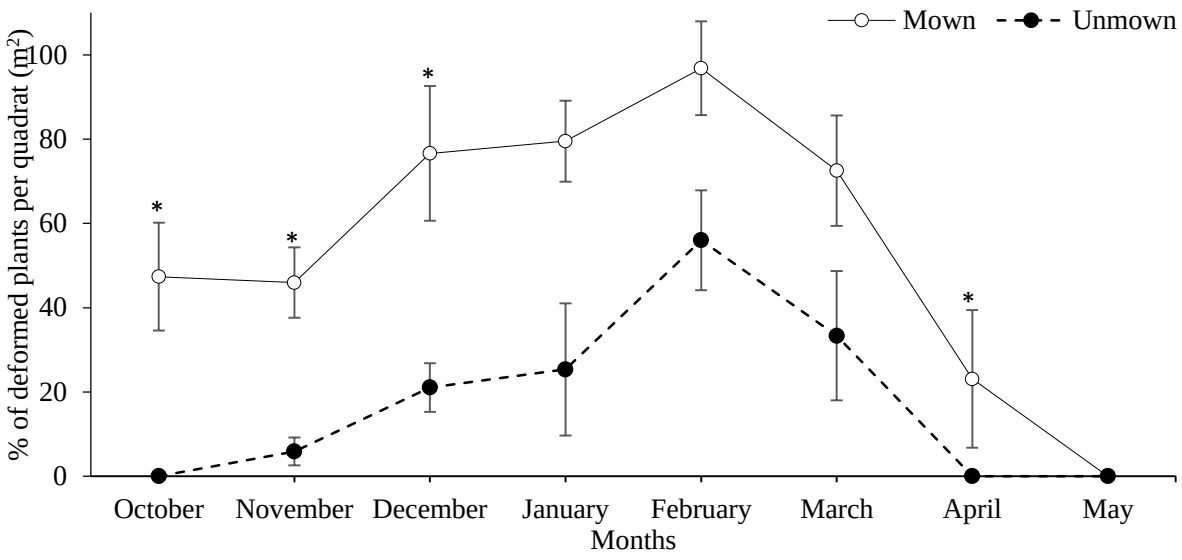


Figure 2.4: The effect of mowing on the percentage of deformed *Campuloclinium macrocephalum* per quadrat (1 m x 1 m) compared to mown and unmown plot from October 2017 to May 2018 (mean ± SE). Deformation was defined as distortion of the stems. For each data point n = 6 plants for each data point. The overall difference between treatments was assessed using a one-way repeated measures ANOVA (Table 2.1). Asterisks (*= $P < 0.05$) indicate significant differences between the mown and unmown plot on corresponding months.

In October 2017, at the beginning of the growing season the height of *C. macrocephalum* plants in the two plots was similar, however, the average plant height increased significantly in the unmown plot from 7 cm in October 2017 to 129 cm in January 2018 ($P=0.0002$; Table 2.1). The change in height on the mown plot was relatively low compared to the height in the unmown plot (Figure 2.5). The maximum average height of *C. macrocephalum* on the mown and unmown plot was 30 cm and 129 cm respectively (Figure 2.5). The overall height of *C. macrocephalum* in the mown plot was significantly lower (by 64%) compared to the unmown plot ($P< 0.005$) (Table 2.1).

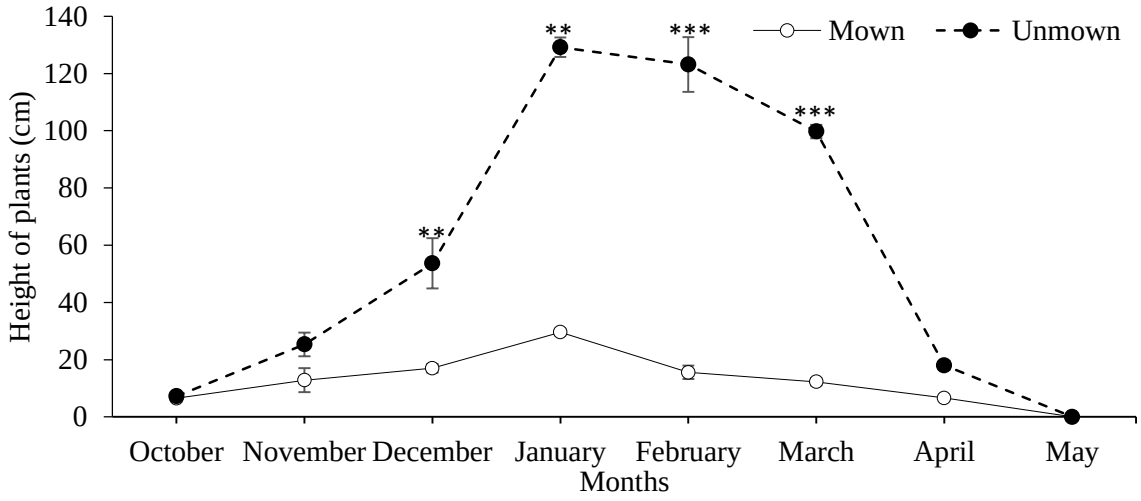


Figure 2.5: The effect of mowing on the height (cm) of *Campuloclinium macrocephalum* plants per quadrat (1 m x 1 m) in mown and unmown plots from October 2017 to May 2018 (mean ± SE). For each data point n = 6 quadrat for each data point. Overall differences between treatments were assessed using one-way repeated measures ANOVA (Table 2.1). Asterisks (**= P < 0.01 and ***= P < 0.001) indicate significant differences between the mown and unmown plot for corresponding months.

The number of flowering plants in the mown plot was significantly lower than in the unmown plot (P= 0.0019; Table 2.1) (Figure 2.6). The number of flowering plants in the mown plot was lower by 74% compared to the unmown plot. The highest number of flowering plants was five per quadrat in the unmown plot recorded in December 2017 and at least one in the mown plot in February 2018. The number of flowering plants declined from December 2018 until there were no flowering plants in either plot in April 2018 (Figure 2.6). Overall, there was a significant difference in the number of flowering *C. macrocephalum* plants between the mown and unmown plots over the flowering months tested (P= 0.0111; Table 2.1). Flowering of *C. macrocephalum* occurred from December to March (4-month period) in the unmown plot with December being the peak month, whereas it occurred only from January to March (3-month period) in the mown plot with February being the peak month (Figure 2.6).

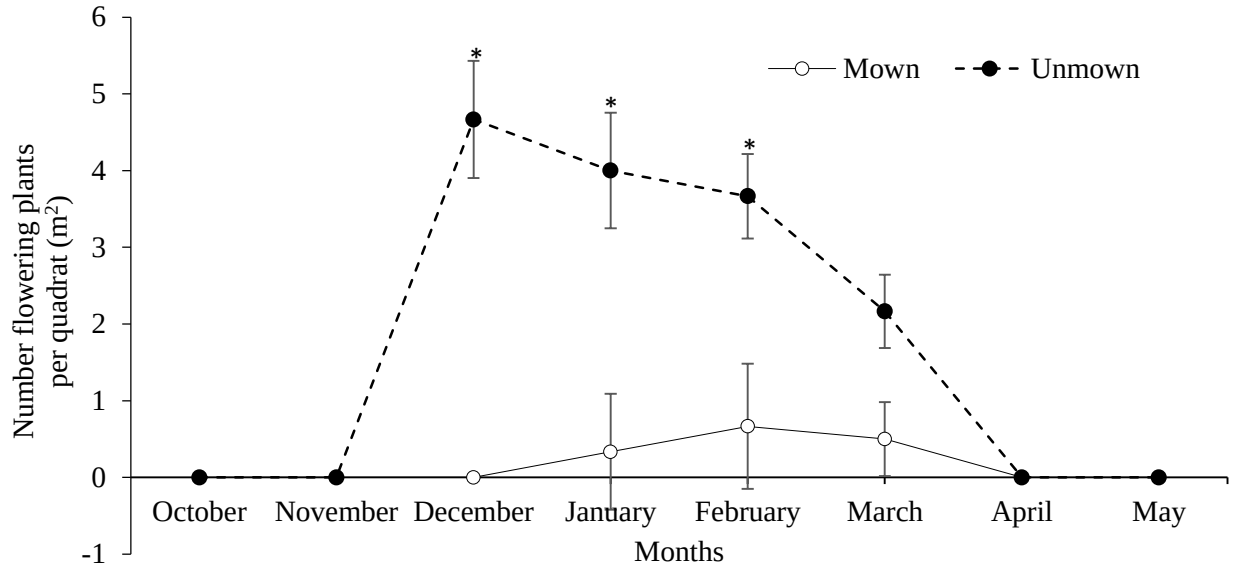


Figure 2.6: The effect of mowing on the number flowering of *Campuloclinium macrocephalum* per quadrat (1 m x 1 m) for mown and unmown plots from October 2017 to May 2018 (mean ± SE). For each data point n = 6 plants for each data point. Overall differences between the plots were assessed using a one-way repeated measures ANOVA (Table 2.1). Asterisks (*= P < 0.05) indicate significant differences between the mown and unmown plot for corresponding months.

The number of leaves per plant was relatively higher on the unmown plot compared to the mown plot. The number of leaves per plant increased from 28 (mown) and 36 (unmown) in October to 37 and 58 respectively in December 2017. After December, the number of leaves per plant decreased for both plots until the plants senesced by the end of May. Overall there was no significant difference between the two plots for the number of leaves per plant (Figure 2.7a) (P= 0.1578; Table 2.1).

The dry mass of leaves per plant was higher for the unmown plot compared to the mown plot (Figure 2.7b). There was no overall significant interaction in leaf dry mass between treatments (mown versus unmown) and time (months) (P= 0.0954; Table 2.1). The leaf dry mass increased from October until peak mass in January at 2 g and 1.5 g for the unmown and mown plots respectively (Figure 2.7b). The increase was followed by decreases in leaf dry mass from January until the plants senesced by the end of May 2018.

There was an increase in stem mass in the unmown plot compared to the mown plot (Figure 2.7c). The seasonal change of stem mass was significant (P< 0.001; Table 2.1). The change in mass of stems from the mown plot was minimal, while there was an increase in mass from October (0.38 g) to February (2.5 g) per plant, followed by a decrease as *C. macrocephalum* senesced (Figure 2.7c). The overall stem mass of *C. macrocephalum* in the mown plot was significantly lower than in the unmown plot (P= 0.0021; Table 2.1).

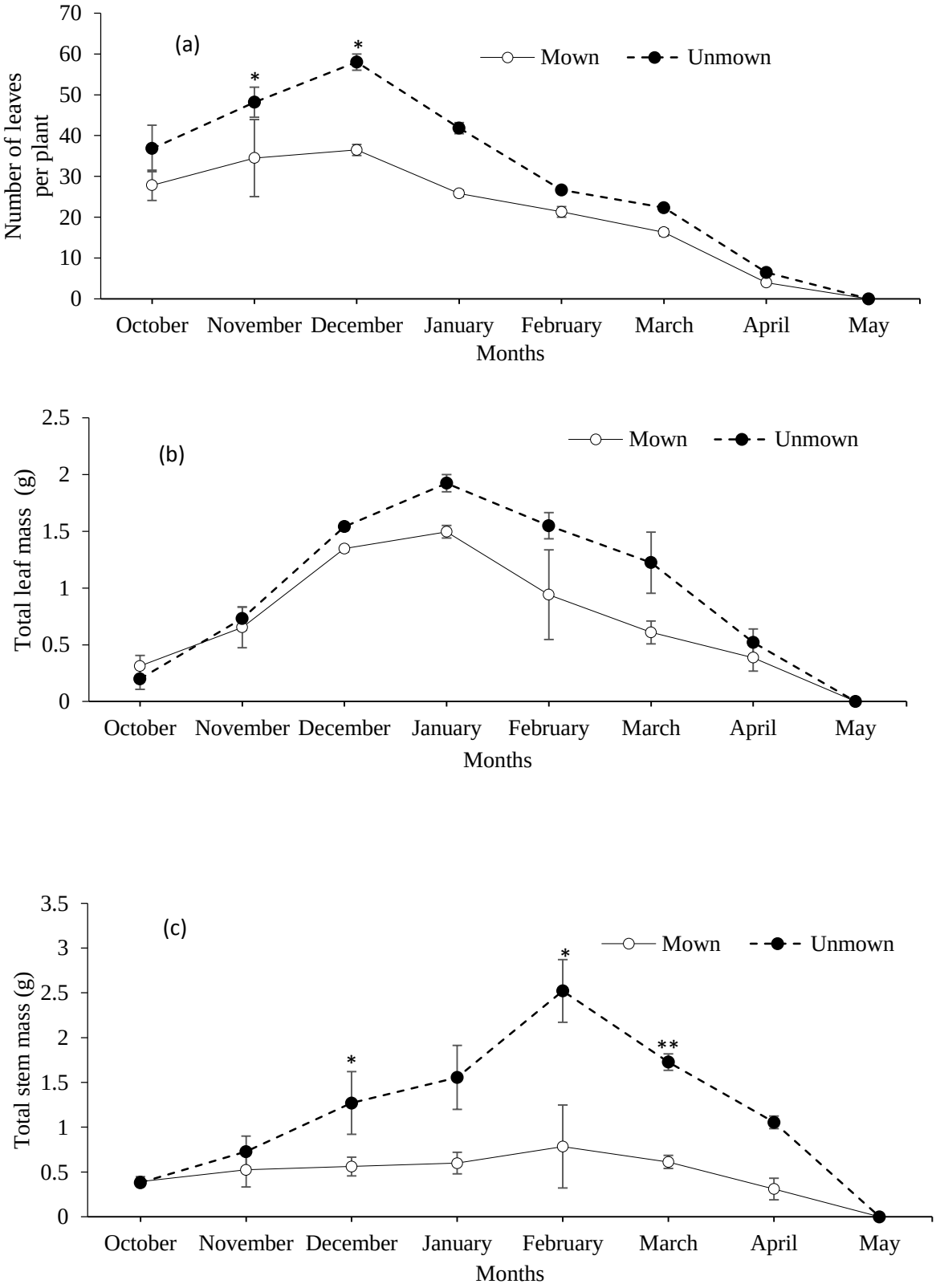


Figure 2.7: The effect of mowing on the number of leaves per plant (a), (b) leaf dry mass (b) and stem dry mass (c) from October 2017 to May 2018 (mean ± SE) on *Campuloclinium macrocephalum* plants compared to unmown plots. For each data point n = 6 plants for each data point. Overall differences between plots assessed using one-way repeated measures ANOVA (Table 2.1). Asterisks (*= $P < 0.05$, **= $P < 0.01$) indicate significant differences between the mown and unmown plot for corresponding months.

The relationship between the standing crop (biomass) and height of *C. macrocephalum* for the unmown plot (a) was significant (P= 0.0101) but relatively weak (r^2= 0.135) (Figure 2.8a). As the biomass in the plot increased due to seasonal change and more summer rains, the height of *C. macrocephalum* plants also increased. From about 4000 to 10 000 kg ha^{-1} the standing crop seemingly imposed a maximum limitation (limiting function: point where the growth or height was limited) on *C. macrocephalum*. The biomass/height relationship in the mown plot was not significant (P= 0.1697) and very weak (r^2= 0.041) (Figure 2.8b)

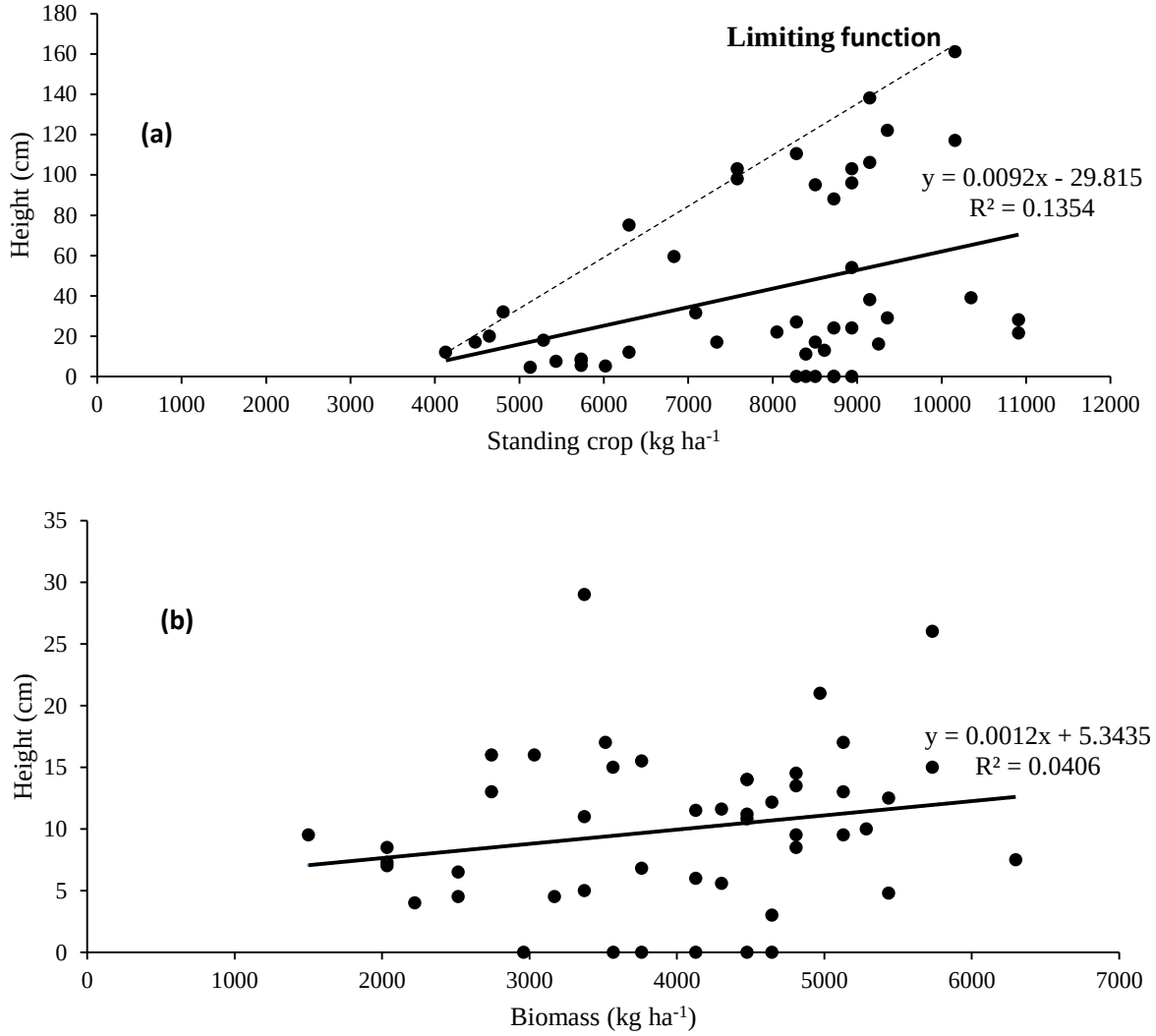


Figure 2.8: The relationship between the height of *Campuloclinium macrocephalum* and biomass (standing crop) of the herbaceous layer from an unmown (a) and a mown (b) plot from October 2017 to May 2018. n=48 (a) and n=48 (b) for *C. macrocephalum*.

There was a rapid increase in the number of adult *L. tractabilis* in the mown plot, from two per plant in October 2017 (early in the growing season) to a peak of 28 adults per plant in

December 2017 (summer), which was followed by a gradual decrease to May 2018 (Figure 2.9). In the unmown plot there was a small increase in the number of adults from October to January, followed by a decrease to April 2018, when no more adults were found above ground on plants in this plot. The seasonal change in the number of adults between the two plots was significant (P= 0.0118; Table 2.1). The highest number of adults per plant in the unmown plot was nine in January 2018 and 28 in December 2017 in the mown plot (Figure 2.9). By May, there were no *L. tractabilis* recorded above ground in either of the two plots because the *C. macrocephalum* plants had senesced (Figure 2.9). The predicted number of *L. tractabilis* generations for both the mown (8.469) and unmown (8.349) plots, between October 2017 and April 2018, was eight generations.

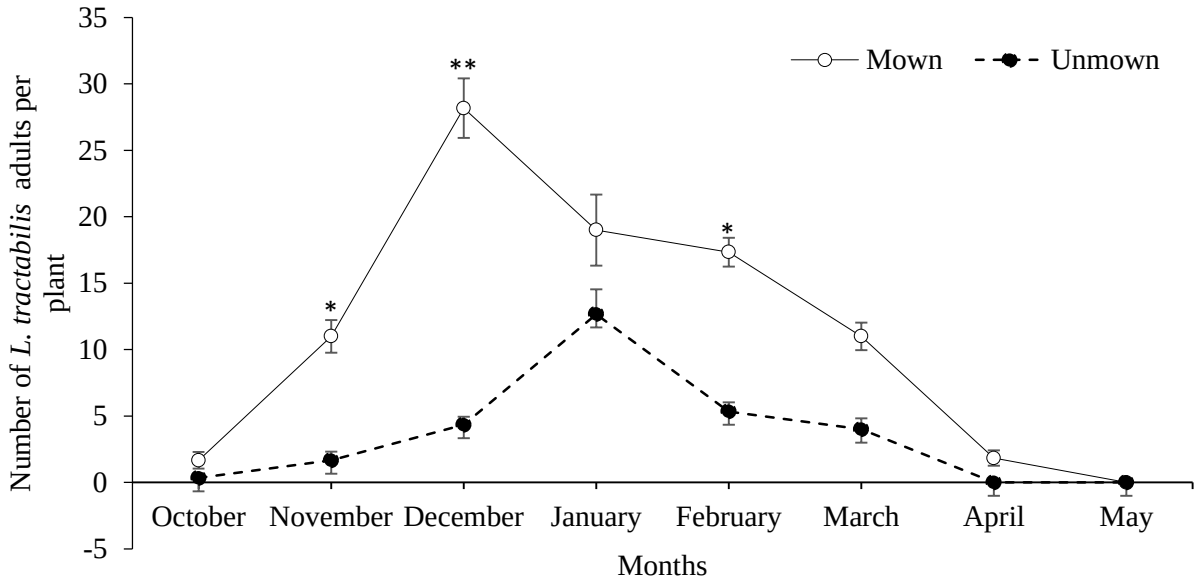


Figure 2.9: The effect of mowing on the number of *Liothrips tractabilis* adults per plant for mown and unmown plots from October 2017 to May 2018 (mean ± SE). For each data point n = 6 plants for each data point. Overall differences between the plots were assessed using a one-way repeated measures ANOVA (Table 2.1). Asterisks (*= P < 0.05, **= P < 0.01) indicate significant differences between the mown and unmown plot for corresponding months.

The highest number of nymphs per plant in the mown plot was 44 in December 2017 and 14 in the unmown plot in January 2018 (Figure 2.10). For both plots, the number of nymphs increased from October until the peak months, December 2017 (mown) and January 2018 (unmown). This was followed by a decrease, until there were no nymphs in April 2018 for plants above ground in the unmown plot. While nymphs were still present in the mown plot for the same time period, nymphs were absent on both plots in May 2018 (Figure 2.10). Overall there was no significant difference between the number of nymphs on the mown plot compared to the unmown plot (P= 0.0654; Table 2.1).

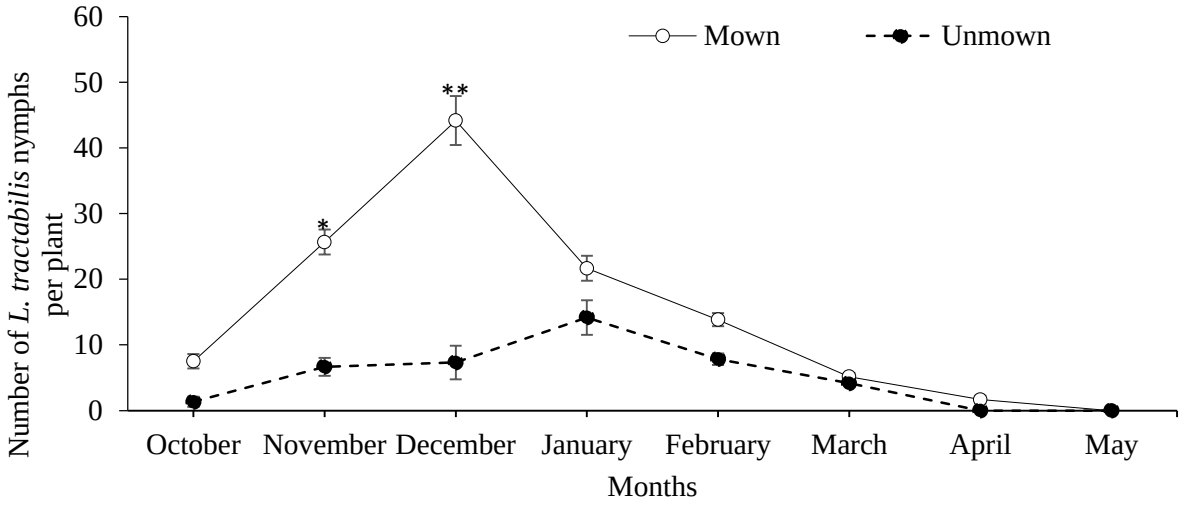


Figure 2.10: The effect of mowing on the number of *Liothrips tractabilis* nymphs per plant between mown and unmown plots from October 2017 to May 2018 (mean ± SE). For each data point n = 6 plants for each data point. Overall differences between plots were assessed using a one-way repeated measures ANOVA (Table 2.1). Asterisks (*= P < 0.05, **= P < 0.01 and ***= P < 0.001) indicate significant differences between the mown and unmown plot for corresponding months.

The mown plot had more *L. tractabilis* eggs per plant than the unmown plot (Figure 2.11). There was no overall significant difference in the number of eggs per plant between the mown and unmown plots over the tested months (P= 0.0921; Table 2.1). The highest number of eggs per plant was 48 for the mown plot, with only eight eggs per plant on the unmown plot, both in December 2017. After December, the number of *L. tractabilis* eggs decreased in both plots until April 2018 when no more eggs were recorded (Figure 2.11).

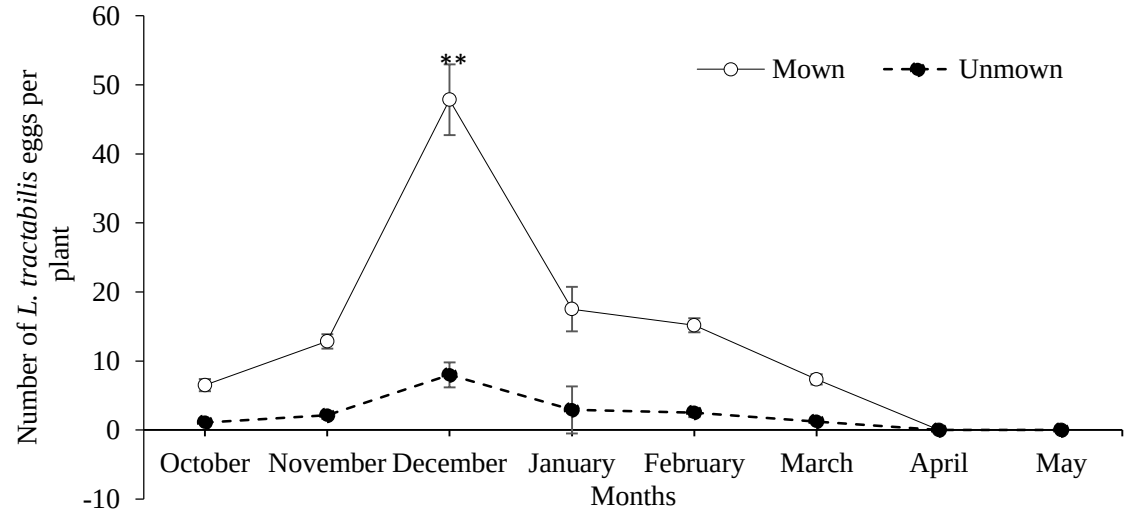


Figure 2.11: The effect of mowing on the number of *Liothrips tractabilis* eggs per plant on mown and unmown plot from October 2017 to May 2018 (mean ± SE). For each data point n = 6 plants for each data point. Overall difference between plots assessed using one-way repeated measures ANOVA (Table 2.1). Asterisks (**= P < 0.01) indicate significant differences between the mown and unmown plot during corresponding months.

2.4 Discussion

Mowing the grass resulted in *C. macrocephalum* in that plot being exposed to relatively higher maximum temperatures compared to the unmown plot. The maximum monthly temperatures in both plots were within the upper thresholds of *L. tractabilis*, but the mown plot had relatively higher temperatures which may have resulted in an increased rate of development of *L. tractabilis* (van Lenteren *et al*., 2006; Ramanand *et al*., 2017). Adults of *L. tractabilis* were more abundant (76%) in the mown plot compared to the unmown plot. The mown plot also had greater abundance of nymphs (74%) and eggs (86%) which meant that most adults of *L. tractabilis* laid eggs on the mown plot. Increased rates of development may have also shortened the duration of development from egg to adult (McConnachie *et al*., 2011). The greater abundance of *L. tractabilis* adults on the mown plot with higher temperature was similar to that seen in *Teleonemia Scrupulosa* (N. Venter, pers. comm.), *Stephanitis pynoides* (Trumbule and Denno, 1995), and *Corythuca arcuata* where the agents were more abundant on sites with relatively high temperature (Barber 2011). *Liothrips urichi* (Nakahara *et al.,* 1992) in open sunny environment resulted in greater impact on the host plant.

Despite the greater abundance of *L. tractabilis* adults in the mown plot, the predicted degree days by Ramanand *et al*. (2017) was sufficient for *L. tractabilis* to complete an estimated eight generations in both the mown and the unmown plots. Ramanand *et al*. (2017) predicted 4-8 generations per year in most parts of Limpopo, Mpumalanga, North West and Gauteng provinces as being possible. *Liothrips tractabilis* may have avoided extreme temperature in the mown plot by retreating within deformed parts of stems, which may have provided a buffered micro-climate (Muth *et al.,* 2008; Ruban, 2009). This was also evident as the eggs and nymphs of *L. tractabilis* were found predominantly on deformed parts of stem sections.

The average height of the main stems of *C. macrocephalum* was also significantly lower in the mown plot because the stems were deformed. *Liothrips tractabilis* have more negative impacts on new apical shoots and buds (McConnachie and McKay, 2015). As the plant develops in the presence of the thrips, instead of normal vertical growth, the stem grows facing downwards thus reducing the stem height. Due to the deformation of the stems, the degree of flowering in the mown plot was greatly reduced, with the agent feeding on leaf tissue primarily at the apical shoot tips (McConnachie *et al*., 2011), thus reducing seed

production and ultimately the spread of *C. macrocephalum* within the area (McConnachie and McKay, 2015).

Normally *C. macrocephalum* reaches an average height of 1.3 m by December (McConnachie *et al*., 2011). Plants were <50 cm on the mown plot, while most of the plants were 1.2 m in the unmown plot. As a result, the main stems of *C. macrocephalum* were longer and heavier in the unmown plot than those from the mown plot, since the stem height was reduced by 64%. Feeding damage by *L. tractabilis* resulted in *C. macrocephalum* plants in the mown plot having fewer leaves (41%) compared to the unmown plot. This may have resulted in less photosynthesis in plants on the mown plot which causes reduction in the growth of plants. Cowie *et al.* (2016) found that there was a reduction in the growth of *Solanum mauritianum* as a result of defoliation by *Gargaphia decoris* by feeding on the leaves of the host plant. Defoliation by the biocontrol agent, *Zygogramma bicolorata* on *Parthenium hysterophorus,* in Australia also reduced plant height, flower production and plant biomass (Dhileepan *et al.,* 2000). Defoliation of *C. macrocephalum* may have also contributed to the reduced height of stems and the proportion of plants in flower in the mown plot.

Results from this study are similar to those of Nakahara *et al.* (1992), where *L. urichi* was found to cause more damage and was more effective in controlling *Clidemia hirta* in open pasture land, than plants growing in shaded environments. *Teleonemia scrupulosa* was also found to cause greater damage to *Lantana camara* plants that were growing in full sun compared to those in shade (N. Venter pers. comm.). Other studies also found greater plant damage in high light environments (McGeoch and Gaston, 2000; Chacon and Armesto, 2006), and there was also greater herbivore damage in similar environments (Fortin and Mauffette, 2001; Levesque *et al.* 2002; Niesenbaum and Kluger, 2006).

The response of herbivores to host plants in direct sunlight versus plants in the shade is species specific with some insect species performing better on plants exposed to sunlight while others do better on those in the shade (Trumbule and Denno 1995; Bentz 2003; Barber 2010; Diaz *et al*. 2011). According to Shrewsbury and Raupp (2006) these trends are not necessarily only caused by light intensity or the quality of the host plant, but other factors such as predation and vegetation type around the host plant, which also play a role. The presence of *P. eupatorii* on *C. macrocephalum* may have contributed towards the results of this study, but its influence was not measured. In other words, no inferences can be made on

P. eupatorii as a contributing factor to the results obtained as pathogen infestations normally peak around March, which is towards the end of the growing season, when *C. macrocephalum* starts to dieback to its rootstocks (Goodall *et al.*, 2011). Further, anecdotal observations were made and found no difference in terms of leaf necrosis and abscission by *P. eupatorii* infestation on *C. macrocephalum* between the mown and the unmown plot.

In conclusion, mowing of grass promoted the abundance of adults, nymphs and eggs of *L. tractabilis* which resulted in greater deformation of the host plant, *C. macrocephalum*. The exact mechanism underlying this result is unknown at this stage. *Liothrips tractabilis* in sufficient numbers significantly reduced the proportion of plants flowering and the height of the host plants. As a result, the reduced proportion of plants flowering will potentially reduce the spread of *C. macrocephalum* over time. Therefore, early season mowing seems to be a potential management tool to enhance the impact of *L. tractabilis* on *C. macrocephalum*. Mowing roadsides that are invaded by *C. macrocephalum* should therefore be implemented before the start of the growing season. Future studies should consider measuring physiological parameters (chlorophyll, leaf quality, phenolic, nitrogen and water contents) of *C. macrocephalum* to further understand the different response of this weed when exposed to *L. tractabilis* in mown and unmown areas, or for plants in shaded or high light environment.

CHAPTER 3:

Which life stages of *Liothrips tractabilis* overwinter and what are the impacts on the fleshy rootstocks of *Campuloclinium macrocephalum* during the dormant season?

Abstract

Insects cope with harsh conditions through behavioural adaptations such as moving underground and possibly feeding on the underground tissues of plants. Diapause is considered a physiological adaptation for overwintering. Dry, hot spells or cold temperatures trigger insects to diapause. Diapause intervenes in a single developmental stage, either as adults, pupae or larvae, depending on the insect species. Under unfavourable conditions such as low temperatures, leaves on perennial plant species become senescent, usually in autumn, and then new leaves grow in the following spring. *Liothrips tractabilis* is a biological control agent on *Campuloclinium macrocephalum,* that feeds on stem and leaf tissue and apical shoot tips of the plant. When *C. macrocephalum* dies back to its rootstocks in winter, *L. tractabilis* moves underground only to reappear, when the weed regrows in spring (October). The aim of this chapter was to investigate which insect life stage of *L. tractabilis* overwinters underground and assess its impact on the fleshy rootstocks of *C. macrocephalum*. *Liothrips tractabilis* had no significant impact on the mass, thickness, length and number of roots of the host between biocontrol and control plants. As the winter progresses the thrips adults move progressively deeper into the soil from May to September, so in September when fires often occur in the Highveld, there are none at 0-2 cm, and 15 of the total 16 (94%) are below 4 cm. This most probably means that 94% of the thrips are safe from the typical September control burns. Thus, integrated control with fire and biocontrol should be trialled more thoroughly.

Keywords: Diapause, fire, integrated control, control life stage, root-feeding, underground

3.1 Introduction

Insects survive harsh conditions through various behavioural and physiological and behavioural adaptations. One behavioural adaptation includes moving underground, with some insects feeding on the rootstocks of plants (Jiang *et. al.* 2010; Terao *et. al.* 2012). Physiological adaptations of insects may include diapause as an overwintering tactic (Saunders, 2004). Diapause enables insects to adapt to changing environments (such as dry, hot spells or cold temperatures) and also increases their survival capacity (Irwin and Lee, 2000). It involves physiological changes such as lowered energy metabolism compared to the active state (Costal, 2006; Dingha *et al*., 2009). It intervenes in a single developmental stage, either the adult, pupae or larvae, in different insect species (Jiang *et. al.* 2010). Insects that diapause as adults must accumulate sufficient fat reserves before entering diapause, as this will be the main source of energy during diapause (Hahn and Denlinger, 2007).

Dry and hot spells may trigger diapause in tropical insects, whereas insects from temperate regions usually diapause in response to cold temperatures during winter (Irwin and Lee, 2000; Saunders, 2004). The initiation and termination of diapause is critical to the survival of some insects (Hahn and Denlinger, 2007). Prolonged diapause may result in insects missing mating opportunities or oviposition periods, while shortened diapause may lead to exposure to harsh conditions that can have adverse effect on the survival of the insects (Han and Bauce, 1998).

Inactivity by individuals during diapause may expose them to predation and depletion of stored energy reserves (Zhou *et. al.* 1995; Han and Bauce, 1998). Even though metabolism slows during diapause, it can draw heavily on energy reserves if it is maintained over an extended period (Jiang, *et. al.* 2010). Generally, in the case of pupal diapause, resources to overwinter are acquired as larvae before entering diapause (Ellers, *et. al.* 2002). Insects in diapause feed less frequently or do not feed at all (Hahn and Denlinger, 2007). Reduced metabolic rates results in higher overwintering survival by saving the already limited energy compared to the active state (Irwin and Lee, 2003; Piiroinen, *et. al.* 2011). The higher body mass and lower metabolic rates of the Colorado potato beetle (*Leptinotarsa decemlineata*) has been shown to be correlated with higher overwintering survival (Piiroinen, *et. al.* 2011).

Under unfavourable weather conditions such as low temperatures, leaves on most deciduous plant species will senesce (Lim *et al.*, 2007). In addition, all the above-ground tissue of some

plant species dies back to rootstocks during the winter (Zhu, *et. al.* 2000). Root-feeding by insect herbivores includes species of Coleoptera, Homoptera, Hymenoptera, Orthoptera, Diptera and Lepidoptera (Brown and Gange, 1990; Johnson and Gregory, 2006). Below-ground herbivory often has a greater impact on the vigour of plants, compared to damage by above-ground herbivory (Barber, *et. al.* 2011). Below-ground herbivory may also reduce food reserves such as carbohydrates (Dintenfass and Brown, 1988). Damage to root tissues by root-feeding insects can directly impact plant fitness and survival (Nunes and Kotanen, 2018). Below-ground herbivory has been shown to negatively affect water and nutrient absorption, synthesis of plant hormones and production of secondary chemicals (Dintenfass and Brown, 1988; Barber, *et. al.* 2000).

In a survey by Blossey and Hunt-Joshi (2003), 34% of the above-ground biological control agents that were released resulted in the suppression of alien invasive plants, while it was 54% for root-feeding agents. A root-feeding flea beetle, *Longitarsus bethae* was released in South Africa against *Lantana camara* to compliment the ineffective above-ground feeding agents (Simelane, 2010). The root-feeding flea beetle, *Longitarsus jacobaeae* (Coleoptera: chrysomelidae) was released in combination with leaf-feeding cinnabar moth, *Tyria jacobaeae* to successfully control *Senecio jacobaeae* L. (Asteraceae) in northern California (Pemberton and Turner, 1990). In agriculture, root feeding by *Diabrotica virgifera virgifera* (corn rootworm) (Coleoptera: chrysomelidae) was reported to have caused 40-50% yield reduction in maize (Godfrey *et al.*, 1993).

Liothrips tractabilis is a biological control agent that was released in 2013 against *C. macrocephalum* in South Africa (McConnachie and McKay, 2015). It feeds on stem and leaf tissue and on the apical shoot tips of the host plant (McConnachie *et al*., 2011). In late autumn (May) *C. macrocephalum* dies back to its fleshy rootstocks which results in *L. tractabilis* not having any above ground plant tissue to feed on. When *C. macrocephalum* senesces in winter, *L. tractabilis* was observed to apparently disappear and then resurface when the host plant regrows in spring (October) (McConnachie and McKay, 2015). *Liothrips tractabilis* retreats to the fleshy rootstock of the host plant until *C. macrocephalum* regrows in spring, it is currently unknown how deep in the soil *L. tractabilis* retreats and whether it has an impact on the roots over winter.

Understanding how deep below the soil surface *L. tractabilis* overwinters will also assist if fire is to be implemented to control *C. macrocephalum*. The soil surface temperature as a

result of grass fires can rise up to 600 ^{o}C, but within six minutes the temperature can go back to a normal temperature of at least 24 °C (Scotter, 1970; Rundel, 1981; Mbalo and Witkowski, 1997). A study by Masson (1949) recorded surface temperatures of 700 to 800 °C during a fire event, but only an increase of 14.4 to 34.4 °C at the 2 cm depth and found no significant increase in soil temperature at 3-4 cm depth. The ambient soil temperature was found to vary from 20 to 25.4 °C at 1 m depth (Scotter, 1970: Sharan and Jadhav, 2002). Hence soil has a great buffering capacity to heat transfer from the surface during vegetation fires. Grassland fires are common and play a vital role in ecosystem functioning (Little *et al.*, 2013). Fire has also been used to control invasive plants, whether they are started intentionally or accidentally.

Therefore, the aim of this chapter was to investigate the insect life stage of *L. tractabilis* that overwinter and to assess their impact on the fleshy rootstocks of *C. macrocephalum*. The three objectives were:

***Objective* 1.** Investigate which *L. tractabilis* life stages overwinters (adults, nymphs or eggs**).**
***Objective* 2.** Record how deep from the soil surface the *L. tractabilis* life stages are observed on the rootstocks.
***Objective* 3.** Assess the impact of *L. tractabilis* on the fleshy rootstocks of *C. macrocephalum*

3.2 Methods and Materials

Study site

The underground winter study was conducted at Waterkloof Airforce Base, Pretoria (25°48'55457"S, 28°13'27.68"E), during the southern hemisphere winter from May to early spring in September 2018. The vegetation of the site was dominated by grass including, *Cymbopogon caesius, Hyparrhenia hirta*, and *Eragrostis curvula*. Invasive plants within the area included *Lantana camara* and *Solanum mauritianum*. The region has severe frost in winter, summer rainfall and an overall mean annual precipitation of 593 mm. The area is classified as Carletonville Dolomite Grassland (Mucina and Rutherford, 2006).

Experimental design and protocol

To achieve the aim and objectives of the study, a plot of 10 m x 10 m was demarcated where *L. tractabilis* had already established. The *L. tractabilis* at the site was released in November 2014 and had made a significant impact on the target weed. A control plot (10 m x 10 m plot) with *C. macrocephalum*, but where *L. tractabilis* was absent was also sampled.

Field work

Each month from the beginning of winter (May) until the beginning of spring (September) 2018, six senesced plants were randomly selected on each plot and dug out monthly within a 1 m x 1 m quadrat. Soil samples were also collected within a 20 cm radius and 30 cm deep to avoid leaving eggs, nymphs or adults behind. After digging, each rootstock and its associated soil was placed in a container to ensure the soil remain compact to avoid disturbing the *L. tractabilis* life stages (eggs, nymphs and adults), and for further observation and analysis in the laboratory. In total 60 senesced plants were sampled, 12 plants for each month, six plants from each of the *L. tractabilis* present and *L. tractabilis* absent plots.

Laboratory work

Each rootstock was examined using a dissecting microscope (S6-BLED Stereo Zoom Dissecting Microscope) and a magnifying glass (Waltex pocket sliding magnifier 3x) for all *L. tractabilis* life stages (eggs, nymphs and adults). The depth from the soil surface where eggs, nymphs or adults occurred was measured using a plastic measuring tape. Other parameters that were measured include numbers of roots from the stem per plant, as well as thickness, length and dry mass of the roots. The thickness of the roots at the base of *C. macrocephalum* was measured using digital calipers, a plastic measuring tape was used to measure the length of roots. Roots were dried at 50 ^{o}C in an oven for 72 hours before they were weighed.

Statistical analysis

T-tests were used to test the monthly difference in the parameters measured between the plot with *L. tractabilis* and the one without (control). A one-way repeated measures ANOVA was used to compare the overall differences between the treatments (presence or absence of *L. tractabilis*) over time (monthly from May 2018 to September 2018) for each of the parameters measured. Analyses were conducted using SPSS Statistics 22.0, and Microsoft Excel 2016. The daily maximum and minimum temperatures were obtained from South African Weather Service (SAWS).

3.3 Results

There were a total of 86 adults and four nymphs of *L. tractabilis* found on the fleshy roots of *C. macrocephalum* during the dry season at different soil depth from the soil surface (Figure 3.1). Three of the nymphs where found in the 0-2 cm zone, while the remaining individual was found in the 2-4 cm zone. Adults were most frequently found at the 4-6 cm depth from

soil the surface (52% of individuals), followed by 24% 2-4 cm zone. The depth wi theth the least number of adults was at 8-10 cm (2% of individuals) (Figure 3.1).

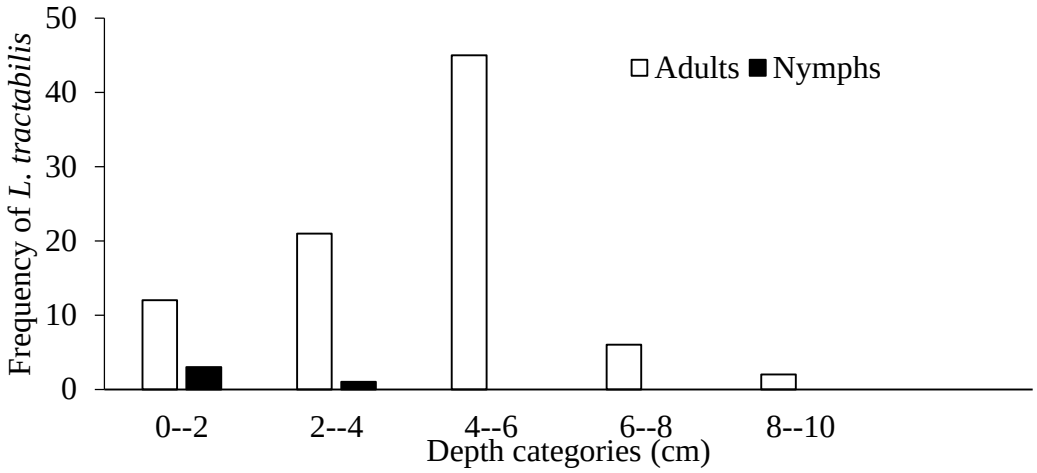


Figure 3.1: The frequency of *Liothrips tractabilis* (adults and nymphs) overwintering on roots of *Campuloclinium macrocephalum* in relation to different soil depths during the dry season (winter). n= 60 senesced plant. Eggs were not found. And individuals were not found deeper than 10 cm.

Adults of *L. tractabilis* were recorded at a depth of 0-2 cm at the beginning of the dry season in May and June, and two more adults were found in July (Table 3.1). As the dry season continued, adults were not found in the of 0-2 cm zone in August and September. As the temperature continued to drop (Figure 3.2) adults were found deeper in the soil profile. Three of the highest numbers of adults recorded were 15, 9 and 11 at a depth of 4-6 cm in July, September and August respectively. Only two adults were found at a soil depth of 8-10 cm.

Table 3.1: Number of *Liothrips tractabilis* (adults) overwintering on roots of *Campuloclinium macrocephalum* in relation to different soil depths during the dry season (winter) from May to September 2018. n= 86 adults. Only three nymphs were found in May 2018. *Liothrips tractabilis* was not found deeper than 10 cm.

Depth categories (cm)	**Months**				
	May	**June**	**July**	**August**	**September**
0--2	6	4	2	0	0
2--4	4	8	5	3	1
4--6	3	7	15	9	11
6--8	0	0	0	4	2
8--10	0	0	0	0	2
Total	**13**	**19**	**22**	**16**	**16**

The growing season of *C. macrocephalum* start in late October when the average temperatures are higher (Figure 3.2). The growing season continues to the end of April when the temperature starts declining again. The mean maximum temperature dropped from 25.7 °C in April to 20.1 °C in July (which was the coldest month of the year) with the mean minimum temperature at 5.1°C. Initiation of diapause by the adults occured in May when the host plant senesced. The host plant grows back from roots stocks in October, resulting in *L. tractabilis* resurfacing.

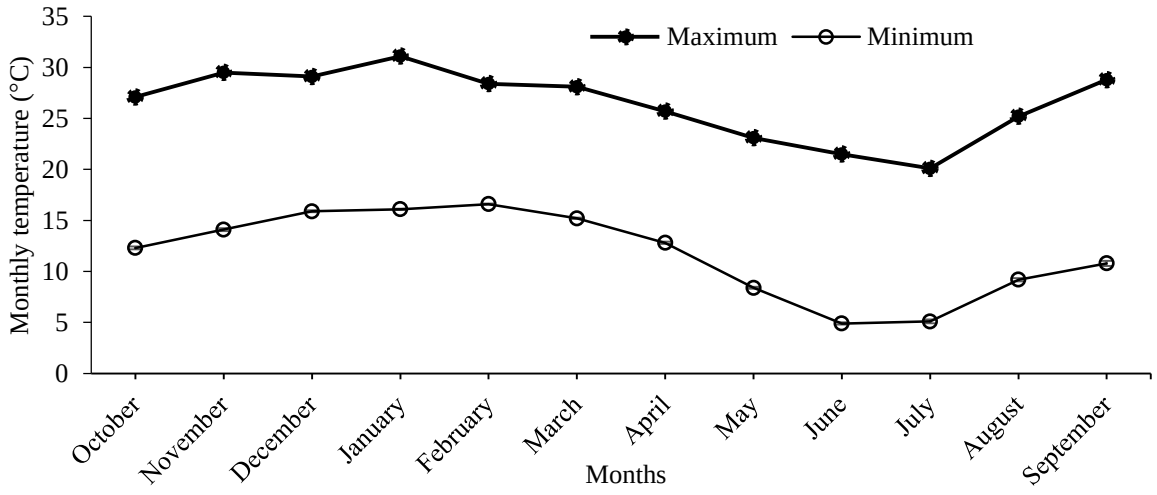


Figure 3.2: Monthly mean maximum and mean minimum temperature (^{o}C) for Pretoria, South Africa from October 2017 to September 2018 (mean ± SE).

There was no significant difference between the number of roots (P= 0.4133; Table 3.2), length (P= 0.6572), diameter (thickness) (P= 0.8784) and mass (P= 0.7214) of roots that were exposed to *L. tractabilis* and the control plants over the tested months (Figure 3.3). Adults of *L. tractabilis* had no significant impact on the mass, thickness, length and number of roots of the host plant.

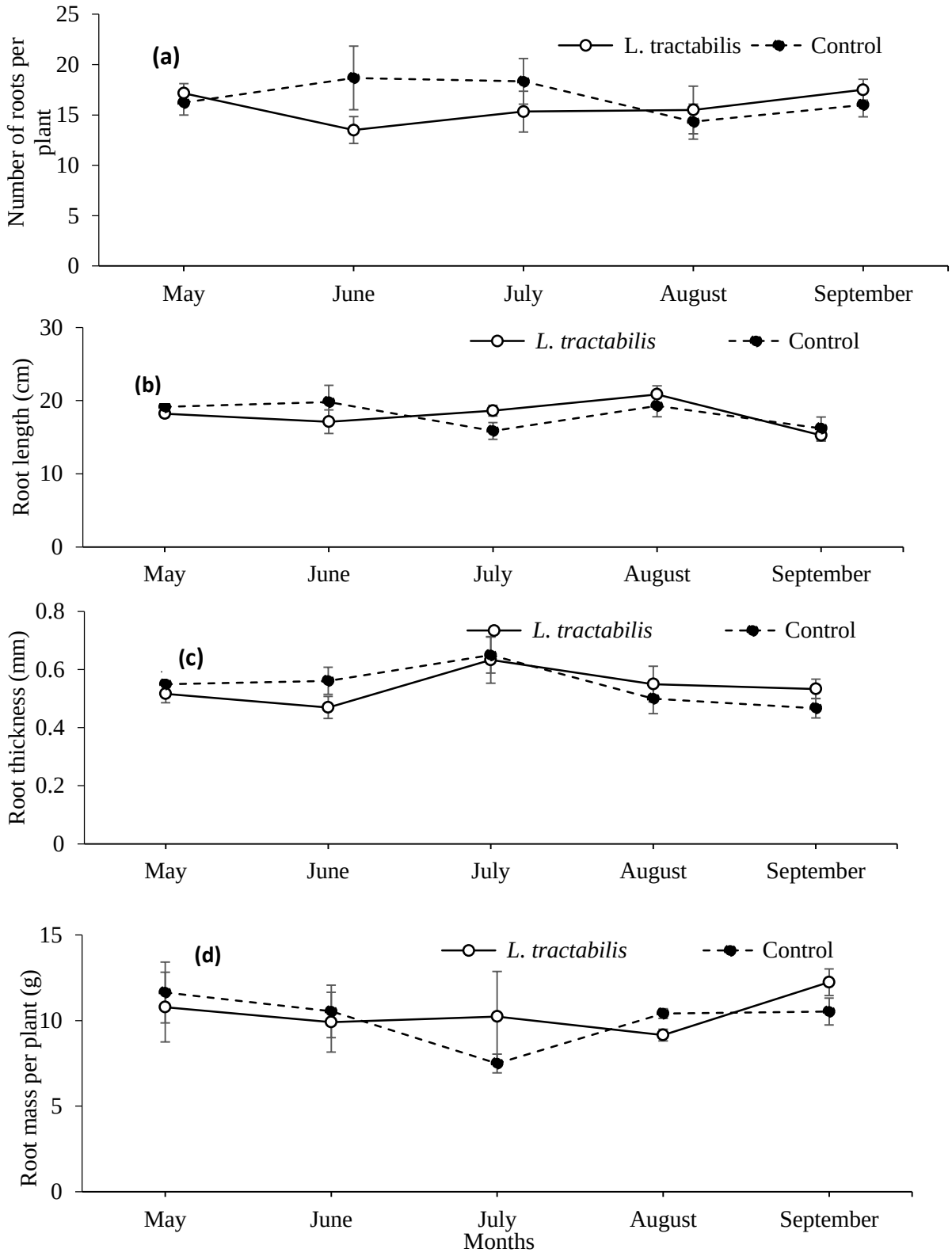


Figure 3.3: The effect of overwintering *Liothrips tractabilis* on the number of roots (a), and the length (cm) (b), thickness (mm) (c) and mass (d) of *Campuloclinium macrocephalum* roots from May to September 2018 (mean ± SE). For each data point n = 6 plants for each data point. Overall differences between the treatment and control samples over time were assessed using a one-way repeated measures ANOVA (Table 3.2).

Table 3.2: Results of a one-way repeated measures ANOVA of *Campuloclinium macrocephalum* variables measured for plants exposed to overwintering *Liothrips tractabilis* and control plants without *L. tractabilis* from May 2018 to September 2018. P-values in bold indicate significant difference.

Variable	**Treatment**			**Time**			**Treatment * Time**		
	F	d.f	P	F	d.f	P	F	d.f	P
Average number of roots per plants (Figure 3.2)	0.7287	1, 10	0.4133	0.2489	4, 40	0.9086	1.2577	4, 40	0.3025
Root length (Figure 3.3)	0.209	1, 10	0.6572	2.917	4, 40	**0.03301**	1.081	4, 40	0.3787
Root thickness (Figure 3.4)	0.025	1, 10	0.8784	2.513	4, 40	0.05668	0.818	4, 40	0.5214
Root mass (Figure 3.5)	0.1344	1, 10	0.7215	1.0261	4, 40	0.4056	0.7438	4, 40	0.5679

3.4 Discussion

Mainly adult *L. tractabilis* were found to be overwintering during the cold and dry winter season on the roots of *C. macrocephalum*. Adults were predominantly (52% of the total) found between 4-6 cm below the soil surface on the roots of *C. macrocephalum*. *Liothrips vaneeckei* (Priesner) overwintered as adults on Lilium species in the United Kindom; overwintering depth and feeding on the host plants were not reported (Morison, 1957). Overwintering without feeding on host plants may also be the case with other *Liothrips* species such as *L. mikaniae*, *L. urichi* (Mound *et al.* 2016), and *L. reuteri* (Bagnall) (Thysanoptera: Tubulifera), collected on *Tamarix* from Mandali (Diyala Province) (Mirabbalou, 2016). Adult Colorado potato beetles (*Leptinotarsa decemlineata*) were found much deeper at 20-60 cm below the soil surface during cold snowy conditions (Lefevere and De Kort, 1989; Milner *et al.*, 1992), they may have dug deeper than *L. tractabilis* in order to avoid the snow. There were three nymphs of *L. tractabilis* that were found within the first 4 cm below the surface during the early overwintering period (May) on the roots of the host plant. This was unusual because in insects, it is typically a single developmental stage that goes into diapause (Terao *et. al.*, 2012). However, as the winter season progressed, the nymphs were not found from June to early September before regrowth of *C. macrocephalum*. The late presence of nymphs may have been due to delayed senesce of some plants.

The trigger for adult *L. tractabilis* to overwinter may be the dry and cold temperatures during the southern hemisphere winter. Dry and cold temperatures also resulted in *C. macrocephalum* dying back to its rootstocks, which may have also contributed to the initiation of diapause of adult *L. tractabilis*. Termination of diapause of *L. tractabilis* happens in the beginning of spring (October) when the temperatures starts to increase and *C. macrocephalum* regrows from the rootstock (Goodall and Witkowski, 2014). When *C. macrocephalum* tissues regrows *L. tractabilis* also resurfaces.

Overwintering of *L. tractabilis* adults had no significant impact on the below ground biomass of their host plant, *C. macrocephalum*. This is consistent with other overwintering species such as *L. vaneeckei* where it only significantly impacted the above-ground biomass (Morison, 1957). During diapause insects feed less frequently or not at all (Han and Denlinger, 2007), which may explain why there was no significant impact of *L. tractabilis* on the biomass of *C. macrocephalum*. There was also no alternation of feeding sites where one life stage feeds on the above-ground organs while another feeds on below-ground organs. For *L. tractabilis*, regardless of the data indicating that adults overwinter underground; adults and nymphs only feed on the above ground tissue of *C. macrocephalum* during the summer period. The root-feeding flea beetle, *L. bethae*, exhibits an alternation of feeding sites, where adults fed on the leaves, while larvae feed on the roots of *L. camara* (Simelane, 2005). Similarly, biological control agents reviewed in literature that feed on below ground biomass did not also feed on the above ground biomass (Brown and Gange, 1990; Pemberton and Turner, 1990; Spike and Tollefson, 1991), therefore the behaviour displayed by *L. tractabilis* is not different from that displayed by other biological control agents.

The use of fire can reduce the seed banks of invasive plant species (Witkowski and Wilson 2001; Dew *et. al.*, 2017) and could possibly also reduce the seeds on the surface of *C. macrocephalum*. *Liothrips tractabilis* may not be adapted for fire- driven ecosystems meaning fire may be incompatible. It is important to consider intensity and scale of fire (Briese, 1996), given the depth at which *L. tractabilis* overwinters, before it is integrated with biological control. Weed density was greater in areas that had a grass basal cover <19% (Goodall *et. al.*, 2011). The use of fire may result in the basal cover being reduced to less than 19%, which can further promote the density of *C. macrocephalum* during the subsequent growing season of the weed. In chapter 2, *L. tractabilis* was found to have greater impact on the target weed in a plot that had a lower standing crop (biomass), therefore reduced basal cover may also favour the agent in sites where it has established. The use of fire is therefore

not advisable in areas where the biological agent has not been released and established, as that will exacerbate the density of *C. macrocephalum*. It is also important to ascertain if fire harmful to agents in grasslands during the dry season.

Unlike forest fires, grasslands fires are less intense and much more brief due to low fuel load (Scotter, 1970: Fuhlendorf and Engle, 2004). Based on studies by Masson (1949) and Tothill and Shaw (1968) on soil temperature under grass fires, they found little or no significant increase in the soil temperature at 3-4 cm depth, and only a small increase of 14.4 °C at 2 cm to 34.4 °C. The increase in temperature at 4-6 cm depth will be less than 14.4 °C. Given the ambient temperature which varies from a minimum of 20 °C in grasslands (Scotter, 1970; Sharan and Jadhav, 2002), this may result in a maximum temperature of up to 34.4 °C at 3-4 cm depth, while at between 4-6 cm the maximum temperature will be lower due to the vertical transfer of heat. This suggests that *L. tractabilis* will likely not be killed during the implementation of fire, because thrips were more frequent at the 4-6 cm depth. Even at 2 cm depth, thrips may not be affected because they can develop at temperatures of up to 32 °C. According to Ramanand *et al*. (2017) temperatures above 32 °C for a few hours will not be lethal for *L. tractabilis*. Determining the LT_{50} of *L. tractabilis* would further assist with determining the potential impacts of fire on the species, especially if the intensity of the fire will be higher than 34 °C below ground. The most ideal time to burn invaded sites would be in either August or September as most of the adults were at least 2 cm below the soil surface and only few adults are most likely to be affected by fire. Fifteen of the total 16 were below 4 cm in September, this most probably means that 94% are safe from the typical September control burns.

Agents that require underground organs of *C. macrocephalum* to complete their life-cycle must be in order to complement the generally effective *L. tractabilis* that impact on the above ground biomass. Successful control of *S. jacobaeae* was because of the release of both root-feeding and leaf-feeding agents (Pemberton and Turner, 1990). Therefore, complimenting *L. tractabilis* with the release of a root-feeding agent may result in the successful control of *C. macrocephalum*.

CHAPTER 4:

General discussion, conclusion and recommendations

The main purpose of this study was to investigate the impact of *L. tractabilis*, a biological control agent, on the growth and reproductive potential of *C. macrocephalum* in South Africa. This chapter summarizes the main findings of the effect of early season mowing on the feeding performance of *L. tractabilis* on *C. macrocephalum* (Chapter 2), underground overwintering and impact on rootstocks/roots (Chapter 3) (Figure 4.1a). Implication of the findings on the biological control of *C. macrocephalum* are also discussed

The invaded range of *C. macrocephalum* in South Africa continues to expand (Henderson and Wilson, 2017) and remains one of the most invasive perennial forbs that threatens the grassland in South Africa (McConnachie and McKay, 2015). McConnachie and McKay (2015) found that *L. tractabilis* causes significant damage to *C. macrocephalum*. In this study early season mowing of grass was found to improve the impact of *L. tractabilis*. In areas where *L. tractabilis* has successfully established, mowing of the vegetation before *C. macrocephalum* regrows resulted in fewer flowering plants (Figure 4.1b), which may reduce the spread of the weed.

Plants in the mown plot were exposed to increased temperatures compared to the unmown plot. This resulted in a greater abundance of *L. tractabilis* (adults, nymphs and eggs) and resulting in greater impact on *C. macrocephalum* due to increased rates of development (Ramanand *et al*., 2017). Light intensity and temperature also affect plant quality (Barber and Marquis, 2011), insect-plant interaction (Roberts and Paul, 2006; Venter *et al.*, 2013), and increased levels of temperature also make plants more susceptible to overheating (Schymanski *et al.*, 2013). Further studies should consider investigating whether *C. macrocephalum* quality in sun and shade has an impact on the *L. tractabilis- C. macrocephalum* interaction.

Mowing may be ideal for restricted areas such as roadsides that are invaded, but the approach may be too costly and unrealistic for larger areas such as game reserves. Therefore, the use of fire before *C. macrocephalum* regrows should be considered as an alternative management tool to reduce the vegetation cover on for more widespread areas that are invaded. Adults of *L. tractabilis* were found to progressively move deeper into the soil, by September none were at 0-2 cm, and 94% were below 4 cm. Thus, integration of fire and biocontrol should be

considered. Before fire is implemented, the simplified mathematical model by Rose (1966) that describes vertical heat transfer must be used to determine the different soil temperatures at different depths that assists in determining whether the biological control agents with a soil dwelling stage will be negatively affected by fire. Fire may also result in the reduction of the seed bank of *C. macrocephalum*, and this should be more thoroughly explored. A simplified mathematical model by (Rose 1966) that describes vertical heat transfer beneath the soil surface can be used to estimate temperature at different soil depths. The equation is:

$$\frac{\partial v}{\partial t} = \kappa \frac{\partial^2 v}{\partial z^2}$$

where *v* is the temperature, *t* is time, *k* is the thermal diffusivity, and *z* the depth.

Puccinia eupatorii a pathogen biological control agent to manage *C. macrocephalum* and that established in most parts of South Africa (Goodall *et. al.*, 2012). The effectiveness of *P. eupatorii* at Waterkloof Airforce Base, Pretoria South Africa was not quantified during the 2017/2018 growing season. Future studies should consider quantifying its effectiveness and whether more releases of *P. eupatorii* are required. The rust has been shown not to have significantly reduced the realised niche of *C. macrocephalum* in savannas, grasslands and wetlands (Goodall *et. al.*, 2012). However, further studies should consider investigating factors that could enhance the impact of to *P. eupatorii*. This may assist in finding areas that are more suitable for *P. eupatorii* that may result in significant impact on *C. macrocephalum*.

The overwintering adults of *L. tractabilis* had no apparent effect on the rootstocks of *C. macrocephalum* compared to when they are on the aboveground biomass. A complimentary agent that will feed on the rootstock of *C. macrocephalum* to maximise the efforts to control the weed is needed. Root-feeding agents have been found to have greater impacts on plant health and vigour than above ground herbivory (Barber, *et. al.* 2011). One of the reasons why there are currently only a few root-feeding agents used as biological control agents worldwide, is that host-specificity tests are not always conclusive due to difficulties in manipulating and observing potential agents during laboratory host range studies (Blossey and Hunt-Joshi, 2003). Therefore, new techniques of testing the host specificity of root-feeding agents should be developed e.g. the use of root observation chambers (Witkowski, 1991b; Wilson and Witkowski 1998). Overall *L. tractabilis* is an effective biological control agent during the growing season, but ineffective during winter. Therefore, an additional root-feeding agent would of great values in controlling *C. macrocephalum*.

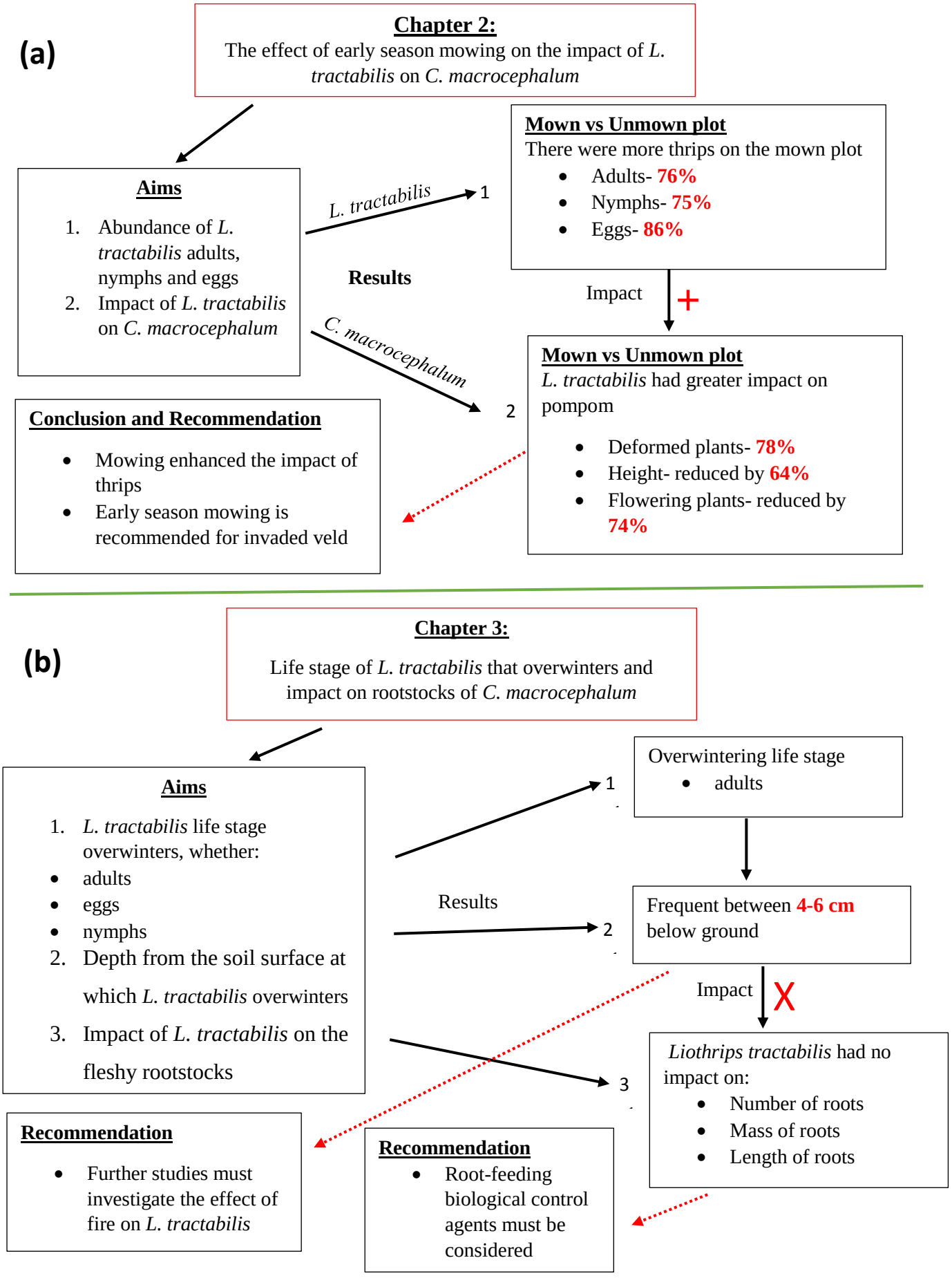


Figure 4.1: Summary of the findings and recommendations from this research study investigating the impact of early season mowing on the impact of *Liothrips tractabilis* on *Campuloclinium macrocephalum* (a); and the life stage of *L. tractabilis* that overwinter and the impact of these on rootstocks of *C. macrocephalum* (b).

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Link to Figure 4:

http://www.waterbergnatureconservancy.org.za/downloads/Waterberg%20Alien%20Invaders%201%20-%20pom%20pom.pdf