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Integrated management of Campuloclinium macrocephalum (pompom weed) (Less.) DC. (Asteraceae) in South Africa

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Integrated management of *Campuloclinium macrocephalum* (pompom weed) (Less.) DC. (Asteraceae) in South Africa



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PhD

2024

Declaration

I declare that this Thesis is my own work. It is being submitted for the Degree of Doctor of Philosophy 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.



October 2024

Supervisors:

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Dr. Jeremy Goodall (Agricultural Research Council- Plant Protection Research)

Dedication

This PhD thesis is dedicated to my family particularly my dad (Takalani Mudau), mom (Gladys Mudau), Madie “Mangie” Mbayela and my brother Tumelo Mudau.

Thank you for your support.

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I would like to thank my supervisors Prof. Marcus J. Byrne, Prof. Ed T.F. Witkowski and Dr. Jeremy Goodall for the constructive comments, continuous input and advice throughout my PhD journey. I am also grateful to Ms. Liame van der Westhuizen for all the advice and support whenever I needed some guidance and input.

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To Sipho Mbonani this journey would have not been possible without the support and collaboration with you, thank you for everything bafo. I would also like to thank members of the B17 lab for their support and company. To Lucas Mlambo, thank you so much for encouraging me to keep going when I felt tired along the way. I would also like to thank Madie “Mangie” Mbayela for the support and technical assistance she provided when compiling my PhD.

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To my mom (Magle) and dad (Skhulu), thank you for the support and for always encouraging me to keep pushing and working hard**.**

Abstract

Campuloclinium macrocephalum (Less.) DC. (Asteraceae) is an invasive perennial forb native to Central and South America and known as pompom weed in South Africa. It has since naturalized and poses a threat to grasslands, wetlands and savannas in many parts of South Africa. The weed is listed as category 1b of NEMBA (National Environmental Management Biodiversity Act), an invasive species which must be controlled and wherever possible, removed and destroyed. It causes significant reduction in grazing capacity of farms and game reserves, as it is unpalatable to livestock and wildlife. The weed has a clustered thick tuber-like root system that enables it to withstand fire and harsh winter conditions such as low rainfall and frost. Pompom has increased its invasion range by 671% between 2006 and 2016. Management strategies to date, such as the application of chemical, fire, mechanical and biological control have not yielded substantial results, hence the increase in invasion range of *C. macrocephalum*.

Application of herbicides alone requires multiple annual applications which may not be feasible, logistically or financially. It is also not ideal to continuously apply herbicides in sensitive environments such as areas that are close to streams, wetlands and dams. Three annual applications of metsulfuron methyl and picloram alone were found to suppress the abundance of *C. macrocephalum* but failed to control the weed. Mechanical control of *C. macrocephalum* through hoeing or digging is ineffective as damage to rootstocks by splitting them leads to further vegetative growth resulting in an increase in pompom density. Multiple biological control agents are often released against a single invasive plant to improve the degree of control. There are three biological control agents released against *C. macrocephalum* in South Africa namely, *Liothrips tractabilis* Mound and Pereyra (Thysanoptera: Phlaeothripinae), *Puccinia eupatorii* (Pucciniales: Pucciniaceae) and a flower-feeder, *Cochylis campuloclinium* Brown (Lepidoptera: Tortricidae). Damage inflicted by *L. tractabilis* and *P. eupatorii* is moderate, and the degree of control provided by the two agents has not resulted in sufficient control of the weed.

A strategy of combining different control methods such as chemical, mechanical, fire or biological control can improve the impact and effectiveness of any combination of the four methods and improve the degree of control of a particular weed. Integrated management of invasive weeds vary to suit a target plant based on invaded habitat, existing control method and management objectives. The first aim of the study (Chapter 2) was to evaluate how burning

alone prior to the growing season of *C. macrocephalum* and burning alongside herbicide application affects the weed density, regrowth/re-sprouting from rootstocks, flowering density and the seedbank of the weed over two growing seasons at Rietvlei Nature Reserve, Gauteng Province, South Africa. The seedbank was estimated by quantifying the number of newly emerging seedlings on each experimental block. During the first growing season (2020-2021) fire in August significantly reduced the seedbank of pompom by over 85% but failed to reduce the density of mature plants within the invaded areas. Herbicide application in December 2020 post fire of the first growing season resulted in over 98% reduction of the weed density during the first growing season. For the second growing season (2021-2022), there was a 9% regrowth from rootstocks of the weed on experimental blocks previously treated with fire and herbicide, which can be managed by a follow up application of herbicide. Integrating fire and herbicide was found to be a successful management strategy and it is therefore recommended as a management approach for the control of pompom weed in South Africa.

The impact of *L. tractabilis* is variable at different sites invaded by *C. macrocephalum*. This is because *L. tractabilis* has established in some open grasslands and failed to establish in more mesic environments particularly seasonal wetlands. A sub-lethal dose of herbicide can be used to suppress the reproductive ability of the weed without killing the plant and sustain the biocontrol population. Therefore, the second aim (Chapter 3) was to identify sub-lethal doses of three herbicides (picloram, impala and metsulfuron methyl) that will not kill but suppress the vegetative growth of *C. macrocephalum* and sustain the *L. tractabilis* population. However, the identified sub-lethal doses of the three herbicides were found to be incompatible with the agent *L. tractabilis*, because of an indirect impact on their feeding sites (young and apical stem sections) which affected the abundance of the agent. Due to lack of feeding sites, *L. tractabilis* was found to feed on old stems which are the least preferred sites. Consequently, considering the indirect impact of sub-lethal doses on *L. tractabilis*, the use of such doses is not recommended given the incompatibility.

In a study in 2018, early season mowing of surrounding vegetation, led to enhanced effectiveness of *L. tractabilis* on pompom weed, resulting in increased levels of stem deformation by the agent. *Campuloclinium macrocephalum* in more sunny vegetation (areas with mown short vegetation) experienced increased light intensity and temperatures compared to areas with

unmown long vegetation (more shady sites). However, it remains unclear how the physiology and growth rate of *C. macrocephalum* were affected by the difference in light intensities and temperature and how subsequent changes in plant growth and ecophysiology affected the biocontrol agents. The third aim of this study (Chapter 4) was to evaluate the influence of two light intensities (full sunlight and reduced sunlight) on chlorophyll content, chlorophyll fluorescence, stomatal conductance, and growth of *C. macrocephalum*, and to assess the reproduction and feeding impact of *L. tractabilis* in a laboratory trial. Reduced light intensity (shade plants) resulted in higher chlorophyll content, chlorophyll fluorescence, stomatal conductance, growth rate and chlorophyll fluorescence than in sun plants suggesting increased photosynthetic rates. This resulted in a significant increase in growth rate, with greater height of *C. macrocephalum* in the shade (108 cm) compared to in the sun (72 cm) over seven weeks. The feeding performance and abundance of *L. tractabilis* was significantly higher in open sun plants. This suggests that light intensity plays a critical role in the performance and establishment of the thrips. Therefore, mowing or burning the surrounding vegetation prior to and post release of the thrips may aid establishment and improve efficacy of the agent.

Liothrips tractabilis is known to prefer young apical shoot stems compared to more mature and old stems. To understand why *L. tractabilis* preferred young stems and plants in short vegetation, the fourth aim of the study (Chapter 5) was to evaluate and compare the nutritional quality of young apical stems compared with old stems (mid-section) of *C. macrocephalum* that grew in long and short vegetation by assessing the nitrogen and carbon levels and C:N ratio. Nitrogen content was greater in apical stems than in mid-section stems from short vegetation, suggesting better nutritional quality for *L. tractabilis* compared to plants from long vegetation. Therefore, the surrounding vegetation has an impact on the nutritional quality of *C. macrocephalum* which affects the performance and population size of *L. tractabilis*. In conclusion, neither fire alone nor herbicide alone significantly reduce the population density of pompom weed, but the application of lethal herbicide to *C. macrocephalum* after fire proved to be the most effective control method.

Keywords: biological control, fire, herbicide, integrated management, light intensity, plant density, plants quality.

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Chapter 1
General introduction

1.1 Invasive alien plants

Biological invasions are the second biggest threat to global biodiversity after habitat loss, (Simberloff *et al.,* 2013; Downey and Richardson, 2016). Alien invasive plants contribute considerably to the global problem of biological invasions which negatively affect ecosystem services and functioning (Blackburn *et al.*, 2014; Flory *et al.*, 2015; Pyšek *et al.*, 2020). They disrupt fire regimes (Gaertner *et al.*, 2014), nutrient cycling (Ehrenfeld, 2010) hydrological cycles (Mugwedi *et al.,* 2015) and displace native indigenous vegetation (van Wilgen *et al.*, 2008; Downey and Richardson, 2016). Disturbances such as habitat transformation and habitat fragmentation increase the invasion rate by creating habitats for alien plants invasions worldwide (Culliney, 2005; Faulkner *et al.,* 2016).

Some of the accidental alien plant introductions are as a result of increased trade, international air travel, human population increase and emigration (Faulkner *et al.,* 2016). Other invasive plants were deliberately introduced either as ornamentals, crops, agroforestry species or fodder hedge plants (Mgidi *et al*., 2007). Hundreds of invasive alien plants that have established in South Africa are native to Australia or South and Central America (Zimmermann *et al.,* 2004). The most vulnerable ecosystems to invasion by alien plants include grassland habitats, disturbed areas, riparian habitats and water ways (Downey and Richardson, 2016). Henderson and Wilson (2017) reported 773 alien plant taxa, which was an increase of 172 from 2006 in South Africa. Invasive plants that displayed the greatest increase in range include *Cylindropuntia fulgida* var. *mamillata* Backeb (Cactaceae), *Opuntia engelmannii* (Cactaceae), *Parthenium hysterophorus* L. (Asteraceae) and *Campuloclinium macrocephalum* (Less.) DC. (Asteraceae) (Henderson and Wilson, 2017).

1.2 Management of invasive plants

Once invasive plants establish and increase their range it is not feasible to eradicate or manage them without management strategies/techniques that will control and reduce their invasion rate (DiTomaso, 2000; Hajek and Eilenberg, 2018; van Kleunen *et al.,* 2020). Principal methods used to control and manage alien invasive plants include chemical, mechanical and biological control, and integrated control which is a combination of a number of these methods (Goodall *et al*., 2011; DiTomaso *et al*., 2017; Hajek and Eilenberg, 2018).

1.2.1 Mechanical control

Mechanical control focuses on physical damage to the invasive plants in order to reduce the growth and invasion range (Cloutier *et al*., 2007; Witkowski and Garner, 2008). The technique includes harvesting, mowing, bulldozing and basal stem cutting (Witkowski and Garner, 2008; DiTomaso *et al*., 2017). These practices can however result in habitat and wildlife disruptions and soil erosion (DiTomaso, 2000; Zimmermann *et al*., 2004). For some invasive plants, mowing and cutting stems may be effective but might result in more vigorous regrowth which increases the density of some weeds (Heisel *et al*. 2001; McConnachie *et al*., 2011).

1.2.2 Chemical control

Chemical control involves using herbicides that block respiration, cell division, photosynthesis, and lipid biosynthesis in plants (Goodall *et al*., 2011; Capinera, 2017; Goodall and Witkowski, 2022). Herbicide applications can be done through hand spraying, paint brushing and aerial spraying (Culliney, 2005; Goodall and Witkowski 2014; Capinera, 2017; DiTomaso *et al*., 2017). The misuse of herbicides can, however, result in the contamination of the soil and freshwater systems and cause non-target effects (Capinera, 2017; DiTomaso *et al*., 2017). In aquatic systems, it was shown that fish are susceptible to some herbicides, such as nitrile, and phenoxy groups, that harm their reproductive and respiratory organs (Pemberton *et al.*, 2002; Fabricius, 2005; Capinera, 2017). Therefore, the use of herbicides must be restricted to areas away from freshwater systems.

1.2.3 Biological control

Biological control is considered the most sustainable, long-lasting and environmentally friendly method of controlling invasive plants (Bean *et al.*, 2012; Hajek and Eilenberg, 2018; Carneiro *et al.*, 2024). Biological control involves the deliberate introduction of host-specific, exotic natural enemies from the country of origin of the weed into the invaded region (Muller-Scharer and Schaffner, 2008; Ramanand *et al.* 2016). Host-specific natural enemies that are used include insects (McFadyen 1998; Barratt *et al*. 2010; Goldson *et al.*, 2014), nematodes and bacteria (Hajek *et al.*, 2007; Goldson *et al.*, 2014), fungi (Klein, 2011; Buccellato *et al.*, 2012) and mites (Mukwevho *et al.*, 2018). Out of all the agents, insects are most frequently used as a result of their host-specific nature (Muller-Scharer and Schaffner, 2008).

Biological control agents are only released once they have been proven to be host specific, meaning not having an impact on economically important non-target plants and native plants (McConnachie *et al*. 2011; Ramanand *et al.* 2016) and having a narrow host range (McFadyen, 1998). Under favourable conditions, successful agents will establish, and the population becomes self-sustaining (Muniappan *et al.*, 2009) so that additional releases are no longer required (Barratt *et al*. 2010). Some of the successful biological control programmes in South Africa include the control of drooping prickly pear (*Opuntia monacantha)* (Cactaceae) (Zimmerman *et al.*, 2004; Paterson *et al.*, 2011), *Opuntia ficus-indica* (Cactaceae) populations by *Dactylopius opuntiae* (Cockerell) (Hemiptera: Dactylopiidae) (Zimmermann and Moran 1991), red waterfern *Azolla filiculoides* Lamark (Azollaceae) by *Stenopelmus rufinasus* Gyllenhal (Coleoptera: Curculionidae) (McConnachie *et al*. 2004), *Acacia longifolia* (Andrews) by the pteromalid gall wasp *Trichilogaster acaciaelongiliae* (Pteromalidae)(Dennill, 1987).

Although biological control has generated great success, some agents have failed to establish and spread due to climate incompatibility in the area of introduction (Senaratne *et al.*, 2006; McConnachie and McKay, 2015). For example, drought, frost and flood drastically affected the population of *Neochetina eichhorniae* Warner (Coleoptera: Curculionidae) weevils released against *Eichhornia crassipes* (against hyacinth) Martius (Pontederiaceae) (Bownes *et al.*, 2013). Lower levels of humidity caused stress to egg development of tortoise beetle *Gratiana spadicea* (Klug) (Coleoptera: Chrysomelidae) (Byrne *et al*., 2002).

1.2.4 Integrated control

Integrated control uses multiple methods, such as chemical, mechanical, or biological techniques, to manage invasive weeds (Goodall and Erasmus, 1996; DiTomaso, 2007; van Kleunen *et al.*, 2020). Given the limitations of mechanical, chemical and biological control that have been highlighted, integrated control is aimed at improving the impact and effectiveness of the three control techniques. Mechanical and chemical control have been reported to be suitable for controlling small and isolated infestations of alien weeds (Paynter and Flanagan 2004). Cutting the stems of *Solanum mauritianum* (Solanaceae) Scop., bugweed and applying herbicide was found to be effective in controlling the trees (Mtetwa, 2019). Early season mowing of the invaded veld before the growing season of *Campuloclinium macrocephalum* (Less.) DC. (Asteraceae) was found to enhance the performance of the biocontrol against it, *Liothrips*

tractabilis Mound and Pereyra (Thysanoptera: Phlaeothripinae) (Mudau, 2019). However, the impact of mowing on the physiology of the host plant *C. macrocephalum* is unknown. A sub-lethal dose of herbicide (glyphosate) and *Neochetina eichhorniae* (Warner) (Coleoptera: Curculionidae), was found to assist in controlling water hyacinth, *Eichhornia crassipes* (Martius) Solms-Laubach (Pontederiaceae) in the field (Katembo *et al.*, 2013).

Therefore, integrated control methods should be considered and investigated, especially because the current control methods on their own are inadequate to manage the target weeds. Adaptive management is another approach that emphasizes learning through ongoing monitoring (long term), allowing weed management strategies to be adjusted or improved based on new data and evolving conditions, promoting more effective and resilient control over time (Shea *et al.*, 2002; DiTomaso, 2007). Risk assessment of specific potential alien invasive species, and the prioritization framework, it assesses the invasiveness potential, current distribution, and habitat suitability of a species, along with its ecological, economic, and social impacts (Kumschick and Richadson, 2013). Management feasibility, including eradication potential and control costs, is weighed alongside the urgency of action and stakeholder input (Shea *et al.*, 2002). Prioritizing early detection, critical habitats, and protected areas, the framework promotes adaptive management, continuously refining strategies based on monitoring results to minimize long-term damage and optimize resource allocation for effective management of IAPs (Cloutier, 2007).

1.3 The target weed: *Campuloclinium macrocephalum*.

Campuloclinium macrocephalum (pompom weed) is listed as category 1b in terms of NEMBA (National Environmental Management Biodiversity Act), an invasive species which must be controlled and wherever possible, removed and destroyed. Any form or trade or planting is strictly prohibited. Pompom weed is an alien invasive perennial plant native to South and Central America (Figure 1.1) (Henderson *et al*., 2006; McConnachie *et al.,* 2011: Calvo and Roque, 2018). *Campuloclinium macrocephalum* has a wide native distribution in South America, it is common in countries such as Argentina, Paraguay and Brazil (Figure 1.1) (McConnachie *et al.,* 2011; Trethowan *et al.,* 2011). According to Henderson *et al*., (2006), the first specimen of *C. macrocephalum* in South Africa was collected in 1962, from Johannesburg. The first wild populations of this weed were found in Pretoria, Fountains Valley in the 1970s (Henderson *et al*., 2006; McConnachie *et al*. 2011).



Figure 1.1: Native distribution of *Campuloclinium macrocephalum* in South and Central America (McConnachie et al., 2011; Trethowan *et al*., 2011).

Since the1980s, *C. macrocephalum* plant has become naturalized in South Africa and has invaded grasslands, wetlands and roadsides (McConnachie *et al.*, 2011; Goodall, 2016). The range of the weed has increased from Gauteng (Highveld grasslands) (Figure 1.2) and it is now found in provinces such as Limpopo, Mpumalanga, and North West (Goodall, 2016). There was also a confirmed occurrence of the weed in the Western Cape near George (Trethowan *et al*., 2011) and it has also been reported in Swaziland (McConnachie *et al*. 2011). *Campuloclinium macrocephalum* poses a significant threat to the conservation of the grasslands in South Africa and other neighboring countries (McConnachie *et al*. 2011). It reduces rangeland condition and grazing capacity of game reserves due to its unpalatability to wildlife (Goodall *et al.*, 2011; McConnachie and McKay, 2015). It also causes displacement of indigenous vegetation resulting in significant reduction in species diversity and richness (Aileen, 2005).



Figure 1.2: Grassland invasion by *Campuloclinium macrocephalum* (pink) at Rietvlei Nature Reserve in Pretoria, South Africa (Photo: P. Mudau).

Campuloclinium macrocephalum is a perennial herb that grows up to 1.5 m in height, growing from fleshy rootstocks below the soil surface (McConnachie *et al*., 2011). Both annual leaves and stems are covered in coarse, bristly hairs, the leaves are dispersed along the length of the stem and forms a rosette at the base (Henderson, 2001; McConnachie *et al*., 2011). Stems and leaves senesce in late autumn (mid-May), as winter approaches the plant dies back to perennial rootstocks and regrows in spring (October) as the average temperatures increases (Goodall and Witkowski, 2014). The weed spreads through its prolific seed production that are easily dispersed by wind and animals (Figure 1.3) (McConnachie *et al.,* 2011). *Campuloclinium macrocephalum* has a clustered thick tuber-like root system that enables it to survive a wide range of conditions such as frost, fire and insignificant amounts of rainfall (Goodall *et al.,* 2012; Trethowan *et al*., 2011).



Figure 1.3: Mature *Campuloclinium macrocephalum* florets (seeds) **(A)** and stalks after seeds were dispersed **(B)** (Photos: P. Mudau).

1.4 Management of *Campuloclinium macrocephalum*

1.4.1 Mechanical control

Campuloclinium macrocephalum can be controlled either through chemical, mechanical, integrated or biological control depending on the scale of invasion (Henderson *et al*., 2006). Mechanical control of this weed includes uprooting the whole plant, including all of its rootstock crown (Goodall *et al.,* 2012). This is only advisable in areas where the infestation is limited to few plants that can be dug out and all of the rootstock crown entirely removed, since if some roots remain the weed is most likely to regrow (Zachariades *et al*., 2021). Furthermore, damage to the primary stems stimulates and promotes vegetative growth and increases the abundance and density of *C. macrocephalum* (McConnachie *et al.,* 2011; Ramanand *et al*., 2016). Repeated cutting of flowering stems throughout the season before the seeds set is the most ideal control method for larger infestations (Goodall 2016). Physical control such as cutting stems and uprooting of *C. macrocephalum* alone are considered unsuccessful and additionally may aggravate the problem through this extra disturbance (McConnachie *et al*., 2011). Mowing of the grass before the growing season (September) of *C. macrocephalum* was found to enhance the performance of the biological control agent, *L. tractabilis*, there was a reduction in the number of flowering plants and stem height and greater abundance of the agent (Mudau, 2019). It remains unclear how mowing affected the physiology (chlorophyll content, chlorophyll inflorescence, stomatal conductance) of the host plant. However, mowing can only be done at a small scale and in areas that are not rocky, instead fire is being considered as an alternative to the mowing method. The effect of fire on the seedbank and the above ground biomass of *C. macrocephalum*

is yet to be quantified, and how fire can be integrated with biological control as *L. tractabilis* was found to overwinter in the top 4-6 cm below the soil surface, where it might be susceptible to fire.

1.4.2 Chemical control

Registered selective herbicides against *C. macrocephalum* include Metsulfuron methyl, Impala and Picloram (Goodall and Witkowski, 2013: Ramanand, 2015; Goodall and Witkowski, 2023). The use of herbicide by the then Department of Water Affairs, Working for Water programme at Voortrekker monument in Pretoria was found to be successful, however, further annual applications were required to eliminate the weed from an area (Goodall and Witkowski, 2013). Known invaded sites in KwaZulu-Natal were controlled using herbicides by the Invasive Alien Species Programme of the Department of Agriculture in the province (McConnachie *et al*., 2011). Regardless of the application of herbicides the invasion range of *C. macrocephalum* continues to expand within South Africa and beyond the boarders (McConnachie *et al*., 2011; Henderson and Wilson, 2017). The use of a sub-lethal dose of herbicide in conjunction with the biological control of *C. macrocephalum* may reduce the spread and density more effectively. A sub-lethal dose disrupts and alter plant growth, development and physiological functions, it suppresses the reproductive ability of the weed without killing it (Ainsworth, 2003; Londo *et al*., 2014). This strategy should leave enough materials on the host plant to sustain the population of the biocontrol agent.

1.4.3 Biological control agents

There are three biological control agents released against *C. macrocephalum* in South Africa, *Liothrips tractabilis* Mound and Pereyra (Thysanoptera: Phlaeothripinae), *Puccinia eupatorii* (Pucciniales: Pucciniaceae) (McConnachie *et al*., 2011; Goodall *et al.*, 2012) and a flower-feeder, *Cochylis campuloclinium* Brown (Lepidoptera: Tortricidae;) (McConnachie and McKay, 2016). The leaf rust *P. eupatorii,* was found suitable for release in 2006 (Goodall *et al*., 2012). However, the rust was discovered in the field near Pretoria on *C. macrocephalum* before the official release and has spread throughout the invaded range in South Africa (McConnachie *et al*., 2011; Goodall *et al*., 2012; McConnachie and McKay, 2015). The leaf rust causes necrosis and abscission on leaves of *C. macrocephalum* resulting in premature death of stems (McConnachie and McKay, 2015).

The application of herbicides was found to be not an option during the peak time of leaf rust, *P. eupatorii,* (around February) because of the start of leaf necrosis and dieback, therefore translocation of the herbicide was not possible (Goodall *et al.*, 2011). Therefore, herbicides must be applied prior the peak rust infestation in December and early January to allow herbicide absorption and translocation. Furthermore, high rainfall and sandy soil also results in incomplete absorption due to herbicide runoff (Goodall *et al.*, 2011).

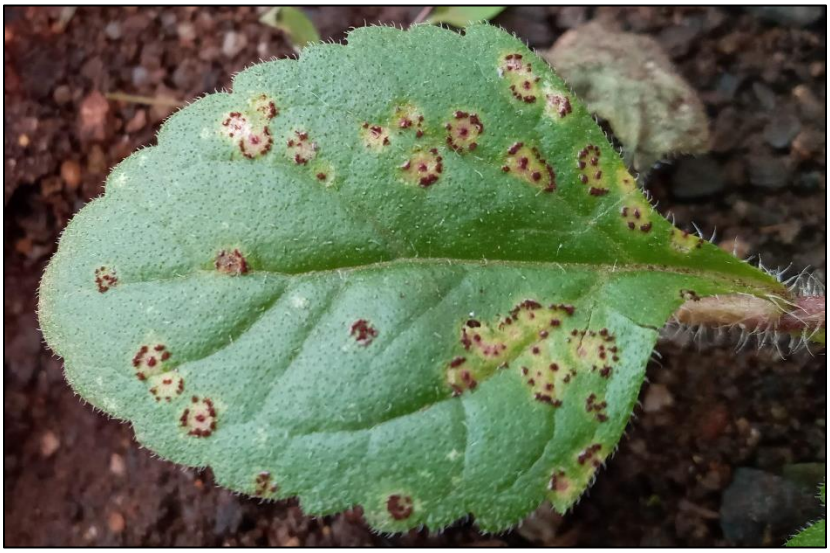


Figure 1.4: A leaf rust (*Puccinia eupatorii*) causing necrosis and abscission on a leaf of *Campuloclinium macrocephalum* (Photo: P. Mudau).

Cochylis campuloclinium (flower-feeder) was first recorded on *C. macrocephalum* in Argentina in 2004 (figre 1.5) (McConnachie *et al.*, 2011). The moth was then imported into quarantine in South Africa in 2006, 2008 and 2013, where host-specificity, host range and impact were evaluated and found to be a suitable agent (McConnachie and McKay, 2015b). Adult moths lay eggs on young flower buds, as the larva develops it feeds on developing florets (McConnachie and McKay, 2016). Laboratory impact studies demonstrated a significant reduction in the number of flower buds (76%) and seeds in mature flowers (54%) (McConnachie and McKay, 2015b). Permission to release the moth in South Africa was applied for and subsequently granted in October 2015 (McConnachie and McKay, 2015). However, the first field release of the moth was only made in January 2022 in Magaliesburg, this was because a laboratory culture was lost because of unfavorable rearing conditions before it was first approved for release (Zachariades *et al*., 2021).



Figure 1.5: Adult flower feeding moth (*Cochylis Campuloclinium*) on *Campuloclinium macrocephalum* flower bud (Photo: A. McConnachie)

Liothrips tractabilis (a stem deformer) was approved for release in 2013 after it was found to be host specific and capable of causing significant damage to the target plant, *C. macrocephalum* (McConnachie and McKay, 2015). Feeding by adults and nymphs deforms primary stems and other growing parts of the host plant, instead of vertical growth the stems bend over downwards (Figure 1.6B) resulting in a reduction of the flowering ability of the host plant (McConnachie and McKay 2015). The agent has been found to prefer young and apical stems of *C. macrocephalum* which is also where optimal damage is inflicted, compared to more mature stems (McConnachie *et al*., 2011). When *C. macrocephalum* senesces and dies back in late autumn (mid-May) till early spring (October) (Goodall and Witkowski, 2014), *L. tractabilis* adults survive the cold and dry winter by retreating underground to fleshy rootstocks. However, there was no significant evidence of feeding or nymph development during this period (Mudau, 2019).

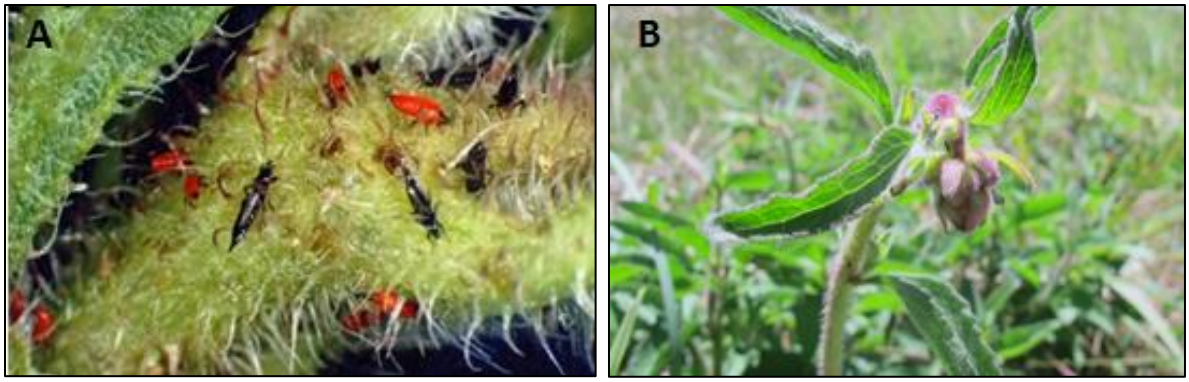


Figure 1.6: A biological control agent *Liothrips tractabilis* **(A)** of *Campuloclinium macrocephalum* and the impact on the host plant **(B)**. **A** is showing the adults (black) and nymphs (red) while **B** shows the impact on the plant (Photo: Besaans, 2014).

In the case of *C. macrocephalum*, *L. tractabilis* had varied success, which appears to be site dependent (Zachariades *et al*., 2021). For example, mowing of the grass before the growing season of *C. macrocephalum* was found to increase the impact and abundance of *L. tractabilis*, and its impact on the host (Mudau 2019); in Reitvlei Nature Reserve *L. tractabilis* was found to have established on elevated well drained sections of the reserve compared to wet and poorly drained soil (Zachariades *et al*., 2021). Anecdotal evidence suggests surrounding vegetation affects the growth form of pompom and nutritional quality of the plant which influences the performance and abundance of *L. tractabilis*. Nitrogen content is a key characteristic that can be used to quantify plant nutritional quality (Awmack and Leather, 2002; Rostami *et al*., 2012) of *C. macrocephalum* growing in long and short vegetation. The difference in nutritional quality of the preferred young plants of *C. macrocephalum* by *L. tractabilis* over older stems could also be associated with the availability of nitrogen, but this needs to be studied further. Despite the use of current management strategies, the invasion range of *C. macrocephalum* continues to increase in South Africa (Zachariades *et al*., 2021). Therefore, integrated weed management, which includes two or more management strategies such as fire, biological control, lethal and sub-lethal herbicide doses could be combined to further improve the management of *C. macrocephalum*.

1.5 Research aims and objectives.

The long-term goal of this study was to contribute new insights which will enable the development of improved management strategies to control *C. macrocephalum* in South Africa, as the invasion range of the weed continues to increase, with large negative impacts on the remaining conserved grasslands and some parts of the savanna biome in the country. Therefore,

the aim of this study was to evaluate the effect of fire alone, fire alongside herbicide and sub-lethal doses of herbicide on *L. tractabilis* and how these affect the persistence, growth and reproduction of *C. macrocephalum*. Secondly, to investigate the effect of reduced and increased sunlight on the physiology of *C. macrocephalum* and the impact of *L. tractabilis*. And assess how the nutritional quality of *C. macrocephalum* affects the feeding performance and abundance of *L. tractabilis*.

Objective 1: Chapter 2: Field study

Assess the interactive effect of fire alone, and fire alongside herbicide on the management of *C. macrocephalum* and evaluate how flowering, seedbank, re-sprouting and plant growth are affected.

Objective 2: Chapter 3: Laboratory study

Identify sub-lethal doses of Picloram as potassium salt (Access 240 SL, 240 g a.i. L^{-1}, Dow AgroSciences), Impala 480 EC and Metsulfuron methyl (Brush-Off, 600 g a.i. kg^{-1}, DuPont) and their impact on *L. tractabilis* (abundance, reproduction, survival and feeding).

Objective 3: Chapter 4: Greenhouse trial

Assess the effect of reduced sunlight and full sunlight on the growth (height, inflorescence, number of leaves, number of flowering stems, number of deformed stems, amount of compensatory growth) and physiology (Chlorophyll content, chlorophyll florescence, stomatal conductance) of *C. macrocephalum* and the impact of biological control agent, *L. tractabilis* under these conditions.

Objective 4: Chapter 5: Nutritional quality of *C. macrocephalum*

The aim of the chapter was to assess and compare the nutritional quality of young stems, apical stems and old stems (mid-section) of *C. macrocephalum* that grew in long and short vegetation.

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Chapter 2

Burning and poisoning the problem: Using fire and herbicide to manage *Campuloclinium macrocephalum* (pompom weed)

Abstract

Campuloclinium macrocephalum is an invasive perennial herb native to South America, commonly known as pompom weed in South Africa. Anecdotal evidence suggests that fire reduces the seedbank of pompom weed without a substantial impact on weed density, while herbicides primarily affect mature plants exclusive of the seedbank, suggesting the need for a more integrated control management approach. Therefore, the aim of this study was to assess the interactive effect of fire and herbicide for two growing seasons on pompom flowering, emerging seedlings, re-sprouting/regrowth, and plant density. During the initial growth period in 2020, fire alone in August substantially decreased the seedbank, as assessed from seedling emergence by more than 85%, but it did not decrease the density of mature plants within the affected areas. However, the application of herbicide in December 2020, following the fire, led to an apparent reduction of over 98% in mature plants during the same growing season. However, in the subsequent growing season of 2021, there was a 9% regrowth of mature plants in areas previously treated with fire and herbicide, necessitating an additional herbicide application in the following growing season. Hence, integrating both fire and herbicide is recommended as an enhanced management strategy for controlling pompom weed in South Africa.

Keywords: Growing seasons, invasive plants, integrated management, regrowth, seedbank, seedlings, weed density.

2.1 Introduction

Campuloclinium macrocephalum (Less.) DC. (Asteraceae) is a highly invasive perennial forb native to South America and known as pompom weed in South Africa (Henderson *et al*., 2006). Pompom weed has increased its invasion range by over 600% from 2006 to 2016 (van Wilgen *et al*., 2020), and dominates much of the Highveld grassland in Gauteng, Mpumalanga and North West Province (Henderson, 2020). Given its prolific seed production, and unpalatability to wildlife and livestock the invasion range is likely to increase further (McConnachie and McKay, 2015). It has perennial rootstocks that enable it to withstand fire and harsh winter conditions such as low rainfall and frost (Trethowan *et al*. 2011; Goodall *et al.*, 2012). Management strategies such as chemical (Henderson and Wilson, 2017), fire (Goodall *et al.*, 2011), mechanical (McConnachie *et al*., 2011) and biological (Zachariades *et al*., 2021) control have not yielded substantial results, hence the increase in invasion range of *C. macrocephalum*.

Numerous herbicide formulations are registered for the control of *C. macrocephalum* but the most widely used contain the active ingredients metsulfuron methyl, picloram, picloram/ fluroxypyr or fluroxypyr/triclopyr (Goodall and Witkowski, 2013). Use of herbicides alone requires many years of annual applications which may not be feasible, logistically or financially (Goodall and Witkowski, 2023). It is also not ideal to continuously apply herbicides in sensitive environments such as areas that are close to streams, wetlands and dams (DiTomaso *et al*., 2017). Mechanical control of *C. macrocephalum* through hoeing or digging is ineffective as damage to rootstocks by splitting them leads to further vegetative growth resulting in an increase in pompom density (McConnachie *et al*., 2011; Goodall *et al.*, 2022).

The stem deforming *Liothrips tractabilis* Mound and Pereyra (Thysanoptera: Phlaeothripinae) was released against *C. macrocephalum* in 2013 in South Africa (McConnachie and McKay, 2015: Ramanand *et al*., 2016). The agent alters the apical shoot tips of stems and significantly reduces floral production, plant height and biomass (McConnachie *et al*., 2011; McConnachie and McKay, 2015). However, *L. tractabilis* has failed to establish in some of the release sites, and in sites where it has established, the rate of spread is very slow (L. van der Westhuizen, pers. comm.). *Puccinia eupatorii* (Pucciniales: Pucciniaceae), a rust fungus is another biological control agent used against *C. macrocephalum* which causes necrosis and abscission of leaves resulting in the premature death of stems (McConnachie *et al*., 2011; Goodall *et al.*, 2012).

Nevertheless, the damage inflicted by the fungus is usually moderate and varies from one year to the next (Zachariades, 2018). Given the limitations of chemical, mechanical and biological control of *C. macrocephalum*, an integrated control approach is worth exploring to improve the effectiveness of these methods.

Integration requires careful undertaking in terms of the timing of each aspect in order that the methods complement each other and do not result in antagonistic outcomes. The timing of herbicide application is critical in any integrated management of invasive plants or vegetation (Ballard and Nowack 2006: Petrice and Haack, 2011). Application of herbicide must be done during the peak growing season, in this case early December to allow optimal absorption of the herbicide (Goodall and Witkowski, 2014). If applied late, around February during the peak *P. eupatorii* infestation stage, leaf necrosis will reduce absorption and translocation of the active ingredient (Goodall *et al.* 2011), lessening the effectiveness of the herbicide. It is also vital that herbicides kill *C. macrocephalum* plants before their seeds become viable, which may restore the seedbank that has been reduced by fire or other control methods.

Integrating different control methods has been found to work against invasive plants, including *Solanum mauritianum* (Solanaceae) Scop. (bugweed), where mechanical control (cutting) and chemical (herbicide) treatment of stumps were integrated (Denny and Goodall 1992; Witkowski and Garner 2008). Juvenile trees (>1-1.5 m) were cut 18 cm above ground level and stumps treated with triclopyr or imazapyr herbicides; seedlings and saplings (<1 m) were treated with triclopyr or picloram (Denny and Goodall 1992; Witkowski and Garner 2008). Water hyacinth *Eichhornia crassipes* (Mart.) Solms (Pontederiaceae) can be managed using a sub-lethal dose of glyphosate herbicide (chemical control) alongside biological control (Katembo *et al*., 2013). The sub-lethal dose retards water hyacinth growth while conserving the population of *Neochetina eichhorniae* (Warner) (Coleoptera: Curculionidae), a biological control agent (Katembo *et al*., 2013). *Chromolaena odorata* (L.) King and Robinson can be managed by combining fire with manual clearing, herbicide application and biological control (Goodall and Erasmus, 1996; Zachariades *et al.*, 2011; te Beest *et al.*, 2012). Combination of clearing, prior to using fire on invaded sites was found to be effective in reducing the density of *C. odorata* compared to a single management strategy (te Beest *et al.*, 2012).

Implementing mechanical control methods, such as early-season mowing of surrounding vegetation, led to the enhanced effectiveness of *L. tractabilis* on pompom weed, resulting in increased levels of stem deformation (Mudau, 2019: Chapter 2). However, this is only possible in areas where the biological control agent, *L. tractabilis* has established, and mowing over rocky terrain and over large areas may not be feasible. A strategy of combining different control methods such as chemical, mechanical, fire or biological control can improve the impact and effectiveness of the four control techniques and improve the degree of control of a particular weed (Ainsworth, 2003; Mosley *et al.*, 2016; Lake and Minteer, 2018). Integrated management of invasive weeds vary to suit a target plant based on invaded habitat, existing control methods and management objectives.

Fire has been used to manage other invasive plant species such as the herbaceous perennial weed leafy spurge (*Euphorbia esula*) (Lym and Nelson, 2002), and the invasive forb yellow star-thistle (*Centaurea solstitialis L.*) (Di Tomaso and Johnson, 2006), with prescribed fires also used to reduce hazardous fuel load, and improve forage for livestock, and wildlife in nature reserves (Di Tomaso and Johnson, 2006). Repeated rather than single burning has been found to be effective in controlling most invasive plants but is however impractical or prohibited in some areas as that may significantly reduce plant basal cover and biomass leading to an increase in soil erosion. Repeated burning alongside the seed-feeding weevil (*Melanterius maculatus*, Curculionidae) significantly reduced the seedbank of the Australian *Acacia* species, *Acacia cyclops* A.Cunn. ex G.Don (Impson *et al.*, 2024). Anecdotal evidence suggests fire also reduces the seedbank of pompom without having a significant impact on the density of mature plants, while in contrast herbicides appear to target mature plants exclusive of the seedbank (L. Van Der Westhuizen, pers. comm.).

Therefore, the aim of this study was to assess whether fire alone, and/or fire with herbicide can be used to successfully manage *C. macrocephalum*. The main objective of the study included evaluating how burning alone prior to the growing season of *C. macrocephalum* would affect the weed density, regrowth/re-sprouting from rootstocks, flowering density and the seedbank of the weed over two growing seasons. The second objective was to assess how burning combined with application of herbicide in mid-summer (December) affected density, regrowth from rootstocks, flowering and number of seedlings of pompom weed. It was expected that fire would reduce

pompom seedling emergence (i.e. reduce the seedbank), while only a herbicide application would reduce the density of flowering (mature) pompom plants.

2.2 Materials and methods

Study area

The study was conducted at an open grassland at Rietvlei Nature Reserve (3 870 ha or 38 km^2), City of Tshwane Metropolitan Municipality, South Africa (25.8825° S, 28.2639° E). This area is classified as Carletonville Dolomite Grassland (Mucina and Rutherford, 2006), characterized by summer rainfall and high temperatures with severe frost in winter. Some of the dominant grass species in the reserve include *Hyparrhenia hirta* (L.) Stapf, *Eragrostis gummiflua* Nees, *Aristida canescens* Henrard subsp., *Digitaria diagonalis* (Nees) Stapf var. diagonalis and *Eragrostis curvula* (Schrad.) Nees. Herbivores within the reserve include black wildebeest (*Connochaetes gnou*), buffalo (*Syncerus caffer*), zebra (*Equus quagga quagga*), wblesbok (*Damaliscus pygargus phillipsi*) and kudu (*Tragelaphus strepsiceros*) (Marais, 2004). Given the grazing impact of the herbivores, reserve management keeps their numbers within its grazing capacity (Marais, 2004). Prescribed fires within the reserve are primarily meant to reduce the fuel load and improve forage, ensuring sufficient grazing for wildlife (Rietvlei Nature Reserve, unpublished). Burning in each management block is done on a three-year cycle, with internal roads dividing the reserve into 58 management blocks (Figure 2.1). Management blocks are burnt late August-September at night after the first thunderstorms of the summer rainy season, prior to the growing season of *C. macrocephalum*. According to the Rietvlei Nature Reserve Management Plan, a block is burnt if it has ≥ 2 tons/ha of dead organic material to sustain or carry a fire (Rietvlei Nature Reserve, undated).

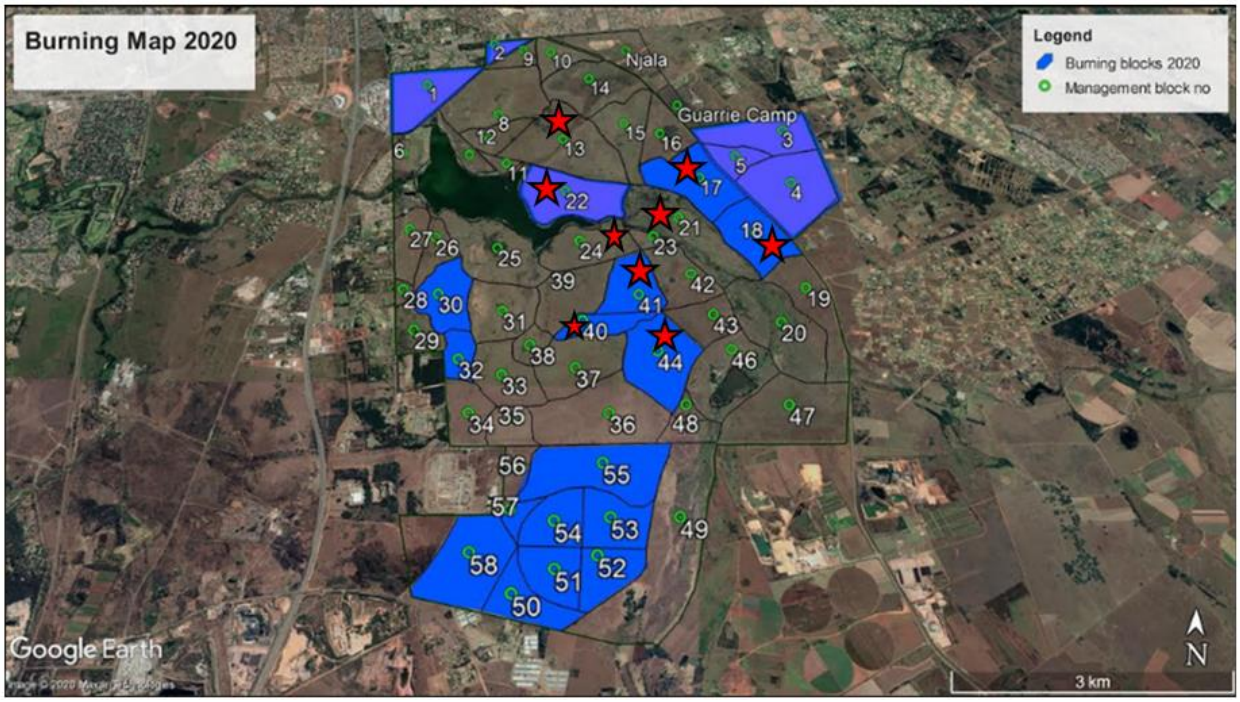


Figure 2.1: Fifty-eight (58) management blocks and the 2020 burning site blocks (blue) of Rietvlei Nature Reserve, South Africa. Red stars represent sampled blocks.

Experimental design

Burning of management blocks was done by the Rietvlei Nature Reserve management team in August-September 2020 by setting alight a long fire-front against the wind that was allowed to burn the entire block with parallel blocks burnt the following year (2021) (Figure 2.2). Blocks were not all burnt on the same night but were burnt alternatively to ensure the fire did not spread to other parts of the reserve. Three specific management treatments were applied to the treatment blocks. In 2020, three blocks (17, 22, 44) were burnt only (August 2020), another three blocks (18, 40, 41) were burnt (August 2020) and then four months later treated with herbicide in December 2020, with a further three blocks (13, 21, 24) left unburnt in 2020 but burnt the following season in August 2021 with no herbicide treatment (Table 2.1).

Table 2.1: Management blocks numbers (n=9) at Rietvlei Nature Reserve that were burnt only (blocks A-17, 22, 44), burnt and treated with herbicide (blocks B-18, 40, 41) or left unburnt (Blocks-C13, 21, 24) over the two growing seasons of *Campuloclinium macrocephalum.* Shaded blocks indicate the year in which each control technique was implemented at different blocks.

Year	**Blocks A** (17, 22, 44)	**Blocks B** (18, 40, 41)	**Blocks C** (13, 21, 24)
2020	survey	survey	survey
2021	survey	survey	survey
2022	survey	survey	survey

Treatment	**Key:**
Burnt only	
Burnt + Herbicide	
Unburnt	



Figure 2.2: Burnt (left) management block (41) and unburnt (right) block (39) in October 2020 at Rietvlei Nature Reserve, South Africa (Photo: P. Mudau)

In blocks that were burnt and sprayed (18, 40, 41) *C. macrocephalum* was spot sprayed by the Reserve's management team with herbicide (Impala 480 EC) using knapsacks (Figure 2.3). Herbicide (0,5 % (500 mℓ / 100 ℓ water) was applied in December 2020 when all pompom plants had emerged before the peak infestation of *P. eupatorii* that causes leaf necrosis which reduces the absorption and translocation of the active ingredient (Goodall *et al.* 2011). This ensured that none of the mature plants were missed during spot spraying. Blocks (12, 21, 24) that were unburnt in 2020 were later burnt in 2021 without herbicide application (Table 2.1). Data collection was done over the two growing seasons of *C. macrocephalum* during 2020-2022.



Figure 2.3: Herbicide (Impala 480 EC) application in December on *Campuloclinium macrocephalum* using knapsacks on a burnt block at Rietvlei Nature Reserve, South Africa (Photo: P. Mudau).

Impala 480 EC is a systemic emulsifiable concentrate herbicide with active ingredients fluroxypyr and triclopyr, both pyridyloxy compounds. The recommended dose for young, immature plants is 250 mℓ / 100ℓ water (0.25%) and 500 mℓ / 100ℓ water (0.5%) for mature plants (300L/ha). A surfactant, Performer (L8069), and a mineral oil was used as a penetrating and spreading adjuvant and a dye was also added to the spray mix to identify plants that had been sprayed. A minimum of 90% of all pompom foliage was covered as recommended while minimizing significant run-off.

Field data collections

Every month during the growing season from October 2020 to May 2021and from October 2021 to May 2022, six 1 m x 1 m quadrats were randomly placed in each 10 m x 10 m plot. This was to ensure resampling of the same quadrat was avoided. For each growing season 48 (6 quadrats x 8 months) per month were assessed on each plot that was chosen within a block. Regrowth or resprouting plants were defined as plants growing back from rootstocks. This was done by assessing the base of the plant they were growing from. In each quadrat, one plant facing north closest to the bottom left of the quadrat was selected and the following parameters were quantified.

- Number of leaves, where only live leaves were counted.
- Height of the main stem from the ground to the tip of the inflorescence.

- Number of stems, defined as number of stems emerging from the base of main rootstock.

The total number of seedlings within each quadrat were also counted monthly and used to estimate how the seedbank was affected by fire and herbicide application. The total number of flowering plants was also counted within each quadrat. The height of surrounding (accompanying) vegetation was measured during the peak flowering period in December 2021 and 2022.

Data analysis

The Shapiro-Wilk test was first used to test if the data were normally distributed. One-way repeated measures ANOVA was used to compare the overall differences between and within the three treatments for each of the above metrics in each of the two growing seasons, from October 2020 to June 2022 and a Tukey's HSD was used for post-hoc tests to identify where differences lay between parameters. A t-test was used to compare the difference between the mean number of stems per plant between burnt and unburnt blocks. Scatter plots and linear regressions were used to assess the relationship between the number of stems and leaves, vegetation height and height of *C. macrocephalum*. Analyses were conducted using SPSS Statistics 22.0, and Microsoft Excel 2016.

2.3 Results

Effect of fire alone on mature plants

The mean number of adult *C. macrocephalum* on burnt blocks was low during the first growing season (2020) after the fire, but then increased during the second growing season after the 2021 dormant period (Figure 2.4a). Despite the occurrence of fire, there was no significant impact on the density of mature plants between the two growing seasons ($F_{1,34}$= 2.64; p=0.347). The mean density of mature plants increased from October till peak density in January on both growing seasons of 2020 and 2021, followed by decline in number of mature plants until all plants senesced by May. During the first growing season after the fire, the peak mean number of plants per quadrat was eight, and this increased to 10 during the second season due to a recovery.

Effect of fire and herbicide on mature plants

The application of herbicide to the burnt blocks during the first growing season was done in December 2020 and resulted in almost all *C. macrocephalum* plants being eliminated on sprayed blocks (Figure 2.4b). There was at least an average of one mature plant per m^2 recorded in January 2021 post herbicide application, and there were no plants recorded on the blocks thereafter. However, up to two plants (9%) per quadrat grew back from rootstock in the following growing season in 2021. The difference in the mean number of plants between blocks with and without (both burnt and unburnt) herbicide application in December 2020 was significant ($F_{2,51}$ = 5.541; p=0.0417; $F_{2,51}$ = 3.833; p=0.0215).

Before and after the fire (no herbicide)

During the first growing season the mean number of plants increased from six /m^2 in October to 11 plants in January 2021, plants then declined on unburnt blocks from February till all the plants senesced in May 2021 (Figure 2.4c). After burning in the second season of the study in August 2021, the mean density of plants per quadrat declined to three in October 2021 and nine in January 2022 compared to the previous growing season. However, there was no significant difference in the number of plants in the season before the fire and after fire was introduced the next growing season ($F_{2,51}$ = 5.389; p=0.0676).

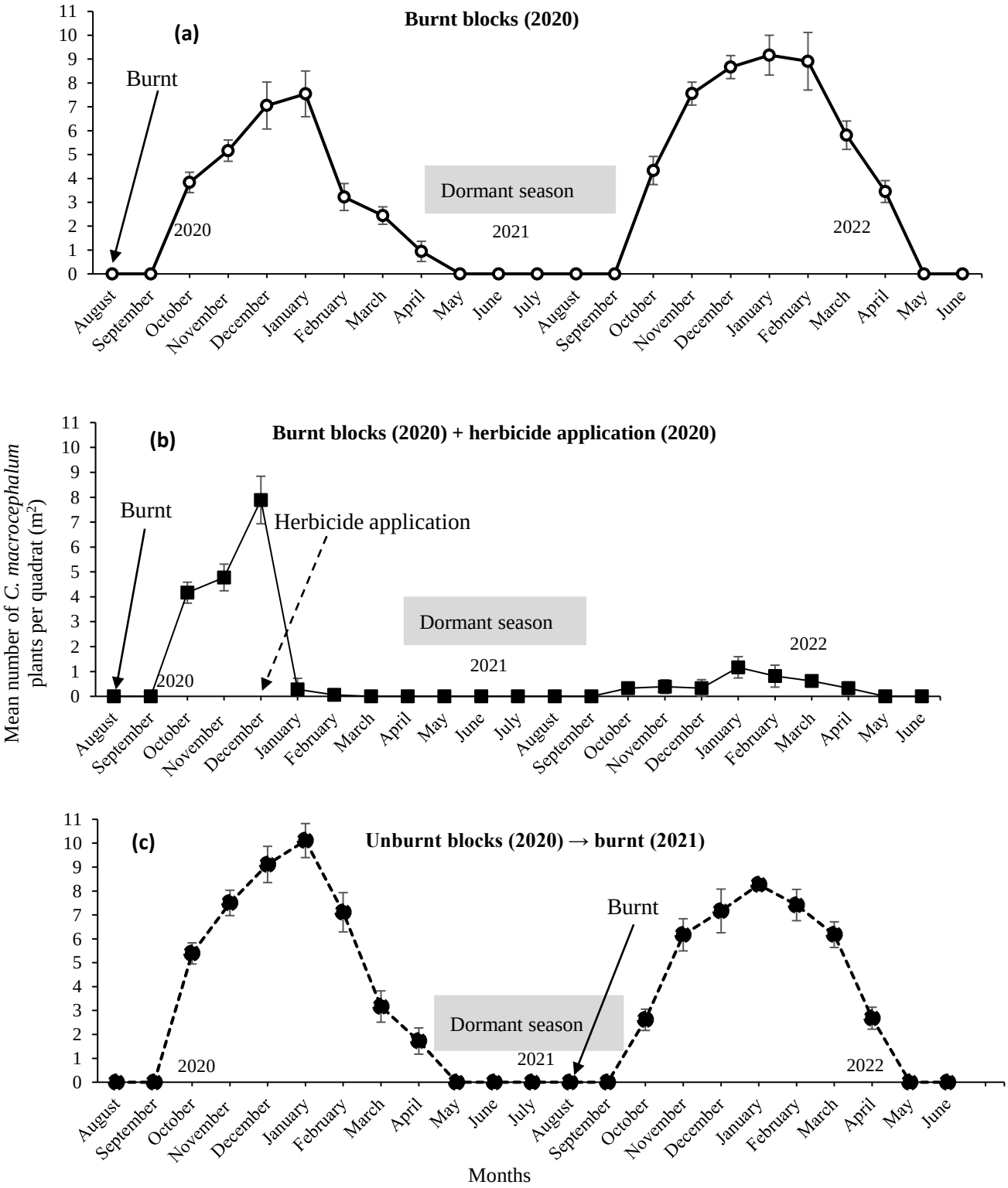


Figure 2.4: Mean number of adult *Campuloclinium macrocephalum* plants/m^2 from October 2020 to June 2022 on burnt blocks in 2020 (a), burnt blocks in 2020 that were treated with herbicide in December 2020 (b) and unburnt blocks that were later burnt in 2021 (c). For each data point n = 18 sampled quadrats (mean ± SE).

Effect of fire alone on seedlings

Fire in the burnt blocks in 2020 resulted in a highly significant reduction in the mean number of *C. macrocephalum* seedlings/m^2 compared to the unburnt blocks during the first growing season ($F_{2,51}$ = 0.556; p< 0.001) (Figure 2.5a). During the first growing season (2020-2021) after the fire, the highest mean number of seedlings recorded was nine in March 2021, the number significantly increased to 18 ($F_{1,34}$ = 9.657; p=0.0267) during the second growing season (2021-2022). There was a 66 % increase in the number of seedlings in the following growing season during peak growth with least 19 seedlings/m^2 the following season in 2022.

Effect of fire and herbicide on seedlings

On blocks where both fire and herbicide were applied, the highest number of seedlings recorded during the two growing seasons was <two seedlings/m^2 in December 2020 and January 2021 and no seedlings were recorded thereafter (Figure 2.5b). During the second growing season (2021-2022) there were no seedlings recorded in response to treatment.

Effect of fire on seedlings on unburnt blocks in 2020 that were burnt in 2021

The peak mean number of seedlings before the fire was 36/m^2 in December (2020). Fire in August 2021 resulted in an over 85% reduction in the number of seedlings during the 2021-2022 growing season compared to the previous season ($F_{1,34}$ = 8.298; p=0.0137) (Figure 2.5c). The peak number of seedlings was five seedlings in January 2022, thereafter the density of seedlings declined until all plants senesced in May 2022.

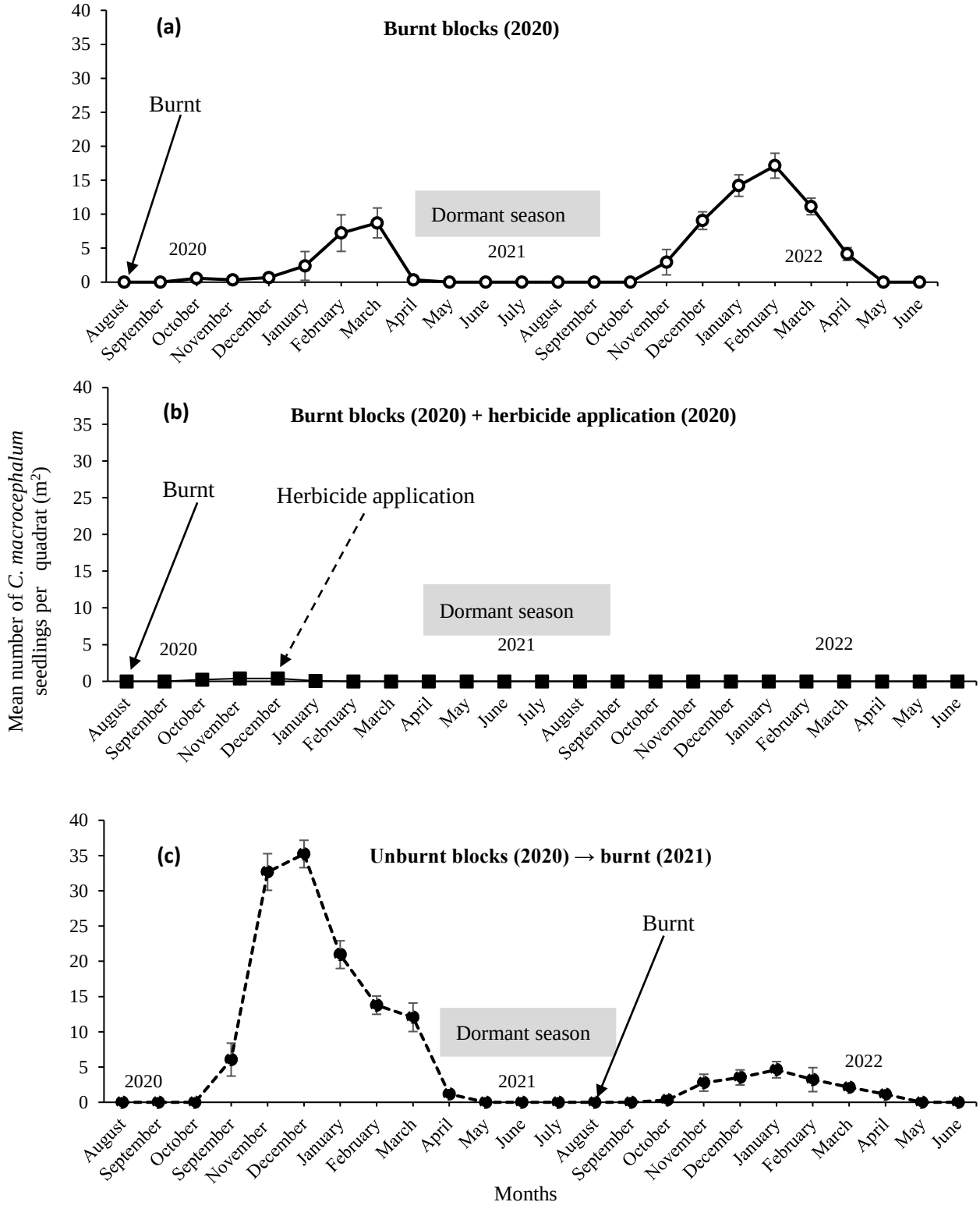


Figure 2.5: Mean number of *Campuloclinium macrocephalum* seedlings/m^2 from October 2020 to June 2022 on burnt blocks in 2020 (a), burnt blocks in 2020 that were treated with herbicide in December 2020 (b) and unburnt blocks that were later burnt in 2021(c). For each data point n = 18 sampled quadrats (mean ± SE).

Effect of fire on flowering on burnt and unburnt blocks

Flowering on both burnt and unburnt blocks during the first growing season in 2020 started in November with peak flowering being recorded in December and January 2021 (Figure 2.6a, c). There was a decline in the number flowering plants from December 2020 on burnt and unburnt blocks until all plants senesced in May 2021. The density of flowering plants on the previously unburnt blocks declined from seven to five/m^2 during the second growing season in 2021. Meanwhile there was an increase in flowering plants on blocks that were burnt prior to the 2021 growing season, peak flowering increased slightly from five to six/m^2, but the increase was not significant ($F_{1,34}$ = 1.727; p=0.0658). Overall, during the two growing seasons fire did not have a significant impact on the flowering of pompom on the blocks.

Effect of fire and herbicide on flowering.

There were no flowering plants recorded during the 2020 growing season on blocks that were burnt and treated with herbicide (Figure 2.6b), but during the second growing season of 2021 to 2022 there was regrowth of *C. macrocephalum* from its tubular root stocks despite the application of herbicides. There was a significant difference in the number of plants flowering between the blocks without herbicide application compared to blocks with herbicide ($F_{2,51}$ = 1.447; p=0.004; $F_{2,51}$ = 0.614; p<0.001, respectively) (Figure 2.6b).

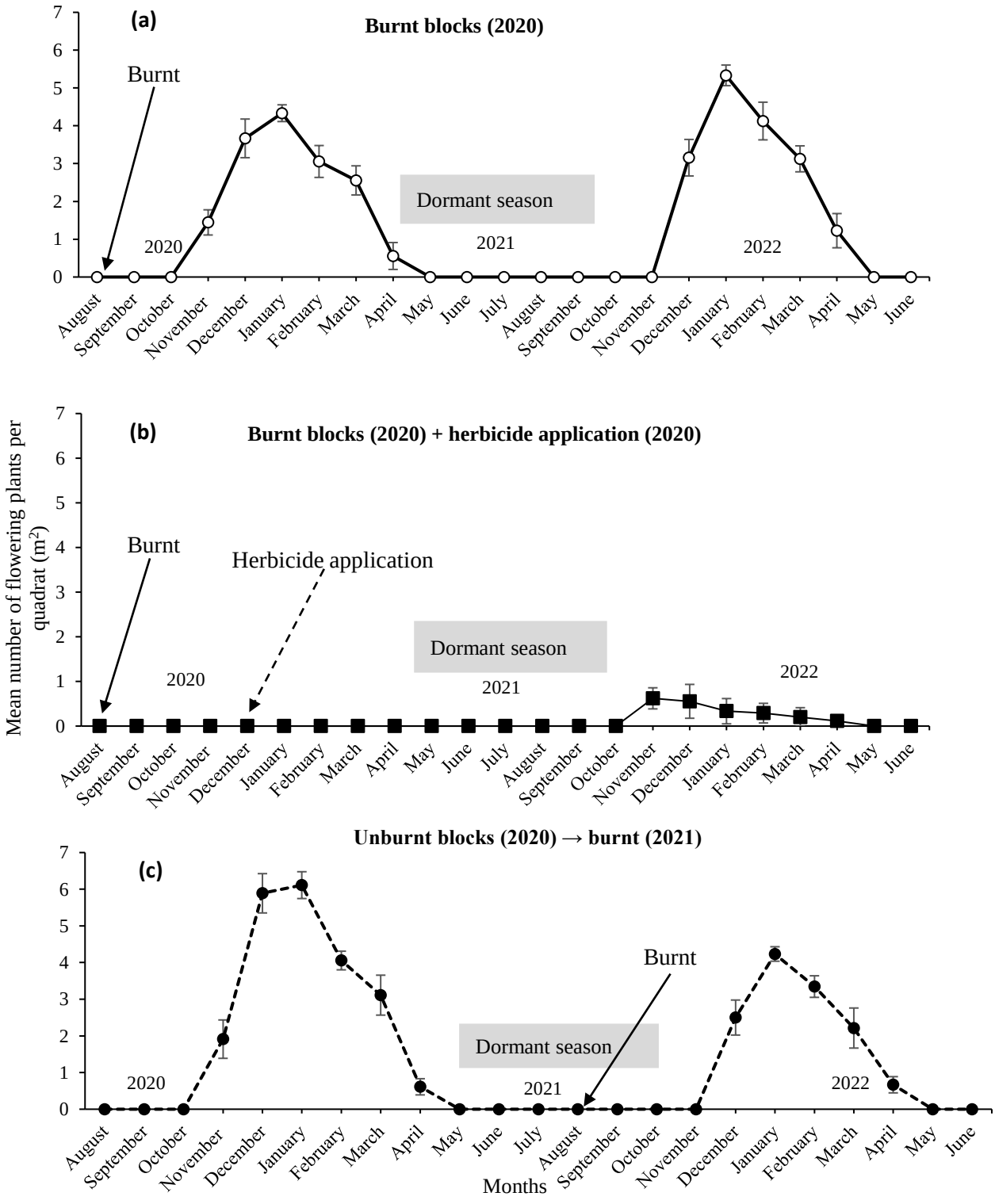


Figure 2.6: Mean number of flowering (mature) *Campuloclinium macrocephalum* plants/m^2 from October 2020 to June 2022 on burnt blocks in 2020 (a), burnt blocks in 2020 that were treated with herbicide in December 2020 (b) and unburnt blocks that were later burnt in 2021 (c). For each data point n = 18 sampled quadrats (mean ± SE).

Change in height of C. macrocephalum before and after the fire

The growth of *C. macrocephalum* on burnt blocks increased from 6.4 cm in October to a peak height of 106.6 ± SE cm in December 2021, and from 12.2 to 124. cm during the same period on the unburnt blocks and the final height difference was statistically significant ($F_{2,51}$ = 12.244; p=0.0138) (Figure 2.7a, c). The peak height of *C. macrocephalum* declined on the previously unburnt blocks (2020) from 124.4± SE to 75.3± SE cm after burning, before the second growing season in 2021 and the difference in height between the two growing seasons was significant ($F_{1,34}$ = 8.196; p<0.001).

On blocks burnt in 2020 where herbicide was applied in December, translocation (uptake) of the herbicide was evident from January 2021 as there were no plants recorded for the remainder of the growing season thereafter (Figure 2.7c). However, in the second growing season in 2021-2022, despite the previous application of herbicide there was plant regrowth, and the height of plants recorded was significantly shorter compared to blocks without herbicide application ($F_{1,34}$ = 7.567; p=0.0107).

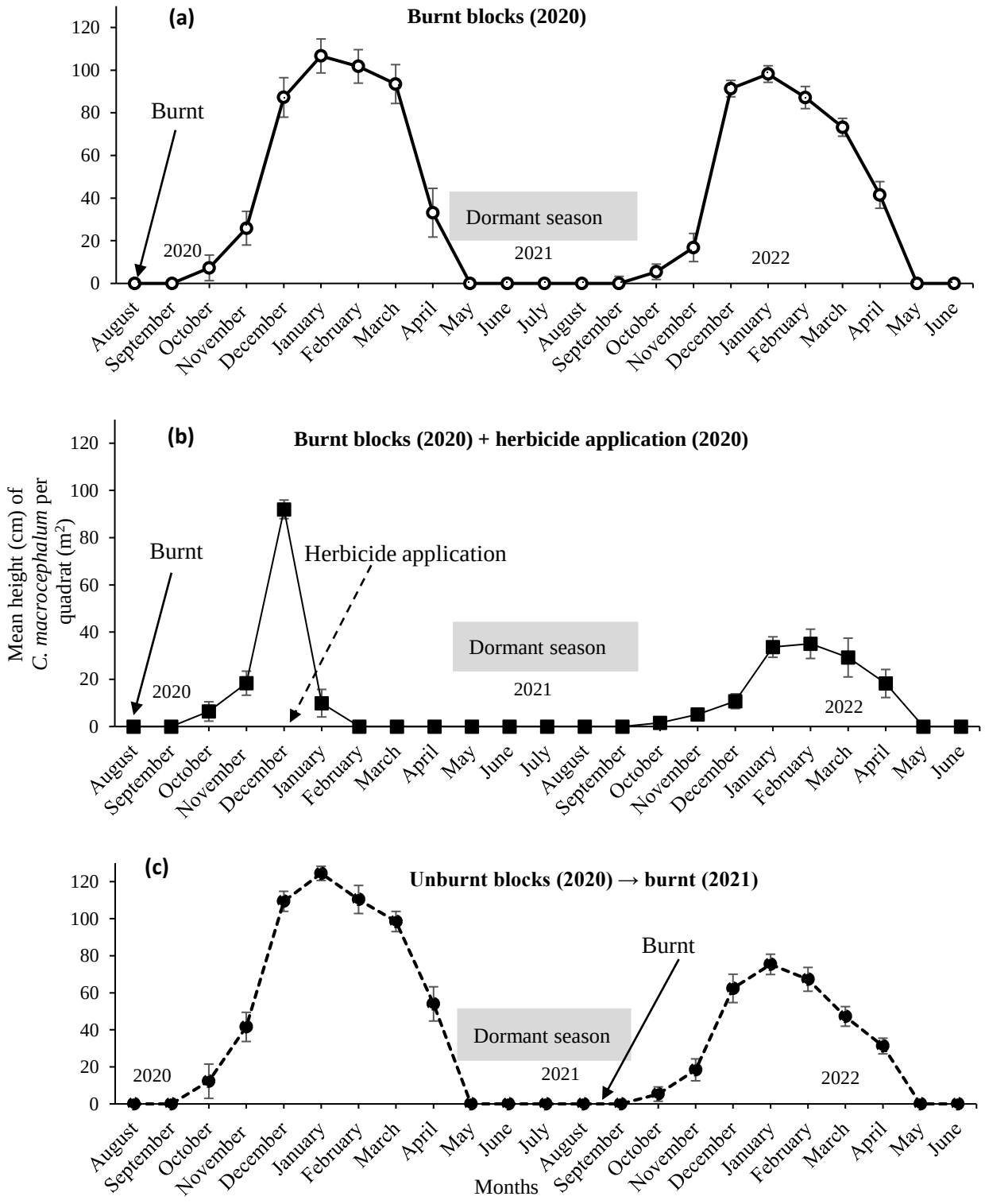


Figure 2.7: Mean height of adult *Campuloclinium macrocephalum* plants/m^2 from October 2020 to June 2022 on burnt blocks in 2020 (a), burnt blocks in 2020 that were treated with herbicide in December 2020 (b) and unburnt blocks that were later burnt in 2021 (c). For each data point n = 18 sampled quadrats (mean ± SE).

The number of *C. macrocephalum* stems per plant on the burnt blocks in 2020 was greater than on the unburnt blocks of 2020 and the difference was significant ($F_{1,34}$ = 1.294; p=0.0403) (Figure 2.8a). The increase in the number of leaves per plant was directly proportional to the number of stems per plant, with a strong linear relationship (R^2= 0.789) (Figure 2.8b).

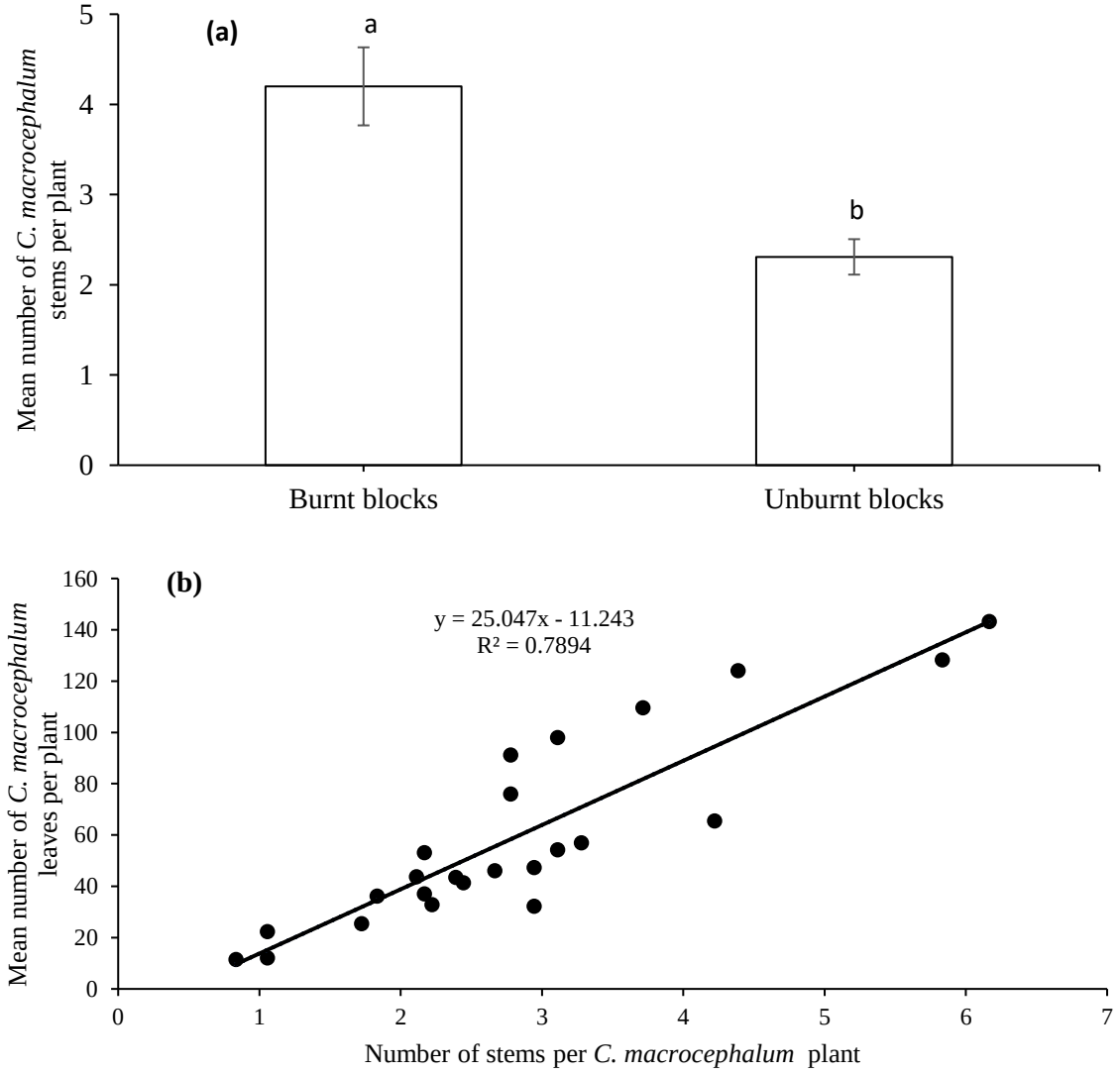


Figure 2.8: The effect of fire on the number of *Campuloclinium macrocephalum* stems per plant (a) during peak growing period of January 2021, and the relationship between the number of *C. macrocephalum* stems and leaves (b) during the same peak growing period of January 2021 (mean ± SE). n=24 (a) plants and n=48 (b) plants.

There was a linear relationship between the height of *C. macrocephalum* and the surrounding vegetation (R^2= 0.8833) (Figure 2.9). The height of *C. macrocephalum* increased as the height of surrounding vegetation increased, but *C. macrocephalum* was taller. *Campuloclinium macrocephalum* height was generally greater than the surrounding vegetation such as grass and shrubs.

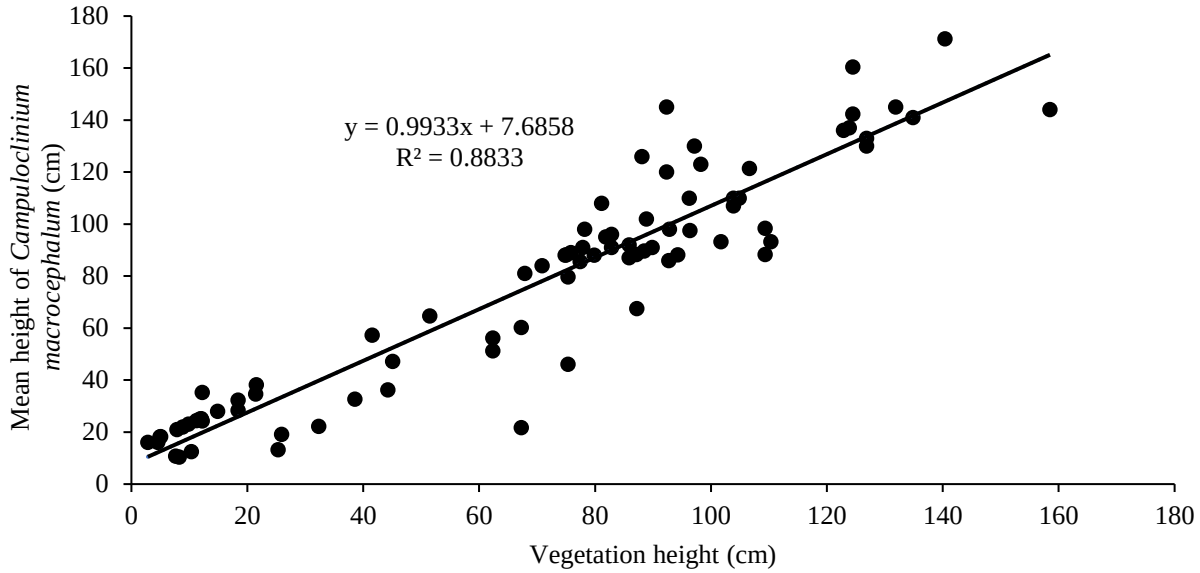


Figure 2.9: Relationship between the height of *Campuloclinium macrocephalum* and surrounding vegetation in infested areas during the peak of the growing period in January 2021. n= 84.

2.4 Discussion

The application of herbicide (Impala 480 EC) following the burning of blocks proved to be effective in reducing the density of *C. macrocephalum*, which is an encouraging outcome for management and control strategies of pompom weed in South Africa. Burning the blocks also decreased vegetation density, making selective herbicide application techniques like spot spraying more practical. This approach also helped minimize unintended impacts on non-target vegetation and reduced the likelihood of missing individual pompom plants. Following the herbicide application in December 2020, the population density of pompom significantly declined, with no plants recorded from February 2021 onwards. Seedlings emerging from seeds that may have survived the fire would have matured by December 2020, making them visible and susceptible to spot spraying, thereby leading to greater weed mortality. Furthermore, no plants on these treated blocks flowered during the first growing season, resulting in the absence of seedlings in the subsequent growing season. Only 9% of the mature plants grew from

rootstocks, possibly surviving the previous herbicide application. This could be attributed to a less than ideal 90% foliage herbicide coverage during the initial application, which may have caused premature senescence of the weed above ground while leaving the below-ground biomass, such as the tuberous rootstocks, less affected, allowing for resprouting. It is crucial for herbicides to reach all growth points of the plants for effective control and substantial mortality (DiTomaso and Kyser 2007; Enloe *et al*. 2018). Plants that regrow can be targeted by a follow-up application of the registered herbicide in December during the next growing season to prevent further proliferation. This highlights the importance of thorough and precise herbicide application in managing and controlling invasive weed species like *C. macrocephalum*, and also the importance of follow-up observations in the next growing season.

Three consecutive years of annual applications of registered herbicides alone at invaded areas was found to suppress the weed but failed to eliminate it (Goodall and Witkowski, 2014). However, it was estimated that at least five annual applications would be essential to lower weed density to <1 plant per 25 m^2 block (Goodall and Witkowski, 2014). Integrating fire and herbicide reduced the required number of herbicide applications from the predicted number of five consecutive annual applications to only two consecutive years of annual applications. Herbicide application in the study was strategically timed during the peak growing season, typically in early December, to optimize herbicide absorption. If applied late, around February during peak *P. eupatorii (*leaf rust) infestation stage, leaf necrosis will reduce absorption and translocation of the herbicide active ingredient (Goodall *et al.*, 2011), which will lessen the effectiveness of the herbicide, and result in regrowth. In any integrated management of vegetation, the timing of herbicide application is critical to ensure effective control of the target weed (Ballard and Nowack 2006: Petrice and Haack, 2011). It is also crucial that herbicides kill all plants especially flowering *C. macrocephalum* plants before their seeds reach maturity and becomes viable. Failure to do so could potentially result in the restoration of the seedbank, which may have been depleted by the fire, especially the seeds close to the surface and in the litter (Witkowski and Wilson 2001). Nonetheless more deeply buried seeds will likely still be viable, and these may be moved to more shallow surface soil and litter layer by burrowing animals. Hence ongoing vigilance is required for long term control.

The impact of fire on the seedbank of pompom was profound, resulting in a significant reduction of over 85% as determined by assessing the number of emerging seedlings. This substantial decrease indicates the effectiveness of fire in suppressing the seedbank of the invasive species, potentially contributing to long-term management and control efforts. The morphological similarities between *C. macrocephalum* and *C. odorata* seeds, including size, shape, texture, and dispersal abilities, suggest that pompom seeds may share similar characteristics, such as intolerance to high soil temperatures during burns (Witkowski and Wilson 2001; te Beest *et al.* 2012). Similarly, the density of *C. odorata* seedlings was also significantly decreased by a single fire treatment (te Beest *et al.*, 2012), and the seeds were found to be intolerant of exposure to fire temperatures and high post-fire soil surface temperatures (Mbalo and Witkowski 1997: Witkowski and Wilson, 2001), this may also be the reason for the decline in emerging seedlings of *C. macrocephalum* in this study.

Fire as a single treatment has also been found to be an inadequate control strategy against other invasive plants such as *C. odorata*, a herbaceous scrambling shrub (te Beest *et al.*, 2012), an annual perennial herb *Centaurea solstitialis L.* (DiTomaso and Johnson, 2006), and a herbaceous perennial plant *Euphorbia esula* L. (DiTomaso and Johnson, 2006) because they are all able to resprout from the base as only above ground biomass is affected by fire as with *C. macrocephalum*. One prescribed fire against an invasive forb yellow star-thistle (*C. solstitialis*) was found to reduce the seedbank by 75% and that was considered unsatisfactory to reduce the infestation because mature plants would still resprout from old stems and restore the seedbank (DiTomaso *et al.*, 1999: DiTomaso and Johnson, 2006). In this study, an 85% reduction would have also been considered unsatisfactory if herbicide was also not used after the fire because mature plants will resprout and flower thus restoring the seedbank and maintain or increase infestation levels. In the following growing season after the fire (without herbicide) in 2021-2022, there was a 66% increase in seedlings from the 15% that had survived the fire meaning that 66% of the seedbank was restored from mature plants that flowered and seeds that survived the fire or possibly more deeply buried seeds, or seed dispersed from unburnt sites. This was because plants resprouted from rootstocks and then flowered resulting in the increase. These findings reinforce the conclusion that fire alone is not recommended as a sole control method for managing *C. macrocephalum* in invaded areas.

The slight decline in weed density of *C. macrocephalum* during the first growing season (2021) was due to the reduced seedbank and mortality of some mature plant rootstocks during the fire. Fire promoted lateral growth thus producing more stems (at least 3-4 more stems) from rootstocks compared to plants on unburnt blocks with mainly vertical growth where each rootstocks produced at least 1-2 stems. Greater numbers of stems result in more flowers being produced, resulting in greater number of seeds being produced, and this causes a surge in the number of seedlings during the following growing season, thus exacerbating the infestation levels (McConnachie *et al.,* 2011).

Another notable effect of fire was the difference in height of *C. macrocephalum* on burnt and unburnt blocks, height was significantly greater in plants on unburnt blocks. This may have been because plants on burnt blocks did not have to compete for light as much with the surrounding vegetation, while plants on the unburnt block may have grown long stems to optimize light harvesting (Valladares and Niinemets, 2008), while plants on burnt blocks with open direct sunlight invested in lateral growth. A similar growth pattern was observed on plants that were in open sunlight and plants that were in a shaded area where light was buffered by a net (Chapter 4). There was a strong linear relationship between the height of the accompanying vegetation and height of *C. macrocephalum* (R^2= 0.894). Overall, the difference in height between burnt and unburnt blocks suggests that fire influences the growth and development of *C. macrocephalum*, with competition for light playing a significant role in shaping its growth patterns and distribution of nutrients within the plant (Chapter 4).

These findings have practical implications for invasive species management. They highlight the importance of timely and targeted herbicide application in controlling invasive plant populations, particularly in post-fire environments where invasive species often proliferate rapidly. Understanding the regrowth dynamics and long-term effectiveness of herbicide treatments can inform more effective management strategies to combat invasive species and restore native ecosystems. Overall, the study provides valuable insights into the efficacy of combining fire and herbicide application as control measures for managing invasive species like *C. macrocephalum*, offering potential solutions for mitigating their negative impacts on ecosystem health and biodiversity. The next section (Chapter 3) will identify and test whether sub-lethal doses of three

herbicides (picloram, impala and metsulfuron methyl) can be integrated with the biological control agent, *L. tractabilis*.

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Appendix



Figure 2.10: Burnt (right) and unburnt (left) blocks in October 2020 (**A**) and the comparison of the of the density of *Campuloclinium macrocephalum* (pink flowerheads) (**B**) during peak flowering in December 2021 at Rietvlei Nature Reserve on a burnt and unburnt block.

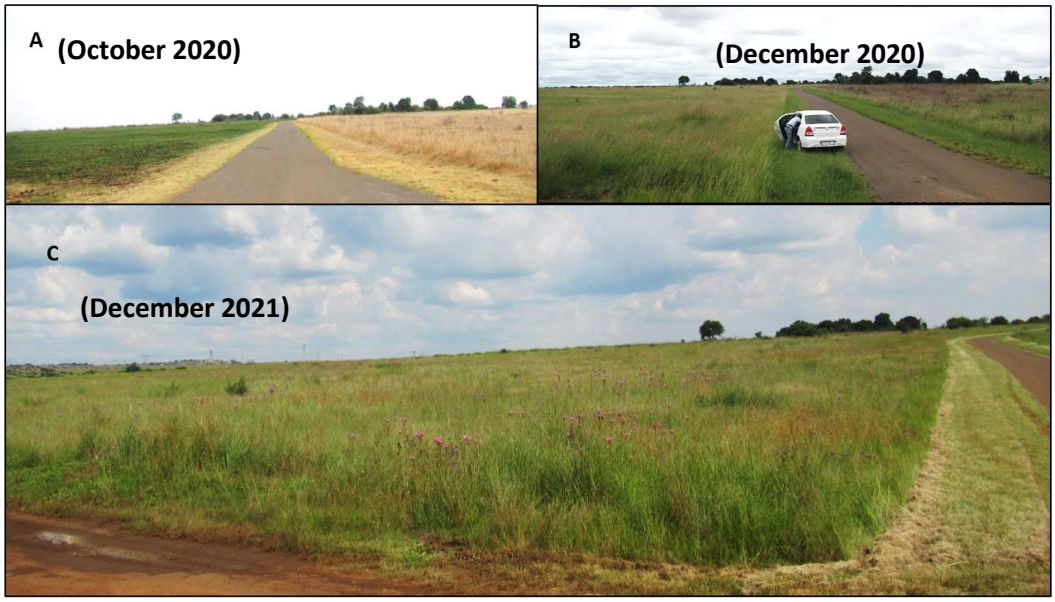


Figure 2.11: Burnt block (**A**, left) with herbicide applied in December 2020. The density of *Campuloclinium macrocephalum* (pink flowerheads) (**B**, left) during peak flowering in January 2021 at Rietvlei Nature Reserve. Regrowth (9%) of mature pompom plants during peak flowering during the second growing season in January 2022 (**C**).

Chapter 3

Testing sub-lethal doses of three registered herbicides and their impact on *Liothrips tractabilis*, a biocontrol agent of pompom weed.

Abstract

Classical biological control does not always result in comprehensive control of the target weed and may have to be integrated with other management techniques. Several biological control agents have been released to improve the degree of control of *Campuloclinium macrocephalum* (Less.) DC. (Asteraceae), pompom weed, a perennial invasive plant in South Africa. *Liothrips tractabilis*, a stem deformer was released against the weed in 2013. A lethal dose of registered herbicides where *L. tractabilis* has established will probably collapse its population resulting in no plant material to sustain the biological control agents. The aim of this study was to identify a sub-lethal dose of each of the three registered herbicides that will suppress the vegetative growth of *C. macrocephalum* without having direct detrimental effects on *L. tractabilis* populations. After eight weeks of plant growth, with weekly sampling, the sub-lethal dose of picloram was identified to be 0.07 ml/100L, 0.05 g/100L for metsulfuron methyl and 0.075 ml/100L for impala (fluroxypyr and triclopyr). Early signs of herbicide application included wilting of leaves and stems, noticeable from weeks three and four. Increased herbicide concentrations also caused a decrease in the number of stems per plant, as the mother plant deteriorated. The sub-lethal doses of the three herbicides indirectly affected the biocontrol agent by causing wilting of the apical stems, which are the preferred feeding sites for *L. tractabilis*. This, in turn, significantly reduced the agent's abundance as the herbicide dosages increased. Consequently, the use of a sub-lethal herbicide dose is not advisable due to incompatibility with the biocontrol agent and its relationship with the weed plant.

Keywords: Biocontrol, *Campuloclinium macrocephalum,* feeding sites, herbicides, integrated weed management, invasive weed.

3.1 Introduction

Classical biological control to manage invasive plants using arthropods is used in natural areas and food production systems around the world and is also considered the most sustainable and environmentally friendly control technique (van Driesche, 2012). However, implementing biological control of weeds does not always result in substantial impact on the invasion range of the weed (Lake and Minteer, 2018). Programs that do not provide adequate control alone can be integrated with other control techniques to improve the degree of control (Lym, 2005; Miller, 2016). Biological control agents can be integrated with other control methods such as mechanical control, grazing, plant competition, herbicides or chemical control and vegetation fires (Ainsworth 2003; Lake and Minteer, 2018).

Multiple biological control agents are often released against a single invasive plant to improve the degree of control (Moran and Zimmermann 1991; Dhileepan *et al.*, 2007; Strathie *et al.*, 2011; Buccellato *et al.*, 2012; Zachariades *et al.*, 2021). For instance, three biological control agents have been released against pompom weed, *Campuloclinium macrocephalum* (Less.) DC. (Asteraceae), a perennial invasive plant native to South America (Zachariades *et al.*, 2021). These include *Liothrips tractabilis* Mound and Pereyra (Thysanoptera: Phlaeothripinae) which was first released in Gauteng and KwaZulu-Natal Provinces of South Africa in 2013, a flower feeder *Cochylis campuloclinium* Brown (Lepidoptera: Tortricidae) released in January 2022 in the Magaliesberg Biosphere (ARC-PHP, 2022) and *Puccinia eupatorii* (Pucciniales: Pucciniaceae) which was an adventive (accidental) release in South Africa (McConnachie *et al*., 2011; Goodall *et al.*, 2012).

The damage inflicted by *L. tractabilis* and *P. eupatorii* on *C. macrocephalum* is moderate, while the degree of control provided by the two agents is insignificant in some field sites (Zachariades, 2018: Zachariades *et al*., 2021). More releases of the flower-feeding moth still need to be done and field impact measured given its recent release (January 2022). Currently, registered herbicides such as metsulfuron methyl, picloram 240 g ai L-1 (Goodall and Witkowski, 2014) and impala are being used to control *C. macrocephalum*. Three annual applications of metsulfuron methyl and picloram alone were found to suppress the abundance of *C. macrocephalum* but failed to control the weed (Goodall and Witkowski, 2014). Goodall and Witkowski (2014) estimated that five (summer) to seven (autumn) annual applications would

reduce the density to <1 plant per 5x5 m^2. Importantly, the two herbicides were found not to achieve the expected 80% average mortality expected of registered herbicides on *C. macrocephalum* rootstocks over the treatment period (Goodall and Witkowski, 2014).

The use of fire and herbicide (impala- fluroxypyr and triclopyr) in combination has been found to yield promising results (see Chapter 2). Fire reduced the emergence of new seedlings of *C. macrocephalum* by over 85%, and fire further ensured that spot spraying of the weed regrowing from below ground parts was possible, which resulted in a 98% reduction of mature plants after application during the peak of the growing season (Chapter 2). There was however a 9% regrowth of the plants that survived the combined fire and herbicide treatment, which could be managed by a follow-up herbicide application (Chapter 2). Application of a recommended or lethal dose in areas where a biological control agent has been recently released or established will negatively affect the efficacy of the agents because herbicides can interfere directly and indirectly with biocontrol agents and can cause the population to crash (Hill *et al*., 2012). Direct impact of herbicides on insects includes the herbicide being toxic when it comes into contact with it, while indirect impact results when the herbicide kills the vegetation that insects feed on (Hill *et al*., 2012; Karouach, *et al*., 2022).

To counter the negative effects of lethal concentrations, the use of a sub-lethal dose (considerably lower dose than the registered dosage) would be ideal if it would only suppress the reproductive capacity and vegetative growth of the weed without killing the plant (Ainsworth, 2003; Jadhav *et al*., 2008). The sub-lethal doses often disrupt and alter plant development, flowering phenology, biomass and reproductive function (Londo *et al*., 2014). *Brassica napus* L. (Brassicaceae) that was exposed to a sub-lethal glyphosate dose displayed delays in its flowering time in relation to control plants (Londo *et al*., 2014). The use of a sub-lethal dose of glyphosate herbicide was used to retard the growth of water hyacinth *Eichhornia crassipes* (Mart.) Solms (Pontederiaceae) while conserving the biological control agents (Jadhav *et al*., 2008). Herbicide resistance in plants occurs more frequently with consistent (annual) herbicide application (Evans, *et al*., 2016).

The aim of this study was to identify sub-lethal doses of three herbicides (picloram, impala (fluroxypyr/triclopyr) and metsulfuron methyl) that will not kill but suppress the vegetative growth and reproductive capacity of *C. macrocephalum* and sustain *L. tractabilis* populations. If

sub-lethal doses are identified and found to be compatible with the biological control agent, a single application will be recommended to reduce the chances of herbicide resistance. The objectives of this study include assessing the number of new stems produced, height of plants, number of flowering stems and leaf production of pompom weed and to further assess the impact of the sub-lethal doses on the survival, reproduction and abundance of *L. tractabilis*.

3.2 Methods and Materials

3.2.1 Experimental design and laboratory trials

Campuloclinium macrocephalum plants used in this study were all sown from seed (collected at Rietvlei Nature Reserve) in the previous growing season of 2021(October) to May (2022) in a nursery at the University of the Witwatersrand, Johannesburg. All plants were allowed to dieback in pots (Bottom diameter: 10 cm, Height: 12 cm, Top diameter: 15 cm) to their rootstocks (senesce) over winter and grow back from rootstocks the following growing season. From October 2022 similar sized potted plants (~ 30 cm tall) were allocated into different treatments. In early November 2022, an equal number of thrips (10) were inoculated on to each of the treatment plants seven days prior to the application of different doses of herbicides, to allow *L. tractabilis* to establish. For this study, establishment was defined as deformation on stems of the host plant by the biocontrol agent. For each concentration of each herbicide a total of eight plants were used, with four plants per aluminum cage (50 (length) × 50 (width) × 100 cm (height), an overall total of 56 plants (including control plants with zero herbicide) per herbicide treatment. Aluminum cages were kept in a controlled environment (Wits insectary), with lights on a 12 hour daylight cycle and temperature of 28 ± 2 °C during the day and of 24 ± 2 °C during the night and watered daily at 6 pm without any use of fertilizer.

The following registered herbicides used were used in the trials: fluroxypyr/triclopyr 320/160 g a.i. L^{-1} (Impala 480 EC, UPL), picloram 240 g a.i. L^{-1} as potassium salt (Access 240 SL, Dow AgroSciences) and metsulfuron methyl 600 g a.i. kg^{-1} (Brush-Off, Dow AgroSciences). The recommended dose of metsulfuron methyl is 25g/100L, picloram 350mL/100L and impala 25g/100L, doses were divided and applied in six concentrations at different percentages of the recommended dose (Table 3.1). Pipets were used to measure volumes of liquid formulations (picloram and fluroxypyr/triclopyr) and the dry formulation (metsulfuron methyl) was measured

using an analytical balance, sensitive (0.0001 g) scale. Mineral oil 363 g L^{-1} (Performer, UPL) was used as an adjuvant in herbicide treatments.

Table 3.1: Six concentrations of three herbicides (picloram, impala and metsulfuron methyl) used to determine sub-lethal doses of *Campuloclinium macrocephalum* at different percentages of the recommended doses per 100L.

% of the recommended dose	Herbicide name		
	Picloram	Impala	Metsulfuron methyl
50	0.1750 ml	0.125 ml	0.125 g
40	0.1400 ml	0.1 ml	0.1 g
30	0.1050 ml	0.075 ml	0.075 g
20	0.0700 ml	0.05 ml	0.05 g
15	0.0525 ml	0.0375 ml	0.0375 g
10	0.0350 ml	0.025 ml	0.025 g

3.2.2 *Identification of C. macrocephalum sub-lethal dose*

Prior to identification of sub-lethal dose for the weed, toxicity testing was also conducted to assess if any of the herbicides had a direct effect on *L. tractabilis* adults and nymphs by directly spraying insects with the different herbicides. For each herbicide concentration there were eight treatment plants of similar age and morphology (height, number of leaves and stems) that were used. Treatment plants were sprayed a week after inoculation with *L. tractabilis* and more than 90% of the foliage was sprayed as recommended to maximize herbicide translocation. The following measurements were taken every week for a duration of eight weeks, height of *C. macrocephalum* measured on the main stem from the soil surface to the tip of the inflorescence using a measuring tape. Number of leaves, number of compensatory growth points (number of new stems) and number of flowering stems. The number of *L. tractabilis* per plant (adults and nymphs) was counted upon termination of the experiments. A sub-lethal dose in this study was defined as a dose that will not kill the plant but reduces the number of new stems that developed and flowered, and not result in considerable reduction in *L. tractabilis* adults and nymphs compared to control plants.

Statistical analysis

The Shapiro-Wilk test was first used to test if the data were normally distributed. One-way ANOVA (SPSS Statistics 22.0) was used to compare the end point (end of trial) results of plants and insect survival across the different doses of the three herbicides. A Tukey's HSD was used for post-hoc

tests to compare between the different doses to assess the mean difference between parameters at the end of the trial (eight weeks).

3.3 Results

3.3.1 *Effect of herbicide on plant height:*

Application of increasing doses of herbicides resulted in relative reduction of growth of plants after eight weeks across all the three herbicide treatments. As the herbicides concentration increased there was a decline in the final height of *C. macrocephalum* as the apical stem wilted. The final height of the control plants was greater than the height of all the treatment plants, with the highest and lowest doses having the tallest and shortest plants respectively (Figure 1 (a, b and c)).

Translocation of herbicide was evident from week three and four, as plants treated with lower doses of picloram showed an increase in height compared to plants treated with the higher doses. The difference in height after week eight was significant ($F_{7,49} = 2.25$; $p < 0.01$) (Figure 1a). Plants treated with higher doses of 0.175, 0.14 and 0.105 ml showed a decline in height from week four.

The same trend was observed with the height of plants treated with metsulfuron methyl, where plants treated with doses over 0.05 g showing a reduction in height from week three ($F_{7,49} = 6.71$; $p < 0.01$) (Figure 1b). Those treated with doses 0.05 g and 0.0375 g showed some increase in height but significantly different to the height of control plants ($F_{7,49} = 7.87$; $p < 0.01$).

Plants treated with impala (c) also displayed a similar trend with control plants being significantly taller ($F_{7,49} = 6.63$; $p < 0.01$) (Figure 1c). From week three, as the herbicide doses increased the height of plants treated with 0.125, 0.1 and 0.075 ml doses declined gradually compared to other treatment plants that were treated with lower doses ($p < 0.05$).

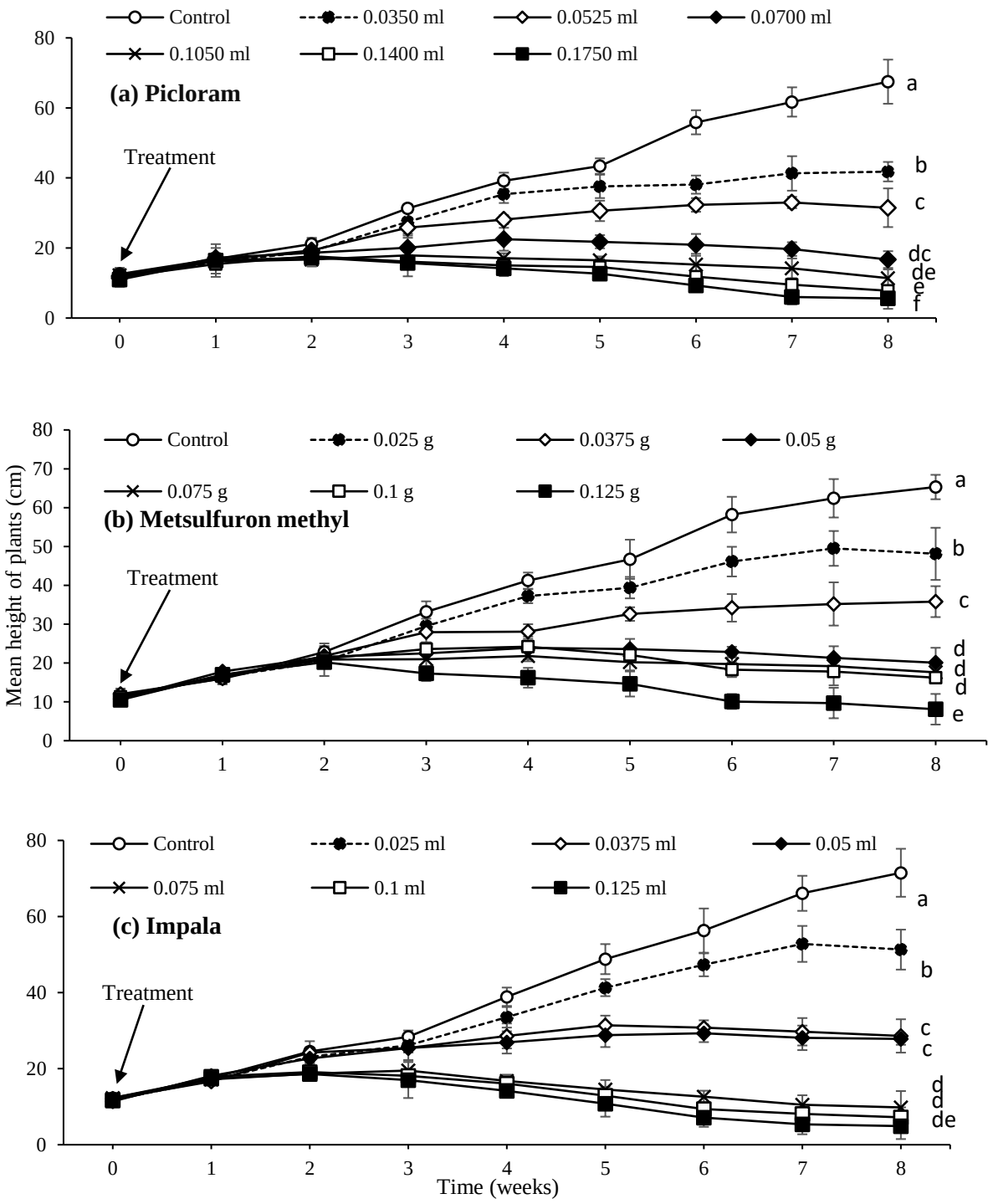


Figure 3.1: Height of *Campuloclinium macrocephalum* plants that were treated with *Liothrips tractabilis* and sub-lethal doses of Picloram (**a**), Metsulfuron methyl (**b**) and Impala 480 EC (**c**) over eight week trial. For each data point n= 8 plants (mean ± SE). Different lowercase letters indicate significant differences between doses ($p < 0.05$) for each of the three herbicides separately at the end of the trial.

3.3.2 *Effect of herbicide on number of leaves:*

Early symptoms of herbicide application included wilting of apical stems and loss of leaves which was evident from week three and four (Figure 2 a, b and c). Despite the application of herbicides most treatment plants showed a slight increase in number of leaves until week four and five, thereafter more leaves started to wilt on all plants treated with one of the three herbicides.

For the picloram treatment the number of leaves on control plants increased from 14 at week zero to at least 68 leaves by week eight which was significantly greater than all treatment plants ($F_{7,49} = 4.95$; $p < 0.01$) (Figure 2a). Plants treated with the higher doses of 0.175, 0.14 and 0.0105 ml (0.105, 0.14 and 0.175%) picloram showed a decline in the number of leaves after week four, while there was an increase on plants treated with 0.07, 0.525 and 0.035 ml (0.07, 0.00525 and 0.0035%).

Plants treated with the lowest metsulfuron methyl dose of 0.025 g had at least 14 leaves at week zero which increased to 33 leaves by week eight (Figure 2b). Leaves of control plants also increased over time from 14 to 66, and the difference was significant in relation to all treatment plants ($F_{7,49} = 5.32$; $p < 0.01$). Plants treated with doses of 0.125, 0.1 and 0.075 g (0.0125, 0.01 and 0.0075%) did not show an increase in the number of leaves after week three but in fact had significant decrease compared to other treatment plants ($p<0.05$).

After week eight, the number of leaves on plants treated with the lowest dose of impala increased from 14 to 39, while the highest dose (0.125 ml) resulted in a significant decrease ($F_{7,49} = 3.75$; $p < 0.05$) from 14 to at least four leaves per treatment plant (Figure 2c). The number of leaves on control plants significantly increased, from 15 at week 0 to 73 by the end of week eight ($p < 0.01$).

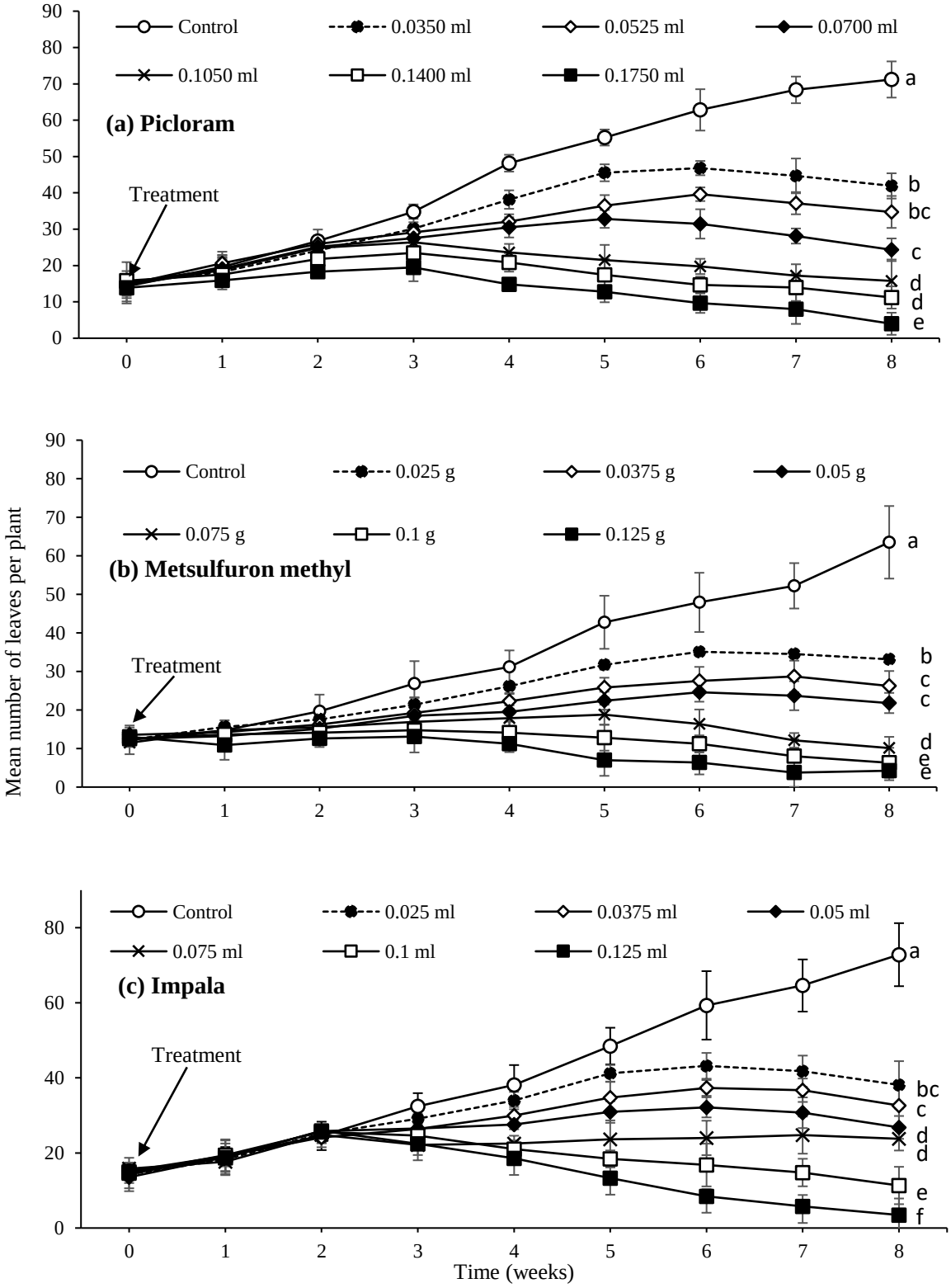


Figure 3.2: Mean number of *Campuloclinium macrocephalum* leaves with *Liothrips tractabilis* post application of sublethal doses of Picloram (**a**), Metsulfuron methyl (**b**) and Impala 480 EC (**c**). For each data point n= 8 plants (mean ± SE) Different lowercase letters indicate significant differences between doses at the end of the trial.

3.3.3 *Effect of herbicide on development of stems:*

On plants treated with picloram the initial number of stems on control plants at week 0 was at least three stems per plant, and that increased to seven stems per control plant (Figure 3a). The difference in number of stems of *C. macrocephalum* treated with 0.0035 and 0.0105 ml picloram concentration and control plants was not significant ($p > 0.05$). Plants treated with 0.175 ml dose of picloram had at least six stems which was similar to control plants at week 0, and the difference was not significant ($F_{7,49} = 2.82$; $p > 0.05$).

There were significantly fewer stems produced by plants treated with 0.125 g/100L (0.125 %) of metsulfuron methyl compared to plants treated with other doses (Figure 3b). The number of stems on plants that were treated with 0.025 g/100L dose and control plants were not significantly different by week eight ($F_{7,49}=2.49$; $p > 0.05$) (Figure 3b).

Similar trends were observed with plants treated with impala where plants that were treated with higher concentrations such as 0.175 ml/100L concentration produced lower number of stems compared to plants treated with a 0.1 g/100L dose ($F_{7,49} = 6.35$; $p < 0.01$) (Figure 3c).

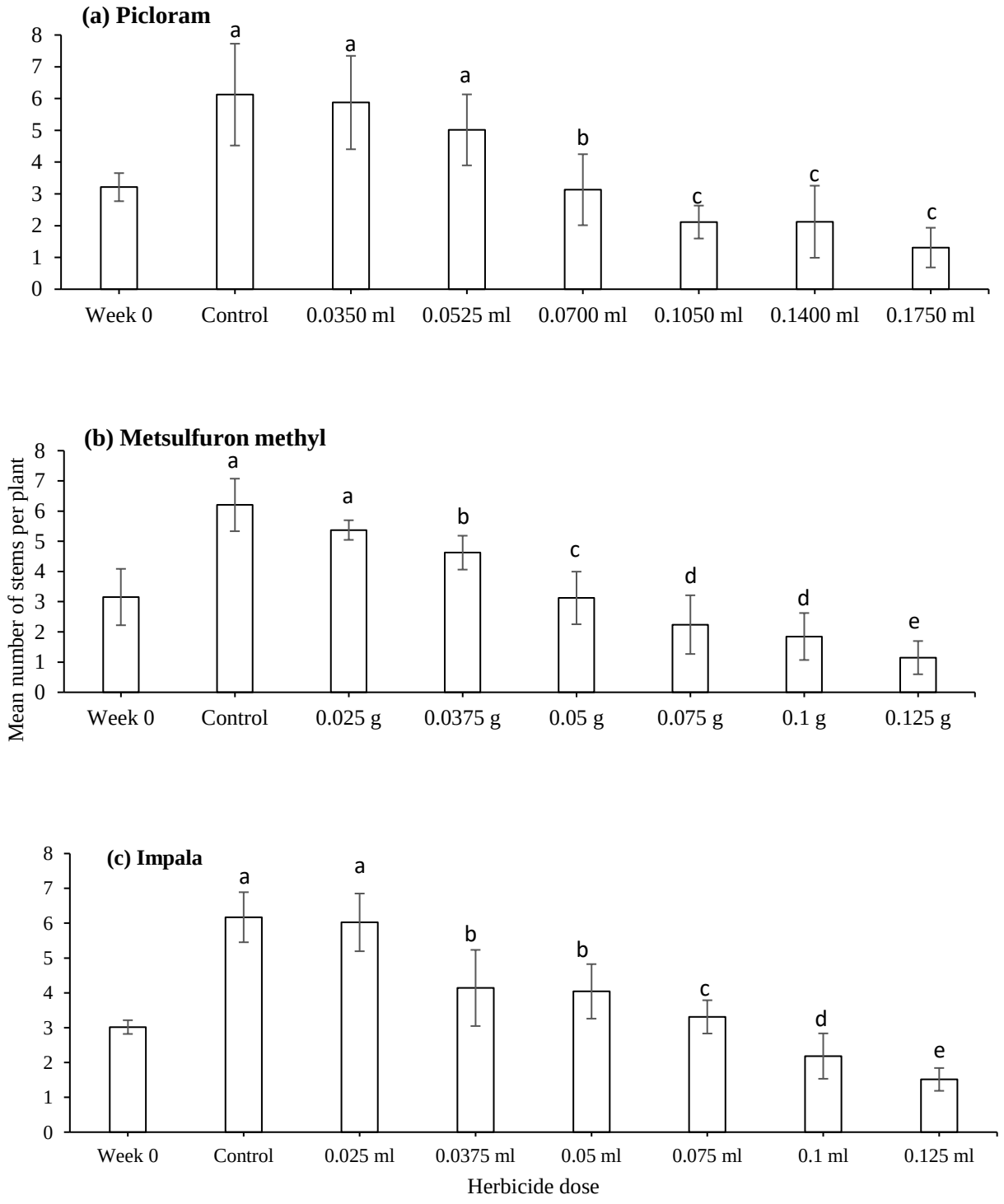


Figure 3.3: Mean number of *Campuloclinium macrocephalum* stems per plant at week eight with *Liothrips tractabilis* post application of sublethal doses of Picloram (a), Metsulfuron methyl (b) and Impala 480 EC (c). n= 8 plants (mean ± SE). Different lowercase letters indicate significant differences between herbicide doses ($p < 0.05$) for each of the three herbicides separately.

3.3.4 *Effect of herbicide on flowering stems:*

On plants treated with picloram the number of stems that flowered declined as the herbicide dose increased. Plants treated with 0.175 and 0.14 ml of picloram did not flower at all (Figure 4a). Plants that were treated with the 0.035 ml dose had at least two stems that flowered which was significantly lower than control plants which had four stems that flowered ($F_{7,49} = 6.37$; $p < 0.01$).

The number of stems that flowered on control plants were significantly higher compared to plants treated with the lowest dose of 0.025 ml of metsulfuron methyl ($F_{7,49} = 5.29$; $p < 0.01$) (Figure 4 b). There was no flowering on plants that were treated with the highest dose of 0.125 g of metsulfuron methyl (b). As the herbicide concentrations increased the number of stems that flowered declined.

Control plants had three stems that flowered, which was significantly higher compared to all the plants treated with the other different doses of impala ($F_{7,49} = 7.81$; $p < 0.01$) (Figure 4 c). Plants that were sprayed with high concentrations of 0.1 and 0.125 ml of impala did not flower at all.

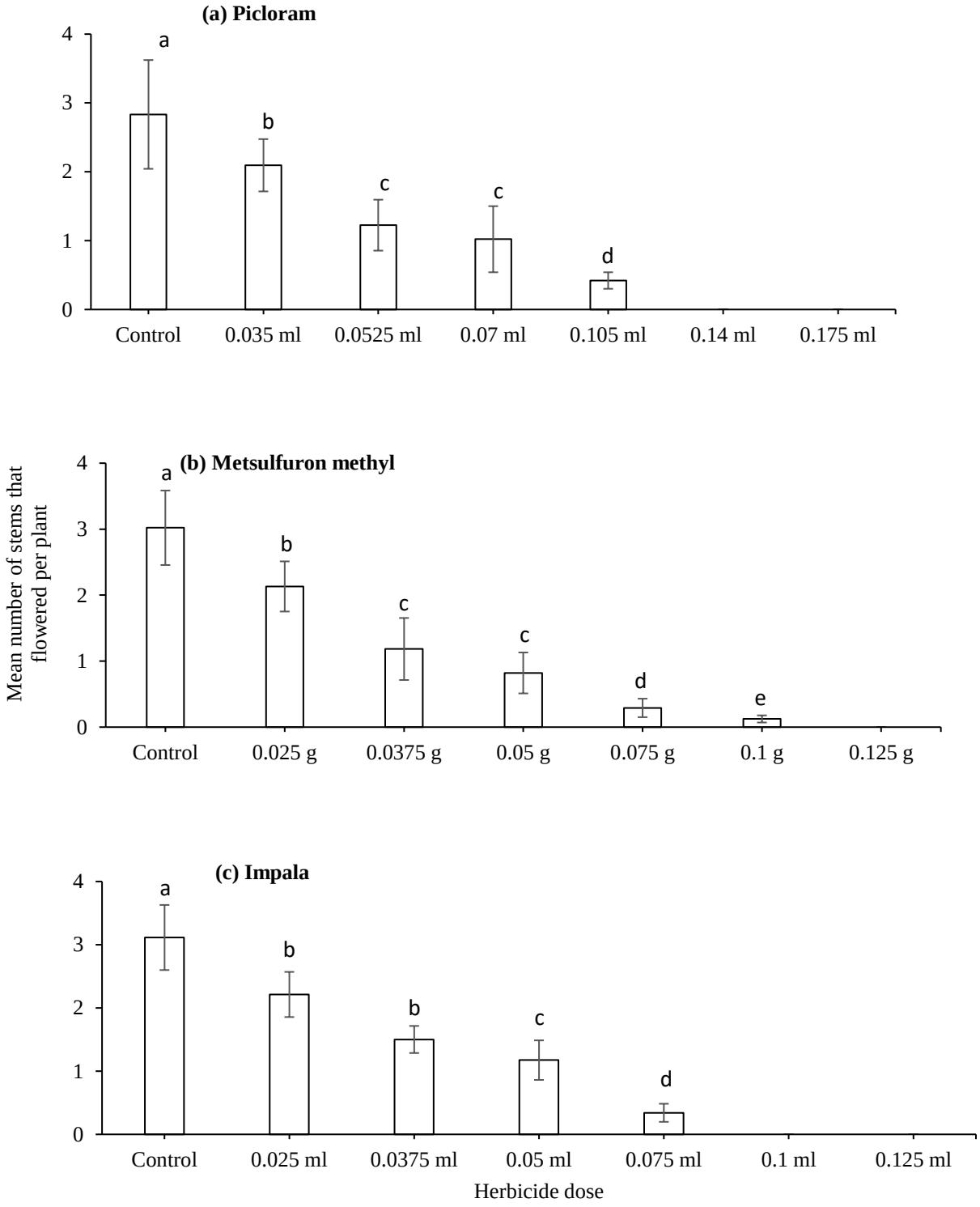


Figure 3.4: Mean number of stems that flowered per *Campuloclinium macrocephalum* with *Liothrips tractabilis* and after application of sublethal doses of Picloram (**a**), Metsulfuron methyl (**b**) and Impala (**c**) after eight weeks. n= 8 plants (mean ± SE). Different lowercase letters indicate significant differences between herbicide doses ($p < 0.05$) for each of the three herbicides separately.

3.3.5 *Effect of herbicide on Liothrips tractabilis adults:*

On plants treated with picloram the number of adult thrips on control plants increased from 10 at week zero to at least 53 adults per plant by week eight, the increase was significantly different from plants treated with the lowest concentration of 0.035 m1/100L picloram ($F_{7,49} = 9.79$; $p < 0.01$) (Figure 3.5a). As the picloram doses increased the number of adults thrips per plant declined across the treatments.

Plants treated with the lowest dose of metsulfuron methyl (0.025 g/100L) had 47 adult thrips per plant which was not significantly lower than on control plants which had an average of 51 adults ($F_{7,49} = 1.79$; $p > 0.05$) (Figure 3.5b). By the end of week eight there was a decrease in the number of adults on treated plants with an increase in herbicide concentration.

Similar results to the two other herbicides trends were observed with plants treated with impala whereby an increase in herbicide concentration resulted in a decrease in number of adults/plant (Figure 3.5c). Plants treated with the 0.125 ml/100L dose did not show any increase in the number of adults, they declined from 10 that was inoculated at week zero to seven by the end of week eight which was significantly compared to other doses ($F_{7,49} = 11.53$; $p < 0.01$).

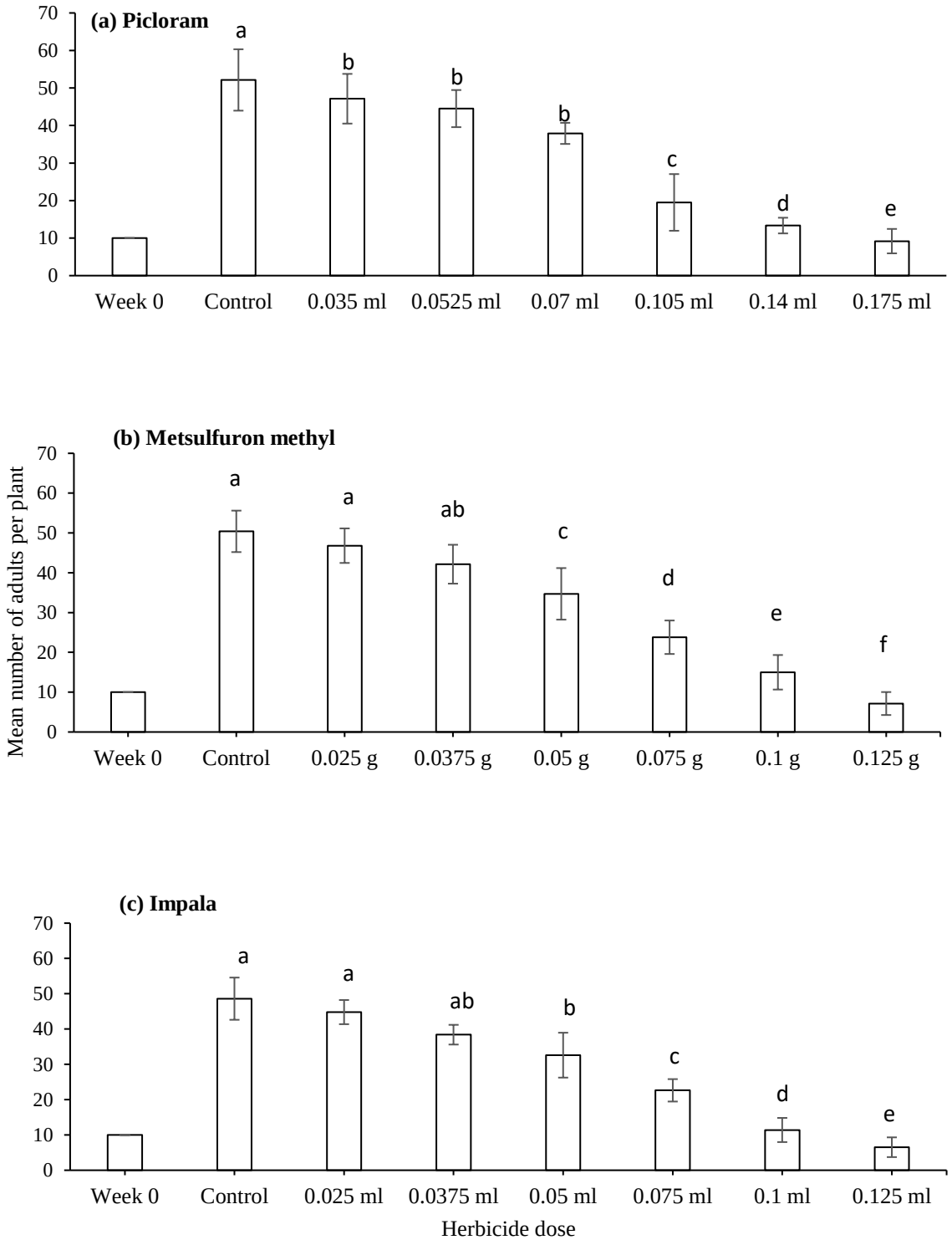


Figure 3.5: Mean number of *Liothrips tractabilis* adults per plant after application of sublethal doses of Picloram (**a**), Metsulfuron methyl (**b**) and Impala (**c**) after eight weeks. n= 8 plants (mean ± SE). Different lowercase letters indicate significant differences between herbicide doses ($p < 0.05$) for each of the three herbicides separately.

3.3.6 *Effect of herbicide on L. tractabilis nymphs:*

Control plants had a greater number of nymphs compared to all the treatment plants ($F_{7,49}$ =9.14; $p < 0.05$) (Figure 6a).

Control plants had an average of 73 nymphs per plant, with plants treated with the lowest dose of Metsulfuron 0.025 g/100L having 53 nymphs per plant, which was significantly lower ($F_{7,49}$ = 7.37; $p < 0.05$) (Figure 3.6b). As the doses were increased the number of nymphs declined, with the 0.0125 g/100L dose having the greatest negative effect on the nymphs.

The same trend was observed with plants treated with impala, where control plants had an average of 63 nymphs per plant which was significantly higher compared to other treatment plants ($F_{7,49}$ = 6.53; $p < 0.05$) (Figure 3.6c). Plants treated with the 0.1 ml and 0.0125 ml/100L doses had the lowest number of nymphs by the end of week eight.

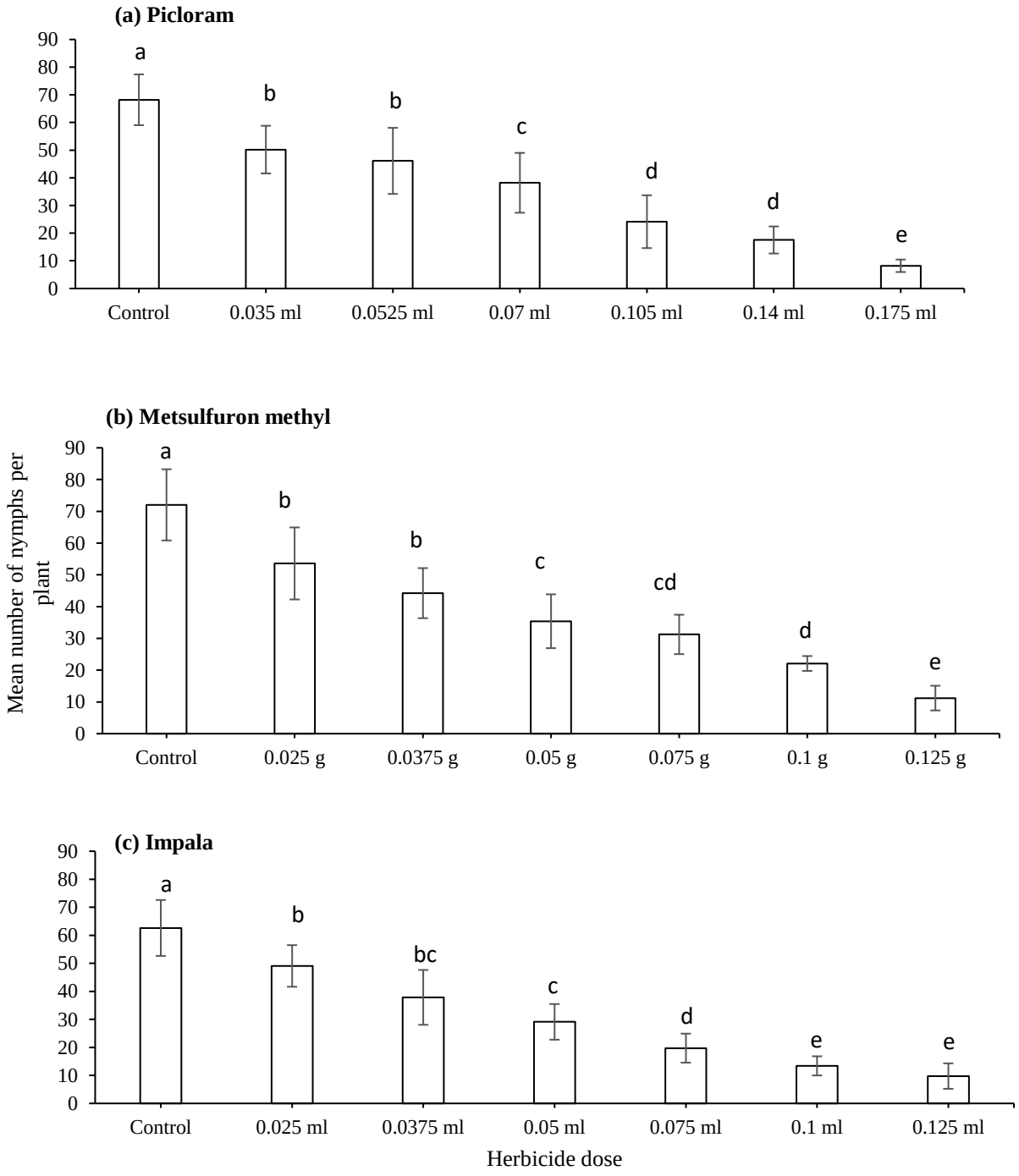


Figure 3.6: Mean number of *Liothrips tractabilis* nymphs per plant after application of sublethal doses of Picloram, Metsulfuron methyl and Impala 480 EC after eight weeks. n= 8 plants (mean ± SE). Different lowercase letters indicate significant differences between herbicide doses ($p < 0.05$) for each of the three herbicides separately.

There was a strong linear relationship between the mean number of *C. macrocephalum* stems per plant and the number of *L. tractabilis* (R^2=0.8885) (Figure 3.7). As the number of stems of *C. macrocephalum* per plant increased, the number of *L. tractabilis* adults on plants also increased.

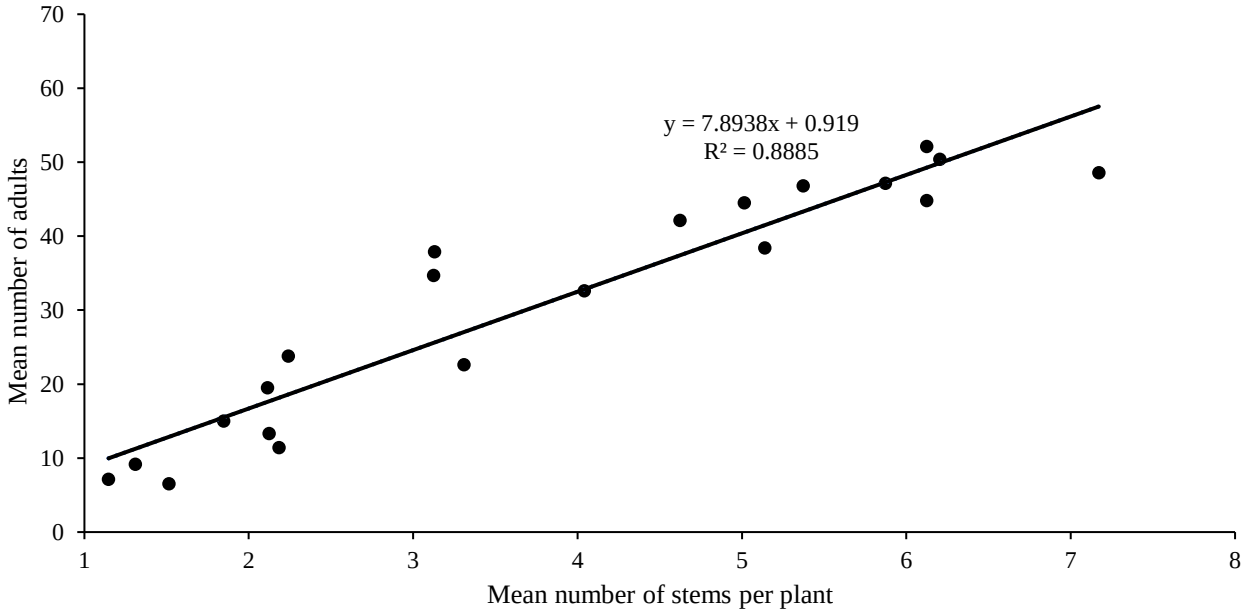


Figure 3.7: The strong linear relationship between the number of *Campuloclinium macrocephalum* stems per plant and number of adult *Liothrips tractabilis* per plant after eight weeks. n=21 plants.

3.4 Discussion

Campuloclinium macrocephalum exposed to non-lethal doses of three different herbicides showed changes in vegetative growth and flowering. The sub-lethal dose of picloram was identified to be 0.07 ml/100L, 0.05 g/100L for metsulfuron methyl and 0.075 ml/100L for impala. The recommended doses of picloram, metsulfuron methyl and impala herbicides did not have any direct impact by directly killing the biological control agent *L. tractabilis*. Multiple studies have also shown that many herbicides do not directly affect the biological control agents of invasive plants (Rees and Fay, 1989; Lym and Carlson, 1990; Lindgren *et al.*, 1999, Nelson and Lym; 2003). Jadhav *et al*. (2008) also found similar results when a non-lethal glyphosate dose was used against water hyacinth alongside *N. eichhorniae* and *N. bruchi*, and when picloram was integrated with the root-feeding flea beetle (*Aphthona nigriscutis*) a biocontrol agent of leafy spurge (*Euphorbia esula*) (Nelson and Lym, 2003). *Galerucella Calmariensis* L. (Coleoptera: Chrysomelidae) a biological control agent of purple loosestrife (*Lythrum Salicaria* L.,

Lythraceae) was also found to be compatible with glyphosate as oviposition was not affected and third instar larvae were able to pupate and mature (Lindgren *et al.*, 1999).

Integrating a non-lethal dose of glyphosate with biological control using weevils, *N. eichhorniae* and *N. bruchi* offered a potential control strategy for managing water hyacinth because the agent's reproductive capacity was not compromised by the application of herbicide (Jadhav *et al*., 2008). This was, however, different in this study, because the reproductive success of the thrips was reduced owing to indirect effects caused by the herbicide. The main effect of the three herbicides was wilting of the young pompom weed apical stem sections which are the preferred feeding sites of *L. tractabilis* where maximum damage is inflicted by the agent (McConnachie and McKay, 2015: Ramanand *et al*., 2017; Zachariades *et al.*, 2021). Unlike *L. tractabilis* that predominantly feeds on stems and lay eggs on deformed apical stems, the two agents against water hyacinth utilize different parts of the host plant with adults feeding on leaves, females laying eggs on fully expanded leaves and larvae feeding endophagously on the petioles making water hyacinth more likely to control (Julien *et al.*, 1999: Jadhav *et al*., 2008).

In North Dakota, USA, the combination of picloram, and the root-feeding flea beetle (*A. nigriscutis*), a biological control agent, resulted in a significant reduction in the density of invasive leafy spurge (*Euphorbia esula*) in a notably shorter timeframe of at least three years compared to insects alone (Lym and Nelson, 2002; Nelson and Lym, 2003). Despite the foliar application, picloram did not have substantial impact on the beetles feeding sites such as the roots, where herbicide was found to not affect the nutritional quality of the roots. The density of the flea beetle was not negatively affected; hence it was an effective integrated management of the weed (Nelson and Lym, 2003). In contrast in this study, the onset of leaf and apical stem wilting caused thrips to migrate and feed on the lower and older sections of the stem which are the least preferred feeding sites of *L. tractabilis*. As the number of stems per plant increased (which are the feeding sites) the abundance of adults also increased. The indirect impact of the herbicide on *L. tractabilis* resulted from an increase in herbicide dosage which reduced the number of stems per plant.

This shift in feeding sites of thrips resulted in a decrease in the number of adult and nymphal thrips. As the concentrations of herbicide were increased the reproduction of the agent declined because the number of stems declined. Numbers of adults and nymphs were not only lowered by

lack of feeding sites such as live apical stems which is where females lay their eggs. Despite the numbers of *L. tractabilis* being negatively affected by a non-lethal dose in relation to the control plants, it was still able to complete its life cycle on the treated plants because there was still enough plant tissue, even though the abundance decreased with an increase in dosage on host plant to sustain thrips populations. Some plants treated with non-lethal dose flowered during the sampling period suggesting that the seedbank of *C. macrocephalum* may still increase in the field.

In a study by Ainsworth (2003), herbicide application was suggested to have inhibited the synthesis of secondary metabolites such as phenylalanine, which is a feeding deterrent in many plants, making plants unpalatable to insects. There was an increase in feeding intensity of *N. eichhorniae* and *N. bruchi* on water hyacinth that was sprayed with herbicide (Jadhav *et al.*, 2008), suggesting a reduced synthesis of phenylalanine Ainsworth (2003). Levels of phenylalanine may have also been reduced in stems of pompom plants by the three herbicides which may have reduced the unpalatability of the least preferred old stems to *L. tractabilis* although deformation was limited.

Integration of a lethal herbicide dose and the leafy spurge gall midge *Spurgia esulae* Gagne (Diptera: Cecidomyiidae) against leafy spurge (*Euphorbia esula*) was successful because there was 15-20% of the weed that was left untreated to sustain the agent population while the herbicide significantly reduced the spread of the weed (Lym and Carlson, 1994: Lym, 2005). Combining herbicides with the gall midge also effectively reduced the spread of leafy spurge from wooded areas and decreased seed production beneath the tree canopy, where herbicide application was impractical (Lym, 2005). Considering the incompatibility of *L. tractabilis* with sub-lethal herbicide doses, a similar management strategy could be employed against *C. macrocephalum*. In this approach, a lethal dose of herbicide would be used in areas where thrips have failed to establish, while more populations of *L. tractabilis* could be released into sensitive areas where herbicide application may lead to contamination of water bodies. It’s worth noting that the usage instructions for picloram specify restrictions in areas where there's a potential for groundwater contamination (Goodall and Witkowski, 2023).

In the field, the recommended application period (November to early December) of herbicide (Goodall *et al.* 2012) may further undermine the efficacy of *L. tractabilis*. During this period, the

thrips population also establishes on the plants after overwintering, and the indirect impact of herbicide application may negatively affect the population of the agent. Additionally, spot spraying may not be feasible due to the high vegetation density of other vegetation during the application of the sub-lethal dose, resulting in some *C. macrocephalum* plants being missed. This could lead to ongoing pompom weed seed production and further contribute to the existing seedbank. It is also important to note that despite herbicide application along with the thrips, some plants were still able to flower in this laboratory trial, which may be the case in the field. Therefore, a more practical and feasible management strategy would involve targeting both the seed bank and mature plants. Such a strategy should include methods like fire, which targets the seed bank, along with a lethal herbicide dose aimed at mature plants before they flower, as discussed in Chapter 2.

Based on the laboratory findings of this study, integration of sub-lethal doses of any of the three herbicides (picloram, metsulfuron methyl and impala) and biological control using *L. tractabilis* in managing *C. macrocephalum* is not feasible and may not yield desired results given the indirect effects of the herbicides on *L. tractabilis*. It is worth highlighting that *L. tractabilis* establishment is variable (Zachariades *et al*., 2021), and the use of sub-lethal doses of any herbicides will negatively affect any population. Future field studies should explore the possibility of integrating the recently released flower feeder, *Cochylis Campuloclinium*, with *L. tractabilis* when they are released alongside each other. A sub-lethal dose trial on the flower feeder could also be undertaken, and if successful could greatly reduce pompom seed production.

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Chapter 4

Impact of different light environments on *Campuloclinium macrocephalum*, and its biological control agent, *Liothrips tractabilis*

Abstract

Campuloclinium macrocephalum is an invasive perennial herb native to Central and South America, commonly known as pompom weed in South Africa. Habitat management through mowing of the grassland vegetation before the growing season of *C. macrocephalum* improves the feeding and abundance of *L. tractabilis*, a biological control agent of the weed. Temperature and light intensity have variable effects on physiological parameters of plants such as stomatal conductance, chlorophyll content, chlorophyll florescence, intercellular CO_2 concentration and evapotranspiration. Temperature also has a direct influence on insect herbivory, reproduction, abundance and insect-plant interactions which may affect insect establishment on the target weed. The aim of this study was to investigate the growth response of *C. macrocephalum* plants when exposed to reduced sunlight (shade) and full sunlight. Secondly, to assess the direct and indirect effects of these on its biological control agent, *Liothrips tractabilis*. Chlorophyll content, chlorophyll fluorescence, stomatal conductance, growth rate and chlorophyll fluorescence were higher in shade pompom plants, suggesting increased photosynthetic rates compared to plants that were exposed to full sunlight. As a result, there was a significantly greater increase in height of pompom in the shade (maximum height =108 cm), relative to the sun (72 cm) over seven weeks. There was also a greater number of *L. tractabilis* on sun plants which resulted in 100% deformation compared to only 75% deformation of stems in shade plants. Light intensity has a significant impact on the growth of *C. macrocephalum* and the feeding performance of the biological control agent. An increase in light intensity resulted in increased feeding, insect numbers and plant deformation.

Keywords: Chlorophyll, deformed stem, insect establishment, feeding site, growth rate, grass mowing, light intensity.

4.1 Introduction

Campuloclinium macrocephalum (Less.) DC. (Asteraceae), is a perennial invasive herb from Central and South America, commonly known as pompom weed in South Africa (Henderson *et al*., 2006: Goodall *et al.*, 2010: McConnachie *et al.*, 2011). It has naturalized here and poses a threat to grasslands, wetlands and savannas in many parts of South Africa (McConnachie *et al.*, 2011: Henderson and Wilson, 2017). It causes significant reduction in grazing capacity of farms and game reserves, as it is unpalatable to livestock and wildlife (McConnachie and McKay, 2015). Pompom has increased its invasion range by 671% between 2006 and 2016 (O'Connor and van Wilgen, 2020).

The herb has a clustered, thick, tuber-like root system that enables it to survive a wide range of conditions such as frost, fire and low rainfall (Goodall *et al.*, 2012; Trethowan *et al*., 2011). In late autumn, stems and leaves senesce and the plant dies back to the perennial rootstock from which it regrows again in spring (Goodall and Witkowski, 2014). Given the weed's rapid rate of spread and negative impacts, a stem-deforming thrips, *Liothrips tractabilis* Mound and Pereyra (Thysanoptera: Phlaeothripidae), was released in 2013 against *C. macrocephalum* (McConnachie and McKay, 2015). *Liothrips tractabilis* adults and nymphs feed on the apical shoot tips resulting in deformation of the *C. macrocephalum* stems. This significantly reduces the growth, height, biomass, leaf and floral production of the host plant (Ramanand *et al.*, 2016; Mudau, 2019).

The new, spring growth of *C. macrocephalum* is more susceptible to *L. tractabilis* feeding than the mature foliage found later in the growth season (McConnachie and McKay, 2015: Ramanand *et al*., 2017). The impact of *L. tractabilis* is variable at different sites invaded by this weed (Zachariades *et al.*, 2021). It has established in some open grasslands and failed to establish in more mesic environments particularly seasonal wetlands (Zachariades *et al.*, 2021). There are several factors that may affect establishment of biological control agents include plant quality (Bala *et al.*,2018), and climatic conditions and elevation (Senaratne *et al.*, 2006).

In a study done in 2018, the feeding damage and abundance of *L. tractabilis* was improved through habitat management by mowing of a *C. macrocephalum* invaded grassland (Mudau, 2019). *Campuloclinium macrocephalum* plants on a mown plot experienced 1.4 °C higher temperature compared to plants on unmown plots, where they instead experienced reduced sunlight, being shaded more by long unmown vegetation. However, it remains unclear how the

physiology and growth rate of *C. macrocephalum* is affected by the difference in temperature and light intensities and how subsequent changes in the plant affect the biocontrol agent. Light intensity and temperature influence all characteristics of plant growth (Mahan and Yeater, 2008: Kim *et al.*, 2011) and have variable effects on physiological parameters such as chlorophyll content, chlorophyll florescence, intercellular CO_2 concentration (Morrow and Slatyer, 1971; Ball, 1987), stomatal conductance (Zhang and Zhang, 2019) and evapotranspiration (Irmak *et al.*, 2000; Ajayi and Olufayo, 2004). A higher chlorophyll content is a potential indication of higher photosynthetic rates (Uddling *et al.*, 2007; Porra, 2002).

Chlorophyll fluorescence measures plant photosystem *(PSII)* health in the leaves and higher chlorophyll fluorescence values are an indicator of a higher photosystem efficiency resulting in increased growth and plant quality (Maxwell and Johnson, 2000; Van Kooten and Snel, 1990: Guidi *et al.*, 2019). Nutritional quality and defensive compounds of plant tissue are key aspects that affect the abundance and feeding performance of phytophagous insects (Moniruzzaman *et al.*, 2009). Nitrogen is the main nutrient needed by insects for growth and increased reproduction (Rostami *et al.*, 2012).

Stomatal conductance regulates gas exchange such as CO_2 uptake and water evaporation through the opening and closing of stomata in response to environmental factors such as light, temperature, soil moisture, water availability and herbivory (Roberts and Paul, 2006: Boccalandro *et al.*, 2012: Djebbar *et al.*, 2012). Stomatal conductance generally increases as light increases and lead to increased photosynthesis and the demand for carbon dioxide. However, excessive light can lead to stomatal closure to prevent water loss (Kumar *et al.*, 2013). Elevated temperatures often lead to increased stomatal conductance as metabolic processes, including photosynthesis, are increased. Reduced soil moisture triggers stomatal closure to prevent excessive water loss through transpiration (Kim *et al.*, 2011). Different plant species and genotypes may have varying stomatal conductance in responses to environmental factors due to their physiological adaptations to light, temperature and humidity (Kusumi *et al.*, 2012). Generally, an increase in stomatal conductance promotes an increase in CO_2 uptake, thus enhancing photosynthesis (Kusumi *et al.*, 2012).

Temperature can have significant effects on insect herbivory, influencing both the behaviour and physiology of herbivorous insects as well as the growth and defense mechanisms of plants

(Patrick and Olckers, 2014). Moreover, warmer temperatures show a direct proportional relationship with insect herbivory, reproduction, abundance, and interactions between insects and host plants (Roberts and Paul, 2006; Venter *et al.*, 2013). Elevated temperatures are likely to stimulate insect activity and feeding rates, thereby increasing levels of herbivory (Wagner *et al.*, 1991). Furthermore, such temperature rises can accelerate insect metabolic and developmental rates, resulting in faster population growth and potentially higher herbivory levels (Ramanand *et al.*, 2017; Venter *et al.*, 2013). This study aimed to evaluate the impact of two different light intensities (full sunlight and reduced sunlight) on the growth of C. *macrocephalum*, and how it affects the feeding behavior and reproduction of *L. tractabilis* in a greenhouse setting. The objectives of the study were to examine the influence of varying light intensities on chlorophyll content, chlorophyll fluorescence, stomatal conductance, and growth of *C. macrocephalum*, and to assess how these influenced the reproduction and feeding impact of *L. tractabilis*.

4.2 Materials and methods

Experimental design and greenhouse trial conditions

Campuloclinium macrocephalum plants were grown by germinating seeds (collected at Waterkloof Airforce Base, Pretoria, South Africa in 2019) in trays in a glasshouse at the University of the Witwatersrand, Johannesburg. Individual seedlings were transplanted from trays into pots (Bottom diameter: 10 cm, Height: 12 cm, Top diameter: 15 cm) with soil during the growing season in November 2019. Once they had reached the 4-6 leaf stage 32 similar sized potted plants (~ 16 cm tall) were selected for the trial and then randomly divided into full-sun and reduced sun using shading netting (shade) environments. *Liothrips tractabilis* adults were obtained from the Plant Protection Research Institute (ARC-PPRI) research facility in Pretoria (Roodeplaat), Gauteng, South Africa. Within each light treatment (sun and shade), eight plants were used as controls (without insects) and eight plants as treatments (with insects). All the plants were kept in eight aluminum cages (50 (length) × 50 (width) × 100 (height) cm) each containing four plants of either control or treatment. Treatment plants were each inoculated with six (unsexed) *L. tractabilis* adults (week 0). Plants were watered daily without the use of ant fertilizer and all measurements on each plant were taken weekly for seven weeks.

Photosynthetic photon flux density (PPFD) was measured in each cage using an Apogee Quantum Flux Par Meter (MQ-500). The maximum PPFD in the full sun environment was 981

μmol m^{-2} s^{-1} and 433 μmol m^{-2} s^{-1} for the shade (56% reduction). The temperature of the two light environments was measured every hour using iButtons placed in one cage in sun the treatment (away from direct sunlight) and one cage in the shade, for the duration of the experiment.

Physiological responses of C. macrocephalum to different light conditions

To non-destructively determine the effect of light on different plant parameters, two leaves per plant (one 2nd and one 3rd leaf pairs) were marked with nail polish on their petioles. All measurements, chlorophyll content, chlorophyll fluorescence and stomatal conductance were taken on all control and treatment plants weekly for a duration of seven weeks on the two marked leaves of each plant. Initial fluorescence (F_o) and maximum fluorescence (F_m) were measured, and the maximum quantum yield of PSII ($F_v/F_m=F_m-F_o/F_m$) was calculated using an OS1p Modulated Chlorophyll Fluorometer (Opti-Sciences, Inc. NH 03051 USA).
A Single Photon Avalanche Diode (SPAD) 502 Chlorophyll Meter (Minolta, Osaka 542, Japan) was used to measure the chlorophyll content of the marked leaves. A leaf Porometer (SC-1, Decagon devices, USA) was used to estimate stomatal conductance by measuring the vapour flux of the plant stomata (Ball *et al.*, 1987). Soil moisture was also measured weekly using a HydroSenseII moisture probe manufactured by Campbell Scientific (Utah, United States).

Impact of L. tractabilis on C. macrocephalum under different light conditions

The impact of *L. tractabilis* on the host plant was quantified by measuring the plant height using a tape measure, counting the number of leaves and flowers and the number of deformed plants. Comparison was made between plants with and without insects in the sun and shade. Mudau (2019) defined deformation as plants with altered apical shoot tips that showed inhibited vertical growth of stems. The same criterion was used in this study. After seven weeks, plants were harvested and separated into below and above ground biomass. Soil particles were carefully washed off the roots. All the fresh leaves were photographed to measure their surface area. The number of *L. tractabilis* adults, nymphs and eggs were counted on the above ground tissues using a dissecting microscope. The presence of the fungus, *Puccinia eupatorii* (Pucciniales: Pucciniaceae) was also noted on the leaves across all treatments. The fungus causes necrosis and abscission on leaves of *C. macrocephalum* resulting in premature death of stems (Goodall *et al.*, 2012). All plant biomass was oven dried for five days at 55 °C and weighed.

Statistical analysis

The Shapiro-Wilk test was first used to test if the data were normally distributed. One-way repeated measures ANOVA was used to compare the overall difference between the parameters between sun and shade, for each of the eco-physiological measures taken. Tukey's HSD was used for post-hoc tests to identify differences between the sun and shade with and without *L. tractabilis* (treatment and control). A Fisher's exact test was conducted to test the difference in percentage of deformation and flowering on plants in the sun and shade. T-tests were used to assess the differences between the parameters from the two environments. ImageJ software (Java 1.8.0_345) was used to calculate the surface area of the leaves. Analyses were conducted using SPSS Statistics 22.0, and Microsoft Excel 2016.

4.3 Results

There was a significant difference ($p < 0.0001$) in mean diurnal photosynthetic photon flux density (PPFD) between the sun and shade cages for the duration of the experiment (Figure 4.1A). The mean daytime temperatures (10h00–17h00) were also significantly different ($P=0.031527$) at 28.6 and 27.7 °C (0.9 °C) between respective light treatments (Figure 4.1B). The mean relative soil moisture was higher in the shade compared to the sun with a difference of 8.8% ($p < 0.0001$, Figure 4.1C).

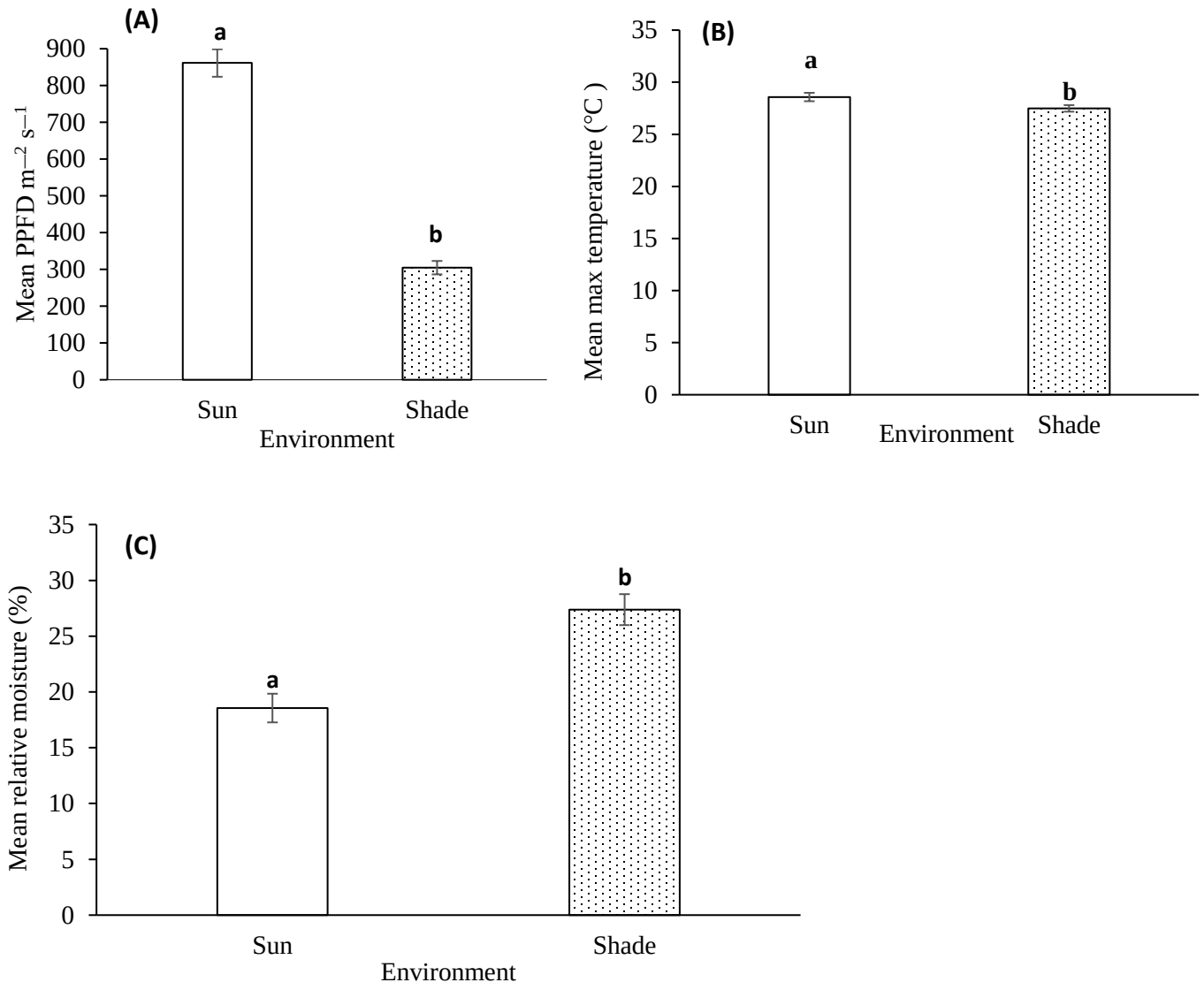


Figure 4.1: Photosynthetic photon flux density (PPFD) (A), average daily temperatures (B) and relative soil moisture (C) (mean ± SE) of *Campuloclinium macrocephalum* plants in sun and shade for seven weeks. n= 16 plants/treatment (mean ± SE). Different lowercase letters indicate significant differences between sun and shade environments (t-test, $P < 0.05$).

Chlorophyll concentrations (SPAD values) were higher in shade plants compared to plants exposed to full sun (Figure 4.2). Within the sun environment, there was a significant response to the presence of *L. tractabilis*, in that treatment plants had significantly higher chlorophyll concentration ($F_{3,60} = 41.36$; $p= 0.028$). After week four, there was a decline in chlorophyll concentrations in both sun and shade plants as the plants aged.

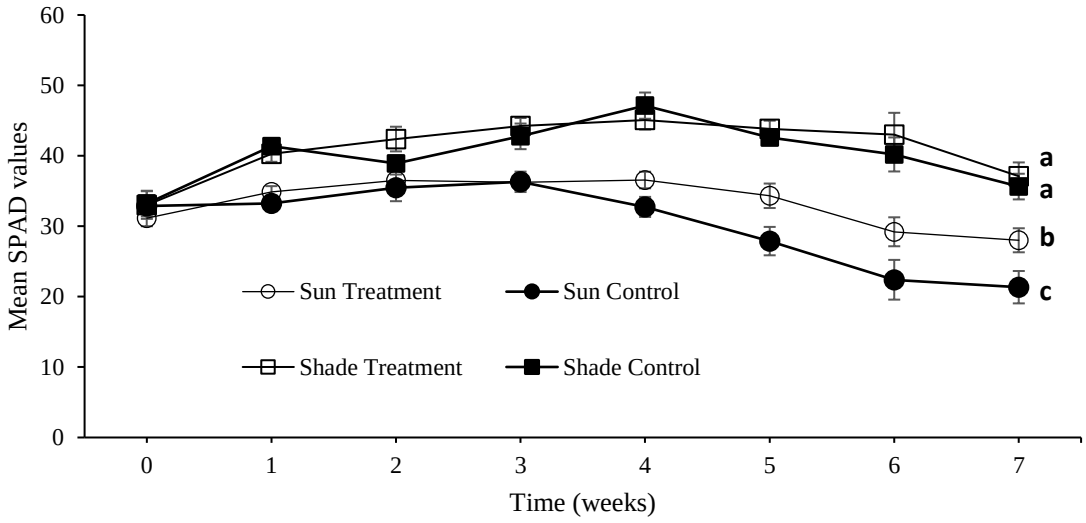


Figure 4.2: Chlorophyll content (SPAD units: mean ± SE) for *Campuloclinium macrocephalum* leaves with (treatment) and without (control) thrips, *Liothrips tractabilis*, in the sun and shade for seven weeks. For each data point n= 16 plants/light treatment (two leaves per plant). Different lower case letters indicate significant differences assessed at the end of the trial period Tukey, $p<0.05$).

Maximum PSII quantum efficiency (F_v/F_m) in the sun was significantly lower than in the shade plants ($F_{3,60} = 0.749$; $p< 0.018$) (Figure 4.3). In the sun the treatment plants had significantly higher F_v/F_m than the control, while the difference in treatment and control plants in the shade was not significant (p=0.715). The presence of *L. tractabilis* resulted in an increase in chlorophyll fluorescence in treatment plants in the sun environment.

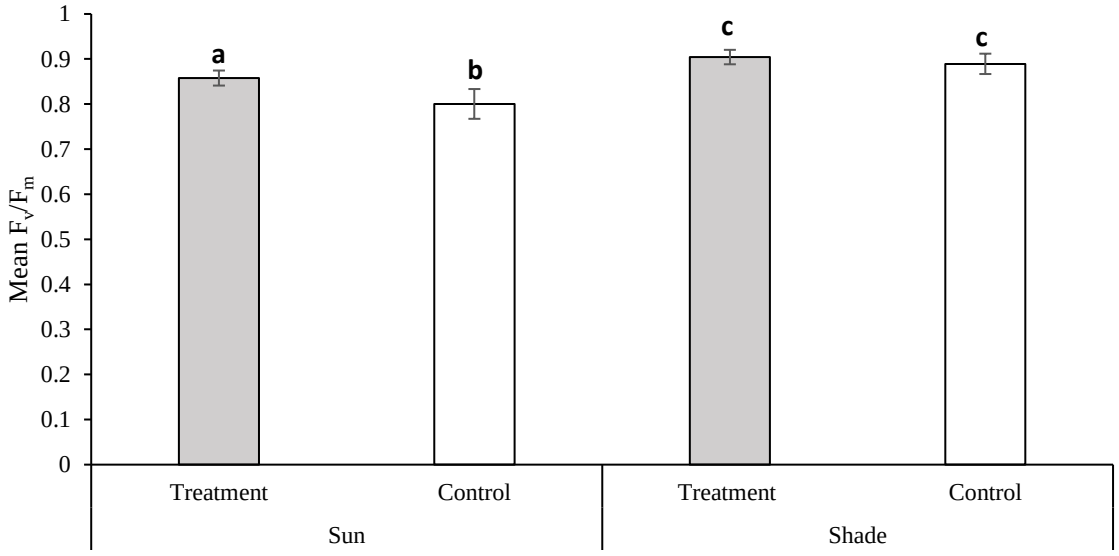


Figure 4.3: Maximum PSII quantum efficiency (Chlorophyll fluorescence (mol m-2s-1n)) of *Campuloclinium macrocephalum* plants with (treatment) and without (control), thrips, *Liothrips tractabilis*, in sun and shade for seven weeks mean ± SE. n= 16/light treatment (two leaves per plant). Different lowercase letters indicate significant differences assessed at the end of the trial period (Tukey, $p<0.05$).

Stomatal conductance of *C. macrocephalum* in the sun was higher after week four on both the control and treatment plants compared to plants in the shade (Figure 4.4), and there was a significant difference in stomatal conductance of plants in the shade and sun for both control and treatment plants ($F_{3,60} = 144.71$; $p< 0.001$). Treatment plants in the sun had higher stomatal conductance from week four compared to control plants. The presence of *L. tractabilis* on treatment plants did not result in an increase or decrease in stomatal conductance.

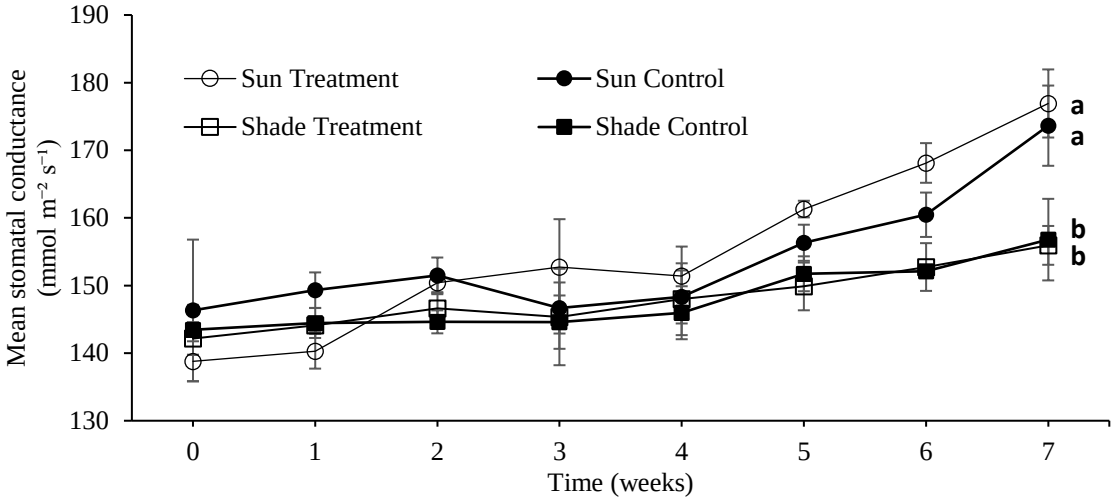


Figure 4.4: Stomatal conductance of *Campuloclinium macrocephalum* plants with (treatment) and without (control) thrips, *Liothrips tractabilis*, in sun and shade for seven weeks. For each data point n= 8 plants/light treatment (two leaves per plant) (mean ± SE). Different lowercase letters indicate significant differences assessed at the end of the trial period (Tukey, $p<0.05$).

The abundance of *L. tractabilis* was significantly higher in the sun plants than plants in the shade (Figure 4.5) ($p< 0.0001$). The averge number of nymphs per plant was over three times greater in sun plants than in the shade. Adult numbers were in week seven 23 and eight per plant in sun and shade. The greater abundance of adults and nymphs in the sun resulted in 100% plant deformation, with only 75% deformation in shade plants ($p< 0.5$).

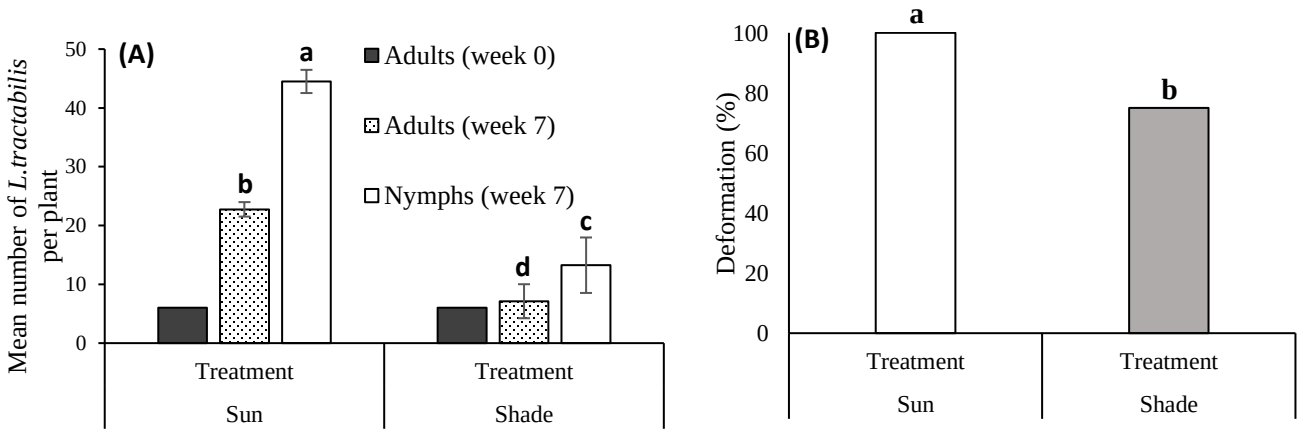


Figure 4.5: Number of *Liothrips tractabilis* adults and nymphs per plant in sun and shade for seven weeks (**A**), percentage of deformed *Campuloclinium macrocephalum* plants with *Liothrips tractabilis* (treatment) (**B**) (n= 8 plants/light treatment, mean ± SE). Different lowercase letters indicate significant differences $P < 0.000$)

The fungus (*Puccinia eupatorii*) first appeared in week four, significantly affecting the leaves of control plants in the sun, followed by the treatment plants (sun) ($F_{3,28}$=5.43; p= 0.0225) (Figure 4.6). The number of leaves affected by the fungus increased over time in both the sun and shade environments, there was however no significant difference between the sun treatment plants and both control and treatment plants in the shade ($F_{3,28}$=144.71; p= 0.25).

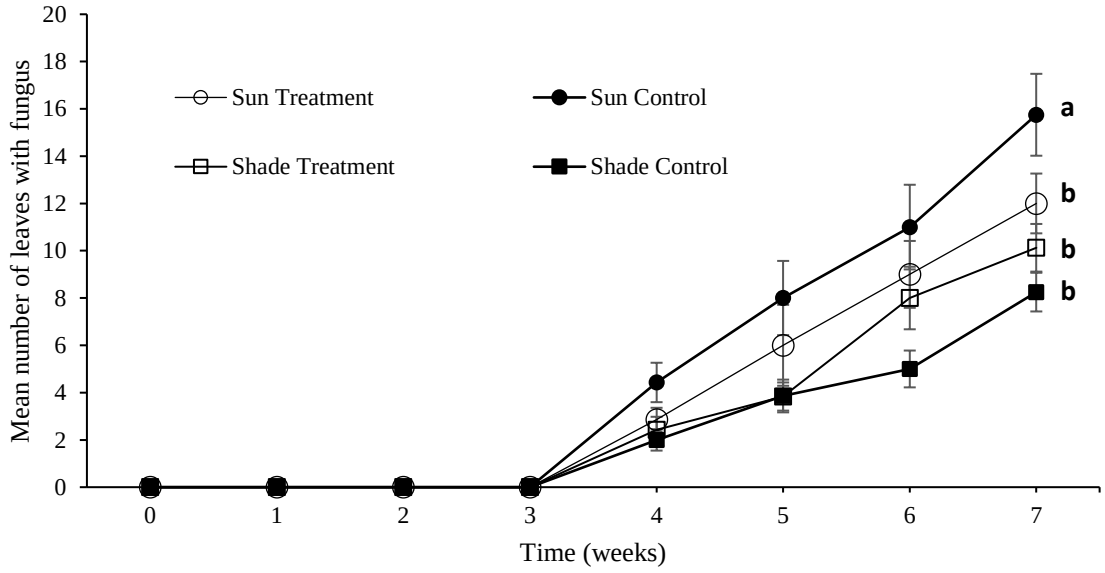


Figure 4.6: Number of *Campuloclinium macrocephalum* leaves per plant with (treatment) and without (control) thrips, *Liothrips tractabilis*, in sun and shade over time that were affected by the fungus (*Puccinia eupatorii*). For each data point n= 8 plants/light treatment (mean ± SE). Different lowercase letters indicate significant differences assessed using one-way repeated measures ANOVA (P < 0.05).

The height of *C. macrocephalum* in the sun and shade were similar at week zero, at 17 cm (Figure 4.7). As the weeks progressed the height of control plants in the shade and sun increased and by week seven and the difference was significant compared to the treatment plants ($F_{3,28}$ = 8.62; p <0.001; $F_{3,28}$ = 6.18; p= 0.015).

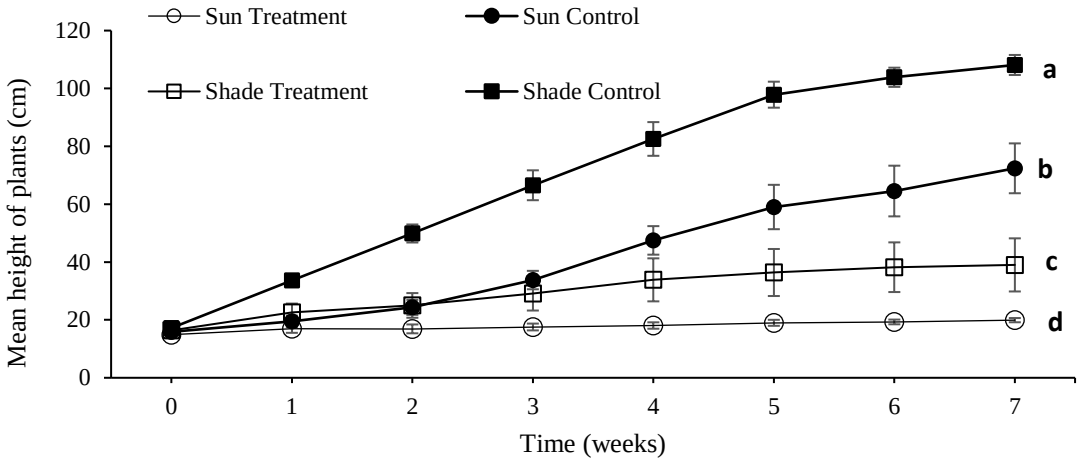


Figure 4.7: Height (growth rate) of *Campuloclinium macrocephalum* plants with (treatment) and without (control), thrips, *Liothrips tractabilis*, in sun and shade over time. For each data point n= 8 plants/light treatment (mean ± SE). Different lowercase letters indicate significant differences assessed using at the end of the trial period (Tukey, $p<0.05$).

The number of leaves per plant in both sun and shade plants increased up to week three (Figure 4.8). Thereafter, there was decline in the number of leaves on treatment plants in both the sun and shade induced by stem deformation, with a continued increase in the number of leaves on control plants. At week seven, there was a significant difference between treatment plants and control plants both in sun and the shade ($F_{3,28}$= 14.8; p= 0.0151).

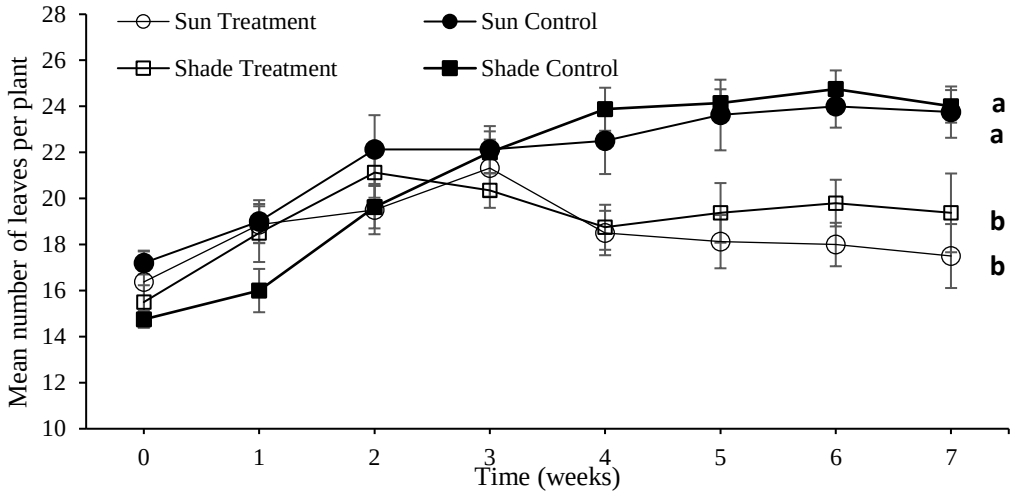


Figure 4.8: Number of leaves per plant of *Campuloclinium macrocephalum* with (treatment) and without (control), thrips, *Liothrips tractabilis*, in sun and shade over time. For each data point n= 8 plants/light treatment (mean ± SE). Different lowercase letters indicate significant differences assessed at the end of the trial period (Tukey, $p<0.05$).

A significant difference in leaf surface area was found between plants exposed to shade and those exposed to sunlight (Figure 4.9A). Specifically, leaves in the shade exhibited a surface area three times larger than those exposed to the sun ($p<0.001$). Additionally, there were noticeable difference in both size and coloration between the leaves of control plants grown in different light conditions (Figure 4.9B).

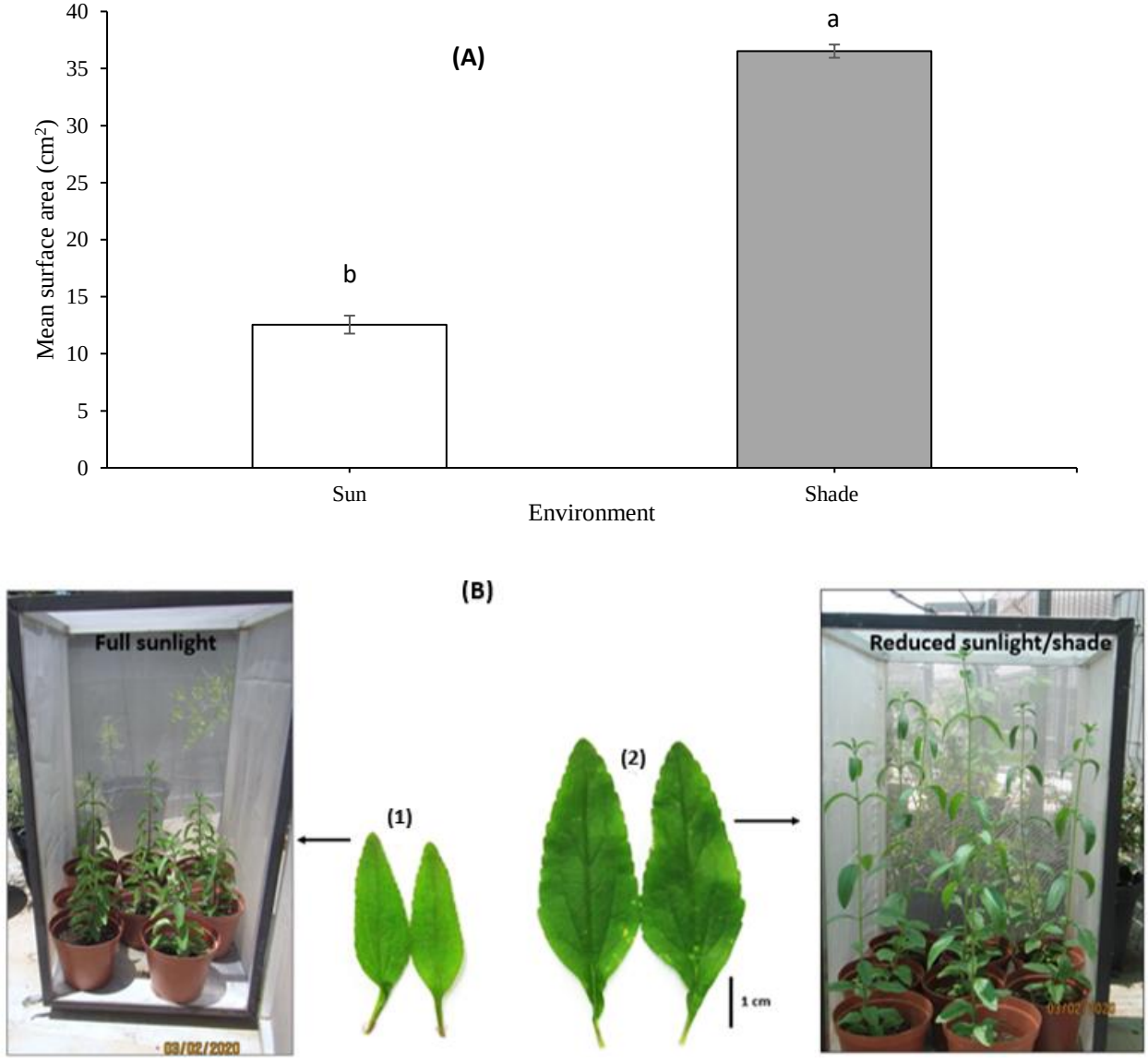


Figure 4.9: The mean surface area of leaves (A) and the difference in height, leaf size and colour in mature, fully developed leaves of *Campuloclinium macrocephalum* under sun (left-1) and shade (right-2) at week six (B). Different lowercase letters indicate significant differences assessed using a t-test.

There was a greater number of flowering plants in the shade, with all the shade control plants producing flowers (100%) versus only 75% in the sun control plants (Figure 4.5). The effect of full sunlight and shade on the flowering of control plants was significant (p=0.0132, Fishers exact test). The abundunce of *L. tractabilis* on treatment plants in the sun had a significant impact on flowering (p=0.0001, Fishers exact test). *Liothrips tractabilis* in the sun inhibited the plants from flowering, resulting in no plants flowering. Despite the presence of *L. tractabilis* on shade treatment plants, 38% (three plants) still flowered.

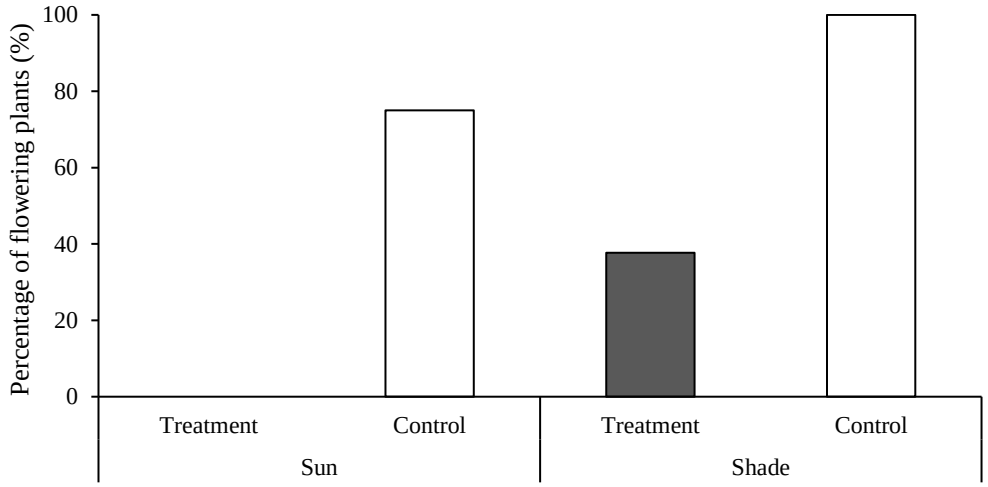


Figure 4.10: Percentage of *Campuloclinium macrocephalum* plants with (treatment) and without (control), thrips, *Liothrips tractabilis* that flowered in sun and shade after 7 weeks. n= 8 plants (mean ± SE).

The above ground biomas of treatment plants in the sun was significantly lower than control plants after seven weeks ($F_{3,28}$= 2.55; p< 0.05) (Figure 4.11). The presence of *L. tractabilis* resulted in significant reduction of the above ground biomass of plants in the sun and shade ($F_{3,28}$= 6.73; p< 0.001). The below ground biomass of treatment and control plants in both sun and shade were not significantly different (p > 0.05). Therefore, presence of *L. tractabilis* on treatment plants in the sun ($F_{3,28}$= 2.03; p=1.21) and shade ($F_{3,28}$= 1.22; p= 0.86) did not have an impact on the below ground biomass of of the host plant.

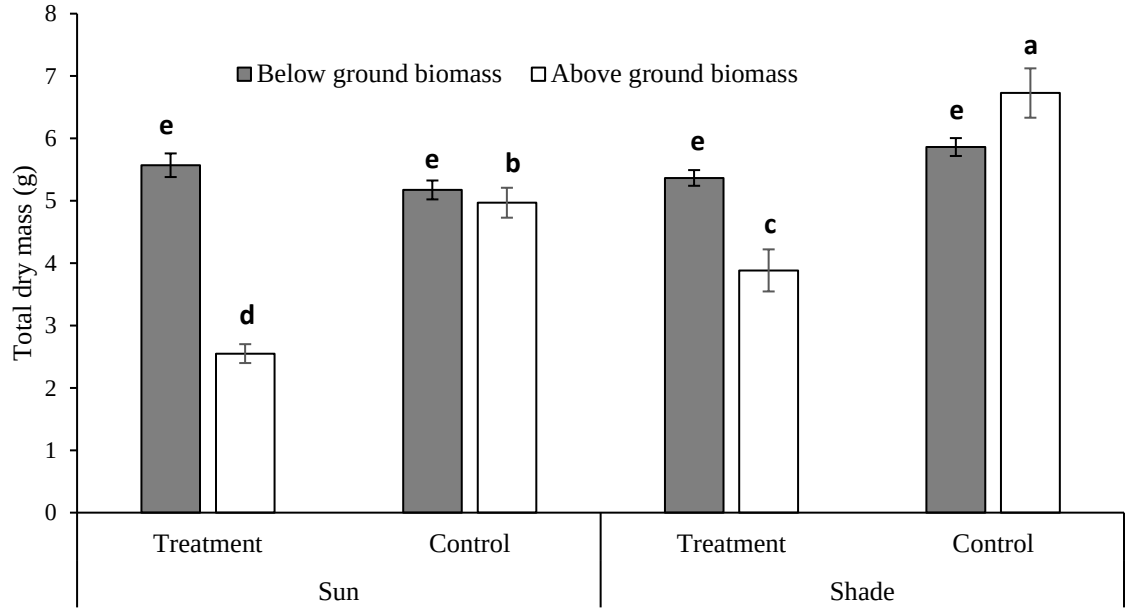


Figure 4.11: The effect of light and thrips, *Liothrips tractabilis*, on *Campuloclinium macrocephalum* below ground biomass (root) and above ground (leaves and stems) biomass in sun and shade after 7 weeks. n = 8 plants/light treatment (mean ± SE). Different lowercase letters indicate significant differences for above and below ground separately (Tukey, $P<0.05$)

4.4 Discussion

Differences in light intensity and temperature significantly influenced the physiology and morphology of *C. macrocephalum*. Plants grown in the shade had longer stems compared to those grown in sunlight. After seven weeks, control plants reached a maximum height of 74 cm in the sun, whereas they reached 108 cm in shade. This phenomenon was observed in various plant species such as bangladhonia (*Eryngium foetidum* L.), common sage (*Salvia officinalis*), and clary sage (*Salvia sclarea*) which tend to grow relatively taller and exhibit apical dominance when exposed to reduced sunlight. This response is also documented by Moniruzzaman *et al.* (2009), Rezai *et al.* (2018) and Kumar *et al.* (2013). Falla *et al.*, (2023) found similar results, where *Solanum mauritianum* Scopoli (Solanaceae) plants grew taller in the shade compared to plants in less shade. Plants adjust their resource allocation in response to light availability, in environments where direct light is limited. Plants invest more energy into vertical growth rather than lateral growth (Taiz and Zeiger, 2002). This allocation strategy allows them to maximize light capture within the constraints of their environment. Therefore, the elongated stems observed in shaded plants may be attributed to this adaptive response to optimize light capture,

as described by studies such as those conducted by Dole *et al.* (2004) and Valladares and Niinemets (2008).

In the shaded environments, *C. macrocephalum* demonstrated rapid growth, leading to the early maturation of its stems. Despite experiencing deformation due to *L. tractabilis* (Figure 4.9), some of the *C. macrocephalum* plants still managed to produce flowers, which reduced the effectiveness of biological control measures in shaded conditions. This ability of *C. macrocephalum* to thrive despite damage is similar to the resilience observed in water hyacinth, *Eichhornia crassipes* (Mart.) Solms-Laubach (Pontederiaceae), which can outgrow damage caused by the beetle *Neochetina eichhorniae* (Coleoptera: Curculionidae) in water bodies with high phosphorus levels surpassing 0.1 mg/L (Katembo *et al*., 2013). This resilience was attributed to the enhancement of plant growth rates by the additional nutrients. Thrips are known to prefer young stems, yet in shaded environments, *C. macrocephalum* stems experienced accelerated growth, mainly vertically, resulting in reduced biomass. Consequently, the abundance of *L. tractabilis* in the shade was low. Conversely, in sunny conditions, *C. macrocephalum* exhibited slower growth rates and less elongation of stems, facilitating greater deformation by *L. tractabilis* in relation to plants from the shade. And despite this, the number of *L. tractabilis* in the sun exceeded those in the shade. The reduced light intensity in the shade favoured greater stem growth rates of the host plant but had a negative impact on the abundance of *L. tractabilis*, thereby promoting pompom growth and resulting in a greater above ground biomass.

The abundance and performance of *L. tractabilis* was not solely influenced by light intensity but were also affected by how the host plant responded to changes in microclimatic conditions, such as variations in light intensity. Differences in microclimatic factors led to variations in the observed morphology and physiology of *C. macrocephalum*. Similar morphological differences were observed in field areas infested with pompom, where accompanying vegetation was either mown or burnt. When *C. macrocephalum* was exposed to more direct sunlight, it reduced its growth rate, favouring the development of *L. tractabilis*, which subsequently had a significant impact as a biological control agent (Mudau, 2019). Therefore, utilizing fire as a management tool before *C. macrocephalum* regrows not only helps reduce the seedbank (Chapter 2) but

effectively mows down accompanying vegetation, further facilitating the establishment of *L. tractabilis*.

In the field, the height of pompom was directly proportional to that of the accompanying vegetation (Chapter 2, Figure 7) with pompom being relatively taller than the surrounding vegetation and displaying apical dominance. This further suggests that light is the key driver in height differences of *C. macrocephalum* in sun and shade. However, the density of leaves on *C. macrocephalum* control plants in both sun and shade was not significantly different, the main difference was plant height, leaf shape and size. Feeding by *L. tractabilis* reduced the density of leaves as a result of deformation of the stems. The surface area of individual leaves in the shade was greater than in full sunlight. A similar pattern was observed in leaf size of common sage (Rezai *et al.*, 2018), blueberry (*Vaccinium corymbosum*) (Kim *et al.*, 2011) and clary sage, *Salvia sclarea* L. (Kumar *et al.* 2013) which all increased with an increase in shade levels. This adaptation ensures that plants capture the maximum amount of sunlight available for photosynthetic activity in a more shade environment where the levels of light are relatively low (Niinemets *et al.*, 1998: Taiz and Zeiger, 2002).

In general, increased leaf size in shade plants often leads to lower stomatal conductance, which helps reduce water loss through transpiration (Holland and Richardson, 2009; Djebbar *et al.*, 2012). Similarly, in this study the stomatal conductance of *C. macrocephalum* in shade plants was relatively lower than in plants in the sun. According to Kusumi *et al*. (2012), an increase in stomatal conductance subsequently enhances photosynthesis, however the role of stomata in regulating phosynthesis may differ depending on the plants species. Higher chlorophyll content generally indicates a greater potential for photosynthetic activity because more chlorophyll molecules are available to capture light energy (Nagaraj *et al.*, 2002; Rezai *et al.*, 2018; Kakoti *et al.*, 2023).

Greater chlorophyll content of *C. macrocephalum* in the shade may have resulted in higher photosynthetic activity which explains the rapid growth rate and larger leaves. Chlorophyll fluorescence and chlorophyll content in mahogany (*Swietenia macrophylla* King) and tonka bean (*Dipteryx odorata* Aubl. Willd) leaves under two light environments (full sunlight and reduced light) were also found to be higher in the shade than sun (Goncalves *et al*., 2001). Similar to our study, this resulted in an improved growth rate in terms of height gained per week in plants in the

shade compared to plants in full sunlight. Leaves of *Lantana camara* L. (Verbenaceae) in the shade were also found to have higher chlorophyll content compared to plants in the sun (Venter *et al.,* 2019). Chlorophyll content in plants however tends to decline with an increase in leaf-feeding and sap-sucking insects (Nagaraj *et al.*, 2002; Venter *et al.*, 2019; Cowie *et al.*, 2016). There was an increase in chlorophyll content on plants with *L. tractabilis* compared to control plants in both sun and shade plants. This may have been because *L. tractabilis* feed and deform stems compared to most insects that feed on leaves. This response may have been due to the impact of *L. tractabilis*, causing deformation of the stem restricting development of new leaves. The higher levels of light intensity promoted the abundance, development and feeding damage by *L. tractabilis*.

The greater abundance of *Liothrips tractabilis* (both adults and nymphs) on sun-exposed plants was influenced by the increased light intensity and temperature in this treatment. The increased developmental rates of insects are typically associated with higher temperatures, as supported by studies such as those conducted by Campbell *et al.* (1974), Logan *et al.* (1976), and Ramanand *et al.* (2017), but can also be affected by the nutritional quality of plants (Awmack and Leather, 2002; Bala *et al.*,2018). Consequently, the impact of *L. tractabilis* was greater in full sun areas, leading to 100% deformations of the plants compared to 75% in shaded areas. In shaded environments, insects generally experience reduced temperatures than those in direct sunlight, which is likely to reduce their metabolic rates, activity levels, and overall performance, as noted by Logan *et al.*, (1976).

The increased deformations observed in plants in the sun resulted in a reduction in the number of flowers and plant height. Although similar reductions in flowering and height were observed in shade plants, the impact of *L. tractabilis* was less significant due to slower development rates, resulting in fewer thrips and thus less stem deformation. These findings align with field observations by Mudau (2019), who noted a higher abundance of *L. tractabilis* and increased levels of deformation in *C. macrocephalum* plants growing in areas with greater irradiance. Other biological control agents, such as *Teleonemia scrupulosa* (Hemiptera: Tingidae) (Venter *et al.,* 2019), *Corythuca arcuata* (Hemiptera: Tingidae), and *Liothrips urichi* (Nakahara *et al.*, 1992), have also been observed to be more abundant in sites with relatively high temperatures.

Liothrips urichi, in particular, has a greater feeding impact on host plants in open sunny environments because of the increased abundance of the agent (Nakahara *et al.*, 1992).

Light intensity, which affected the growth patterns of *C. macrocephalum* emerged as a significant factor influencing both the abundance and performance of *L. tractabilis*. Although there was a slight difference of 0.9°C in the average maximum temperature experienced by *C. macrocephalum* and *L. tractabilis* in sun (28.58°C) versus shade (27.49°C) conditions. Based on a laboratory study on temperature and development of *L. tractabilis* (Ramanand *et al.*, 2017), the estimated number of generations of *L. tractabilis* during the sampling period was very similar, 1.38 and 1. 24 for the sun and shade respectively suggesting that temperature was not a key determinant in thrips abundance. Similar field results were found in a study by Mudau (2019), where the difference in maximum temperature between mown and unmown blocks was 1.4^{o}C. Despite this difference, the estimated number of generations of *L. tractabilis* during the sampling period from October to April remained similar, with 8.47 generations on the mown block and 8.35 generations on the unmown block (Mudau, 2019). Future studies must consider using climate controlled champers (e.g. CONVIRONS) trials, where light and temperature and even CO_2 can be controlled, in order to specifically identify the effect of each factor. It is however unclear how different light intensities affect the quality of the host plant. The next chapter will investigate and compare the nutritional quality of apical stems and old stems (mid-section) of *C. macrocephalum* that grew in long and short vegetation. The objectives of the chapter will also include assessing the nitrogen and carbon content, C:N ratio and δ15N ‰ of apical and old mid-sections.

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Chapter 5

The effect of long and short vegetation on the nutritional quality of *Campuloclinium macrocephalum* stems.

Abstract

Plant nutrients and insect herbivory interact with each other with both positive and negative effects. Nutritional quality of plants can be quantified levels of nitrogen (N) and availability of digestible carbon (C). The feeding performance and abundance of *Liothrips tractabilis*, a biological control agent of *Campuloclinium macrocephalum* showed preference for young apical shoot stems compared to more mature stems. In the field, the abundance and feeding performance of *L. tractabilis* was higher in plants in short vegetation (sun) compared to long vegetation (shade). The aim of this study was to assess nutritional quality of *C. macrocephalum* growing in long and short vegetation to understand the preference for young apical shoot stems over mature stems. The objectives included evaluating and comparing the nutritional quality of young stems, apical stems and old stems (mid-section) of *C. macrocephalum* growing in long and short vegetation. Stems of young *C. macrocephalum* were found to be of higher nutritional quality higher levels of nitrogen content compared to older mid-section stems. Stems from short vegetation were also found to be of high nitrogen content compared to plants from long vegetation making them more favorable to *L. tractabilis*. Therefore, the surrounding vegetation has an impact on the growth and nutritional quality of *C. macrocephalum* which has an impact on the abundance and performance of the biological control agent. For *L. tractabilis* to establish successfully in invaded areas, it is essential to mow the vegetation during its release. Hence management by mowing, and more practically using vegetation fires, can facilitate biological control of *C. macrocephalum.*

Keywords: Carbon, C:N ratio, herbivory, mowing, nitrogen, palatability, plant quality, stable isotopes

5.1 Introduction

Plant quality and insect herbivory are aspects of plant physiology that interact with each other, either positively or negatively (Bala *et al.*,2018). Plant nutrition refers to certain chemical elements and their interactive effects of plant growth, reproduction and development (Bala *et al.*,2018). This in turn can influence the nutritional quality of plants for herbivores and can be quantified by assessing levels of nutrients such as nitrogen (N) essential for protein synthesis, and the availability of digestible carbohydrates (C) as sugars and starches, which are carbon-based compounds that serve as essential energy sources for both plants and herbivores (Bezemer and Jones, 1998; Awmack and Leather, 2002). Nutritional quality and defensive compounds of plant tissue are key aspects that influence the performance of phytophagous insects (Moniruzzaman *et al.,* 2009). The availability and concentration of chemical elements such as N and C have a direct effect on the growth, fecundity and performance of insects (Awmack and Leather, 2002; Erb and Kliebenstein, 2020; Qi *et al.*, 2024).

The nutrition that insects obtain from plants through feeding includes proteins (N), amino acids, carbohydrates, minerals and fats (Boswell *et al.*, 2008; Bala *et al.,* 2018). Availability of these nutrients affects the growth and the fitness of herbivores (Boswell *et al.*, 2008), with development and abundance of insect herbivores improving with an increase in nitrogen content (Wheeler, 2003). Plants that are exposed to high levels of soil nitrogen generally result in increased herbivore population density, feeding performance, reproduction and survival (Room and Thomas, 1985; Bownes *et al.,* 2013; Bala *et al.*,2018). Nitrogen is a key nutrient required by insects for optimal growth, the increase also results in an increase in accumulation of proteins, amino acids and sugars on *Aphis gossypii* (Hemiptera: Aphididae) reared on *Chrysanthemum iindicum* (Asteraceae) (Rostami *et al.,* 2012).

The C:N ratio of plant tissues refers to the ratio of carbon (C) to nitrogen (N) which is an important indicator of plant nutritional quality (Seibt *et al.*, 2008). The C:N ratio in plants varies depending on plant species, tissue type, and environmental condition, and has an impact on insect-plant interactions (Awmack and Leather, 2002). Plants with higher nitrogen content relative to carbon have lower C:N ratios, suggesting higher nutritional quality. Nitrogen isotopic composition (δ15N ‰) can also be used measure nutrients uptake by plants, whether it originate from either anthropogenic or natural sources (Fry, 2006; Thimdee *et al.*, 2002: Gritcan *et al.*,

2016). Elevated δ15N values are the result of an abundance of anthropogenic sources or natural sources in the environment, which leads to heightened isotope fractionation through increased volatilization of ammonia and/or intensified microbial processing (Gritcan *et al.*, 2016).

The feeding performance of a stem deformer *Liothrips tractabilis* Mound and Pereyra (Thysanoptera: Phlaeothripidae), a biological control agent of *Campuloclinium macrocephalum* in South Africa, was found to improve on plants growing in relatively short vegetation compared to plants in longer (greater height) vegetation (Mudau, 2019). *Campuloclinium macrocephalum* displayed different growth patterns when growing in long vegetation compared to short vegetations. The same pattern was observed when they were exposed to full sunlight or shade in greenhouse trial (Chapter 2 and 4). Consequently, these observations and results suggest that the level of shading affects plant nutritional quality. *Campuloclinium macrocephalum* growing in short vegetation is generally exposed to full sunlight, have short and thin stems and display lateral growth by producing more primary and secondary stems. Conversely, plants that grew in long vegetation or grown in the shade had longer and thicker stems and the plants displayed vertical growth, mainly having single or at most two primary stems (Chapter 2 and 4).

The long vegetation or more shaded environment was also found to negatively affect the feeding performance and reproduction of *L. tractabilis* (Mudau, 2019; Chapter 4). *Liothrips tractabilis* feeds more frequently and causes significant damage on young apical shoot stems compared to more mature stems (McConnachie and McKay, 2015). Feeding on these young stems reduces biomass and floral production of the weed (McConnachie and McKay, 2015). Hence this could be advantageous for *L. tractabilis* if they are developing on *C. macrocephalum* plants of better quality. Therefore, the aim of this study was to assess and compare the nutritional quality of apical stems and older stems (mid-section of the stem) of *C. macrocephalum* growing in long vegetation compared to short vegetation. The objective of the study included assessing the nitrogen and carbon content, C:N ratio and δ15N ‰ of apical and old mid-sections.

5.2 Methods and materials

Collection of stem samples

Campuloclinium macrocephalum stems were collected from Rietvlei Nature Reserve, City of Tshwane Metropolitan Municipality, South Africa (25.8825° S, 28.2639° E). The dominant vegetation type in the study area where sampling was undertaken was grassland, dominated by

the *Eragrostis gummiflua* Nees., *Digitaria diagonalis* (Nees) Stapf var. diagonalis and *Eragrostis curvula* (Schrad.) Nees (Marais, 2004). Stems were collected in December 2022 during the peak growing season of *C. macrocephalum* on plots with long vegetation approximately 100 cm in height. Similarly in December 2022, stems from short vegetation, approximately 40 cm in height were collected from plots burnt in August-September 2022, before the start of *C. macrocephalum* growing season. Ten stem samples from each of 10 plants were collected within a 10 m x 10 m plot. Within each plot 1 m x 1 m quadrats were placed at random using a random number table, from which one plant facing north closest to the bottom left corner of each quadrat was dug out and placed in a brown bag. From these plants, apical stem (3 cm) samples were collected at 5 cm from the inflorescence, which is the main section that gets deformed and is most preferred by *L. tractabilis*, the mid-section stems samples were collected (3 cm) from 15 cm from the inflorescence (Figure 5.1).

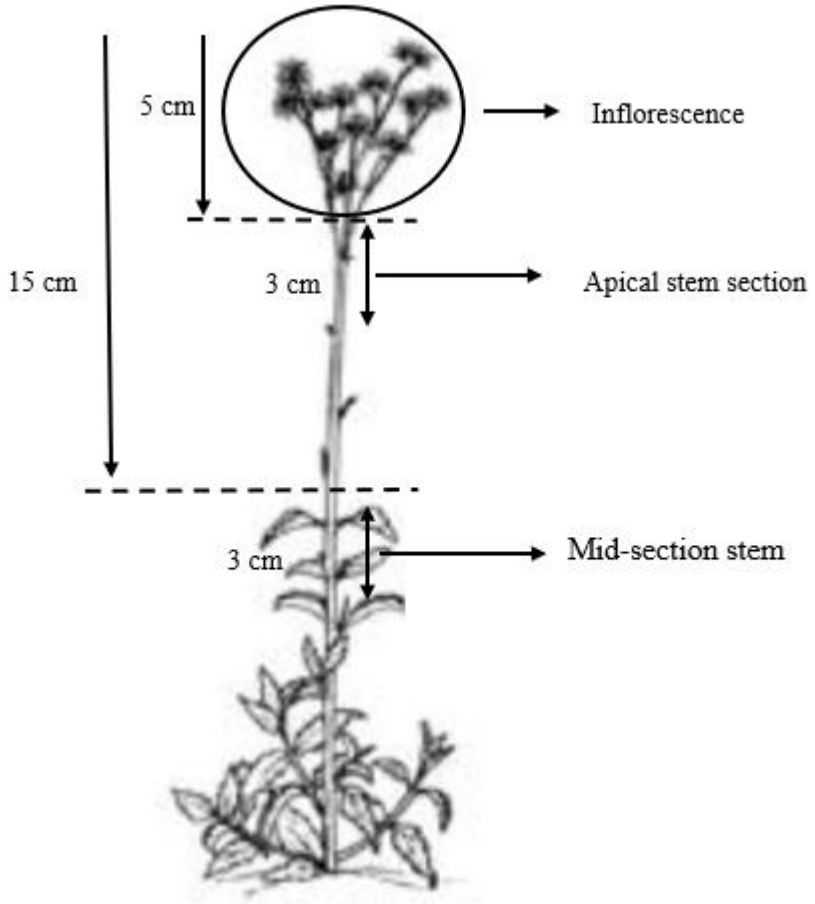


Figure 5.1: *Campuloclinium macrocephalum*, apical and mid-section stem that were sampled and inflorescence (Source: McConnachie *et al.*, 2011).

Sample preparation and analysis

The samples collected were oven dried at 50°C for 72 hours to ensure the stems were completely dry, they were then crushed and homogenized using a pestle and mortar. Approximately 10 mg of each stem sample from short and long vegetation were carefully weighed and using a five-place balance (precision of 0.00001 g) and each placed in tin foil before being sent to Stable Isotope Laboratory, Mammal Research Institute (MRI) at University of Pretoria for analysis. Samples were combusted at 1020°C using an Elemental Analyzer (Flash EA 1112 Series) attached to a Delta V Plus stable light isotope ratio Mass Spectrometer through a ConFlo IV system (all equipment provided by Thermo Fischer, Bremen, Germany). The two laboratory standards were used: Merck Gel ($\delta^{13}C$ = -20.26‰, $\delta^{15}N$=7.89‰, C%=41.28, N%=15.29) and DL-Valine ($\delta^{13}C$ = -10.57‰, $\delta^{15}N$=-6.15‰, C%=55.50, N%=11.86). Data corrections were done using the values obtained for the Merck Gel and DL-Valine during each run. The average of the standard deviations of the nitrogen and carbon values for the Merck and DL-Valine standards provide the ± error for sample $\delta^{15}N$ and $\delta^{13}C$ values.

Carbon and nitrogen ratios (C:N) were calibrated using primary standards using IAEA-CH-3 (Cellulose), IAEA-CH-6 (Sucrose), IAEA-CH-7 (Polyethylene foil), IAEA N-1 and IAEA N-2 (Ammonium sulphate), IAEA NO-3 (Potassium nitrate). All the results have been referenced to Vienna Pee-Dee Belemnite for carbon isotope values, and to air for nitrogen isotope values. Results are expressed in delta notation using a per mille scale using the standard equation:

$$\delta X(‰) = [(R_{sample}-R_{standard})/R_{standard}-1]$$

where X= ^{15}N or ^{13}C and R represents $^{15}N/^{14}N$ or $^{13}C/^{12}C$ respectively.

Data analysis

All analyses were performed using R Commander (Rcmdr: R Commander, R package version 2.9-2; 2024). A general Linear Model (GLM) (Gaussian) was used to test the difference between response (C: N, $\delta^{15}N$, C%, N% and ^{15}N), of predictors (mid-section and apical section) from plants collected in long and short vegetation.

5.3 Results

Percentage nitrogen (%N) of apical stems from short vegetation was higher in stem samples from long vegetation and mid-section stems from short vegetation ($F_{3,37}$=0.86; p>0.05) (Figure 5.2). Apical stems from long vegetation had slightly higher levels of nitrogen compared to mid-section stems from long vegetation. None of the %N values were significantly different.

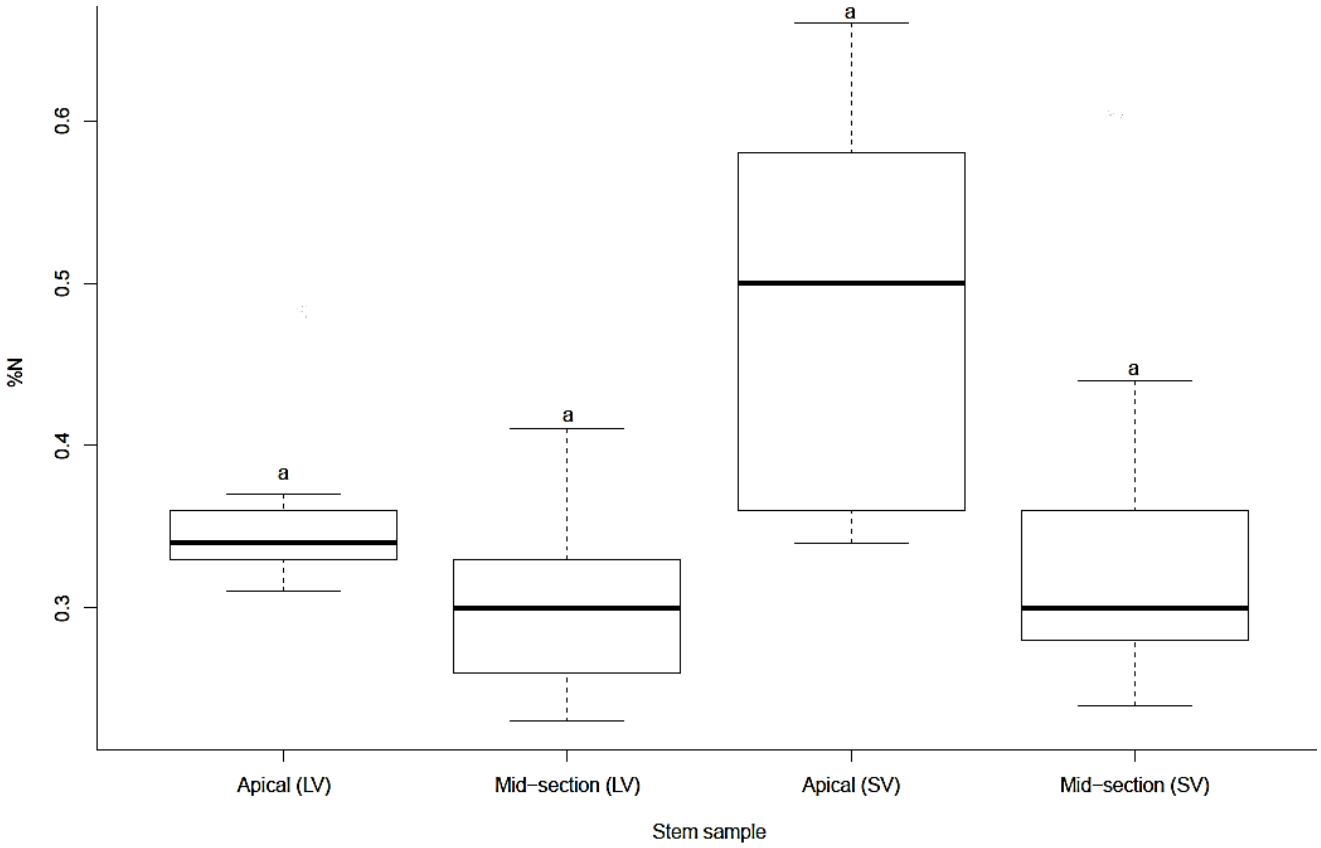


Figure 5.2: Percentage nitrogen (%N) of apical and mid-section stems of *Campuloclinium macrocephalum* plants growing in both long (LV) and short vegetation (SV). n=10 per sample type (mean ± SE). Different lowercase letters indicate significant differences (Tukey, $p < 0.05$).

The percentage carbon of apical and mid-section stems from short vegetation plants was lower than percentage carbon of apical and midsection stems from long vegetation plants ($F_{3,37}$=2.108; p<0.001) (Figure 5.3).

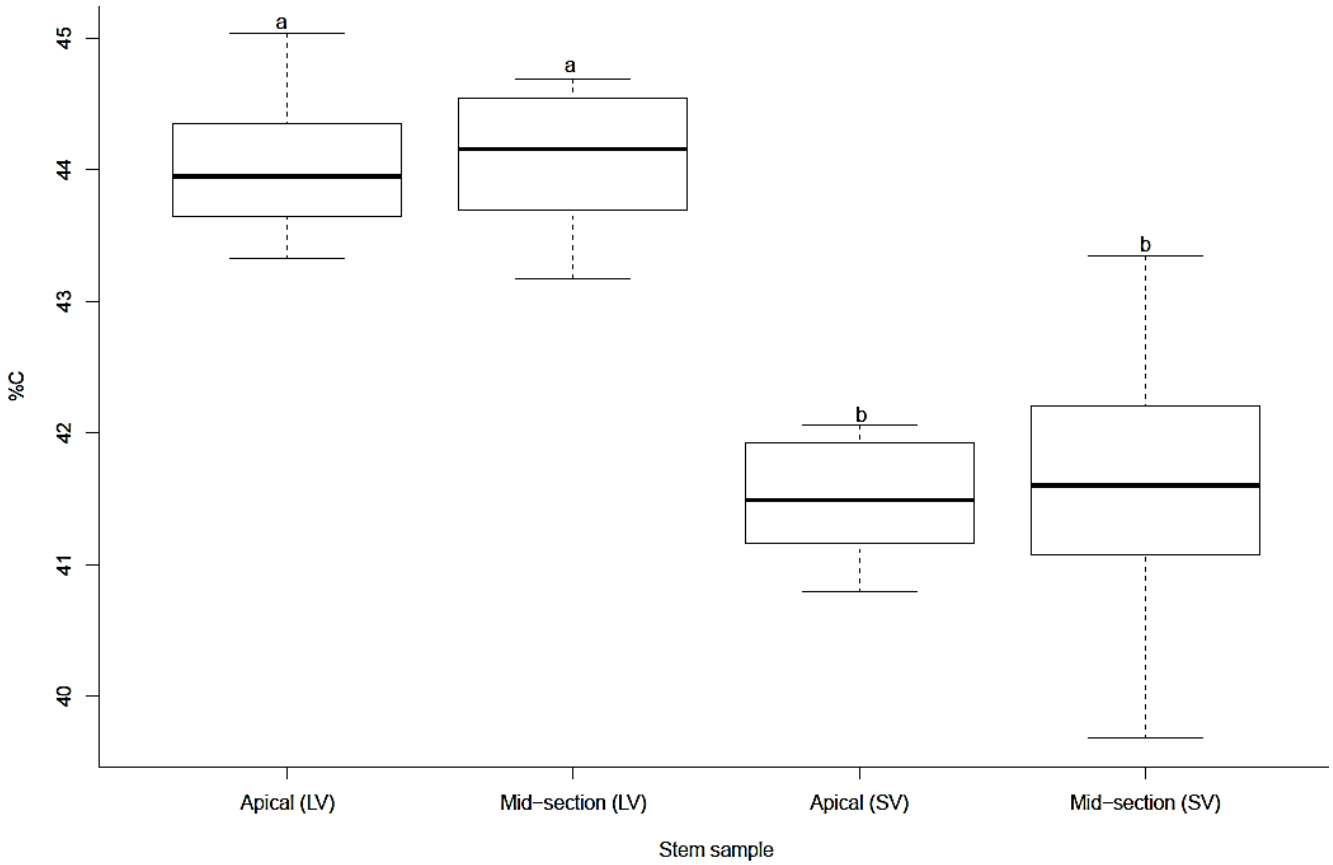


Figure 5.3: Percentage carbon (%C) of apical and mid-section stems of *Campuloclinium macrocephalum* obtained from plants growing in both long (LV) and short vegetation (SV). n=10 per sample type (mean ± SE). Different lowercase letters indicate significant differences (Tukey, $p < 0.05$).

The C:N ratio of short vegetation apical was significantly lower compared to apical and mid-section stem samples from long vegetation and mid-section from short vegetation ($F_{3,37}$=31.7; $p<0.001$) (Figure 5.4). Apical stems from both vegetation type had lower C:N ratios compared to the mature mid-section stems.

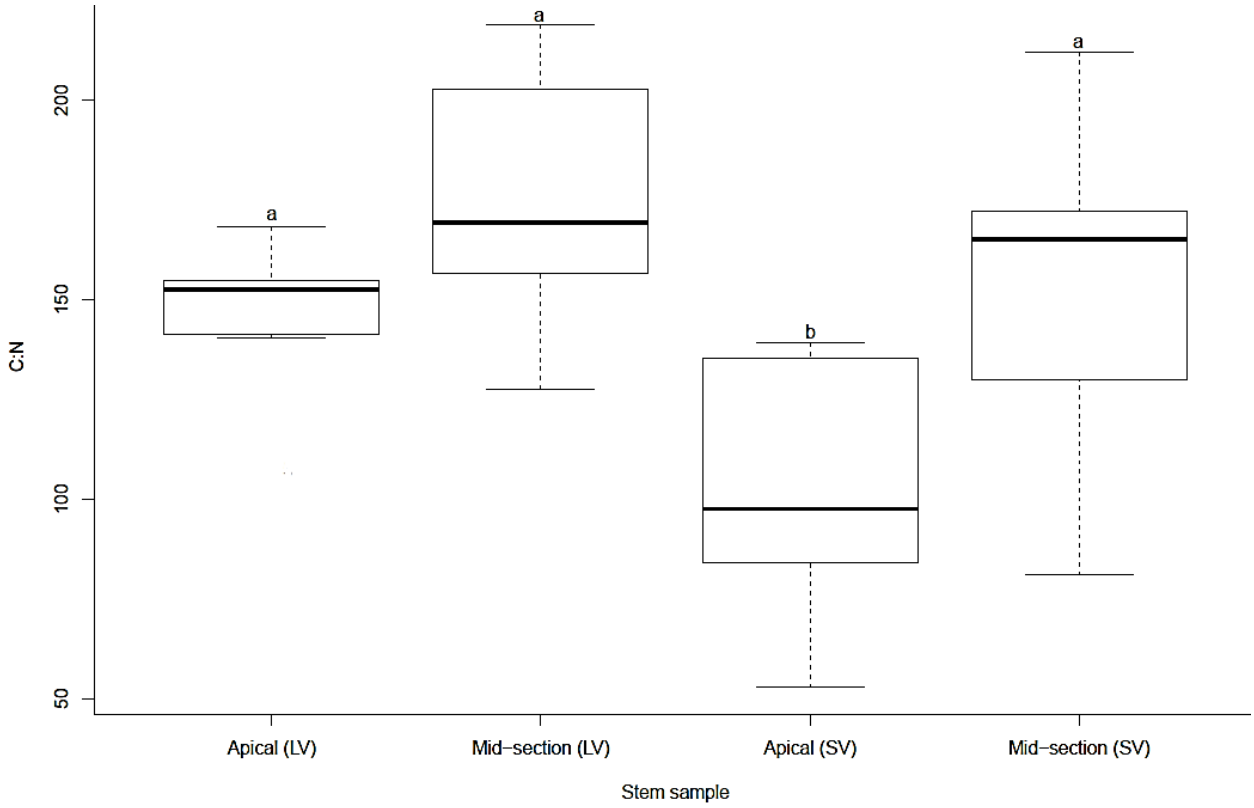


Figure 5.4: Carbon to nitrogen ratio (C:N) of apical and mid-section stems of *Campuloclinium macrocephalum* obtained from plants growing in both long (LV) and short vegetation (SV). n=10 per sample type (mean ± SE). Different lowercase indicates significant differences (Tukey, $p < 0.05$).

The nitrogen isotopic composition (δ15N ‰) of mid-section stem samples from long vegetation plantswas significantly lower than apical and mid-section stem samples from short vegetation plants ($F_{3,37}$=15.14; p<0.001) (Figure 5.5).

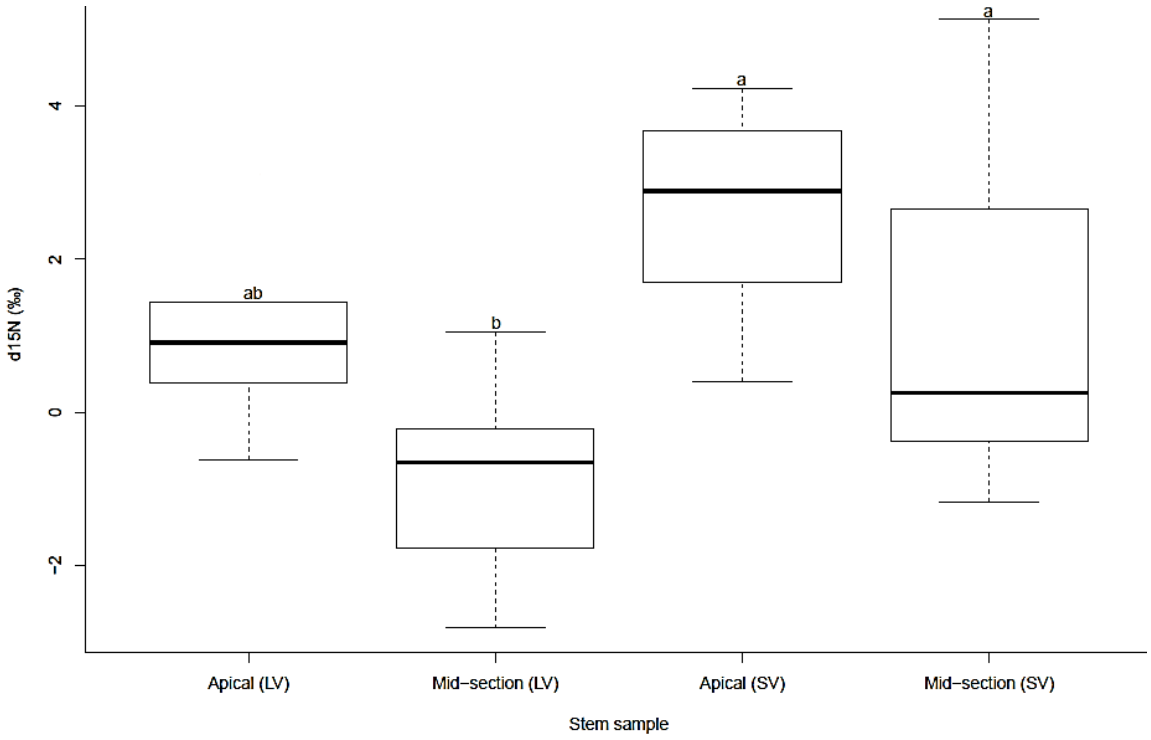


Figure 5.5: Nitrogen isotopic composition (δ15N ‰) of apical and mid-section stems of *Campuloclinium macrocephalum* obtained from plants growing in both long (LV) and short vegetation (SV). n=10 per sample type (mean ± SE). Different lowercase letters indicate significant differences (Tukey, $p < 0.05$).

5.4 Discussion

This study recorded a significant difference in some measures of plant quality between apical stems and mature plant stems, as well as between plants growing in short and long vegetation. Apical stems of *C. macrocephalum* had higher levels of nitrogen content compared to older mid-section stems, probably rendering them more palatable to the biocontrol agent (Awmack and Leather, 2002; Gritcan *et al.*, 2016; Erb and Kliebenstein, 2020). Higher nitrogen content in plants also results in higher feeding and abundance of insect herbivores on the host plant (Mudau, 2019). There was also greater abundance of *L. tractabilis* on *C. macrocephalum* in short vegetation compared to long vegetation (Mudau, 2019), which could be due to the favourable C:N ratio. Similar results were found in Chapter 4, where the abundance and feeding impact of *L. tractabilis* was higher on plants in full sun compared to plants in the shade.

Increase in nitrogen levels of *Salvania molesta* (D.S. Mitchell) (Salviniales, Salviniaceae) also resulted in an increase in abundance of the biological control agent, *Cryptophagous salvaniae* (Coleoptera: Curculionidae) (Room and Thomas, 1985). Consistent with the findings of this study, apical stems generally exhibited higher levels of nitrogen compared to mature stems *C.*

macrocephalum. This reflects the nutrient allocation pattern in actively growing tissue, where nitrogen levels are higher, enabling enhanced protein synthesis, enzyme activity, and various metabolic processes associated with plant growth and development (Bala *et al.,* 2018; Gritcan *et al.,* 2016).

The nitrogen levels in apical stems reflect how plants prioritize nitrogen allocation towards areas of rapid growth and cellular development. Studies on sunflowers (*Helianthus* spp.) (Sheoran *et al.,* 2016) and *Chrysanthemum morifolium* Ramat (Compositae) (Zhu *et al.,* 2009) show that nitrogen is reabsorbed from mature leaves and flowers and then redistributed within the plant to support new growth or reproduction. Adult *Neochetina* spp. preferred feeding on young leaves of water hyacinth which was linked to higher nitrogen concentration (Moran, 2004: Katembo *et al.,* 2013). Similarly, in *Gustavia superba* (Lecythidaceae) (Aide and Londono, 1989) and *Brassica nigra* (L.) Koch (Merritt, 1996), young, immature leaves have levels of nitrogen which enables them to undergo rapid leaf expansion, but which also increases plant quality for generalist insect herbivores. This was also supported by somewhat elevated nitrogen levels in apical stems of *C. macrocephalum* that are still actively growing, compared to the mid-section stems that are older and more mature. As stems mature and lignify, their metabolic demands for nitrogen may decrease, leading to a lower nitrogen level compared to actively growing tissues like young stems (Lambers *et al.,* 2008).

Surrounding vegetation height was found to have a positive impact on the height and growth patterns of *C. macrocephalum*, with a strong correlation between vegetation and *C. macrocephalum* height (Chapter 2). This may have also resulted in *C. macrocephalum* adjusting its nutrient allocation pattern in response to these changes in micro environmental conditions which also results in changes in growth, morphology, and physiology (Taiz and Zeiger, 2002; Valladares and Niinemets, 2008). The changes in morphology and physiology were also observed in *C. macrocephalum* (Chapter 2 and 4), where plants that were exposed to lower light intensity had longer stems and displayed vertical growth rather than lateral growth. Lateral growth results in an increased number of new shoots meaning more feeding sites with more nitrogen availability for the biological control agent. The same growth pattern was also observed in other plants including bangladhonia (*Eryngium foetidum* L.) (Moniruzzaman *et al.,* 2009), and common sage (*Salvia officinalis*) (Rezai *et al.,* 2018) when exposed to different light conditions.

Variations in plant quality between short and long vegetation could indicate plants adaptive plasticity to environmental factors, as they adapt their growth strategies to thrive in their specific ecological conditions. The adaptation of the plants may have a positive or a negative impact on the biological control agent because of the change in nutritional quality, as shown by the growth plasticity of *C. macrocephalum* to different light intensities.

Short vegetation or a more open sun environment has been found to improve the feeding performance and reproduction of *L. tractabilis* (Mudau, 2019; Chapter 4). The performance of *Liothrips urichi*, a biocontrol of *Clidemia hirta* was more effective in controlling the weed in an open sunny environment compared to shaded areas (Nakahara *et al.*, 1992). *Teleonemia scrupulosa* also caused significantly more damage to *Lantana camara* plants in full sunlight than in the shade (Venter *et al.*, 2019). This may be attributed to higher nitrogen levels in *C. macrocephalum* plants, as nitrogen is required for optimal growth of insects (Rostami *et al.* 2012). The difference in nitrogen level between *C. macrocephalum* plants from short and long vegetation may have been caused by the difference in growth rate on plants within the two vegetation types (Mudau, 2019; Chapter 2 and 4). *Campuloclinium macrocephalum* growing in tall vegetation grew faster and exhibited vertical growth in contrast to *C. macrocephalum* plants in short vegetation (Chapter 2).

The observation of significantly lower carbon percentages in stems compared to mid-section stems from both short and long vegetation suggests a distinct patterns of carbon allocation within different stages of plant growth (Sheoran *et al.*, 2016). Mature stems, having completed their primary growth phase, allocate carbon towards structural reinforcement and storage, which are critical for providing mechanical support, water transport, and energy reserves for the plant. Changes in carbon allocation patterns, such as increased investment in structural compounds like lignin or cellulose, can enhance plant resistance to herbivores by making tissues tougher or less palatable (Valladares and Niinemets, 2008). The reduction plant palatability may be due to an increase in carbon (i.e. higher C:N ratios), explaining the lack of feeding on mature stems by *L. tractabilis* as reported by McConnachie and McKay (2015), Ramanand *et al*., (2017) and in Chapter 3.

The differences in nitrogen and carbon allocation in plants at various growing stages of *C. macrocephalum* led to changes in the C:N ratios. As plants grow, their demand for nitrogen decreases relative to carbon, which makes older stem samples exhibit higher C:N ratios (Moniruzzaman *et al.,* 2009). Lower C:N ratios in young stems indicate greater nutrient availability and higher rates of nutrient turnover and palatability to herbivores (Moniruzzaman *et al.,* 2009; Rostami *et al.,* 2012). The levels of δ15N ‰ in young stems, apical (LV and SV) and mid-section (SV) were not significatly different. This may have been because there was no difference in terms of the availability of nutrients in the soil for plants growing in both long and short vegetation (Gritcan *et al.,* 2016). The key difference between plants that were collected from long and short vegetation was vegetation may have not been in soil composition. This further highlight that the difference in *C. macrocephalum* stem quality is driven by nutrient allocation rather than availability of nutrients in the soil. The surrounding vegetation height influences how nutrients are distributed within *C. macrocephalum*, ultimately shaping the nutritional characteristics of its stems, and subsequently affecting the ability of biocontrol, *L. tractabilis* to establish and thrive. Therefore, habitat management such as fire or mowing can be used to manipulate the establishment of biocontrol agents to successfully control invasive plants such as *C. macrocephalum*.

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Chapter 6
Synthesis and conclusion

The key purpose of this study was to establish an improved management strategy against *C. macrocephalum*, given the increase in invasion range of the weed in South Africa (van Wilgen *et al*., 2020). Fire alone, fire alongside herbicide, and a non-lethal dose of herbicide with the biocontrol agent, *L. tractabilis* are the three management strategies that were studied to improve control of *C. macrocephalum*. Fire alone was found to only reduce the seedbank (number of new seedlings) but did not have significant impact on mature plants which enabled mature plants to restore the seedbank. Therefore, fire alone can be ruled out as an isolated management technique. However, when fire and herbicide were integrated there was a 98% reduction in the density of mature *C. macrocephalum* as well (Chapter 2). A follow up application of herbicide administered as a spot spray over the next growing season is critical to kill plants that may have not been killed the previous season, and further follow up inspections would be wise given the wind dispersed nature of the seeds. The integration of fire and herbicide has proven to be an effective management strategy and is recommended for controlling pompom weed in South Africa.

The use of a lethal herbicide dose in sensitive areas such as wetlands is not recommended as that may result in groundwater contamination (DiTomaso *et al*., 2017). Such areas must be prioritized for other control methods using biological control agents such as the flower feeding moth, *Cochylis Campuloclinium*, and/or more additional releases of *L. tractabilis* can be considered after mowing or burning to reduce the vegetation cover to improve their chances of establishing. The use of a sub-lethal dose of the three registered herbicides metsulfuron methyl, impala and picloram with *L. tractabilis* was found to be incompatible given the indirect impact of the sub-lethal doses on the agent through reducing plant quality. The herbicide mainly affected the feeding sites of *L. tractabilis*, causing wilting young and apical stems, leaving only old mid-section stems to feed on. These are the least preferred sites given the lower nitrogen content, with higher levels if nitrogen being critical for the growth and development of these mature insects (Bala *et al.*, 2018). Due to lack of feeding sites, *L. tractabilis* was found to feed on old stems which are the least preferred sites. Consequently, considering the indirect impact of sub-lethal doses on *L. tractabilis*, the use of such doses is not recommended given the incompatibility.

Using a sub-lethal dose of metsulfuron methyl, impala, or picloram herbicide may be more cost-effective than the recommended doses since a larger area can be treated. However, if unintended consequences or production faults occur, the user will not be legally protected, as the applications did not adhere to the label instructions. In addition, sub-lethal doses of herbicides can contribute to the development of resistance in target plants (Londo *et al.*, 2014). Exposure to sublethal doses may select for resistant individuals or strains, leading to reduced effectiveness of treatments and increased difficulty in controlling the target weed (Londo *et al.*, 2014).

The final aim of the study was to provide insight and clarity as to why *L. tractabilis* prefers young stems and plants from short surrounding vegetation over *C. macrocephalum* plants growing in taller vegetation (Mudau, 2019; Zachariades *et al*., 2021). The main reason why *L. tractabilis* prefers young apical stems is due to lower C:N ratio compared to more mature stems (Chapter 5). This finding also explains why a sub-lethal dose does not work when integrated with *L. tractabilis*, the herbicide primarily affects the more nutritious apical stems and prevents the development of new shoots. There was also a difference in C:N ratio of *C. macrocephalum* stems from long and short vegetation. Stems of *C. macrocephalum* from long vegetation had a relatively high C:N ratio which explains the lack of feeding damage and abundance of *L. tractabilis*. Surrounding vegetation plays a significant role when it comes to the establishment of *L. tractabilis*, because the morphology and growth rate of *C. macrocephalum* affected the nutrient allocation (specifically nitrogen, a nutrient commonly limiting plant growth).

Plants growing in long vegetation promoted a more vertical growth pattern of *C. macrocephalum* with at least two stems that grow relatively fast compared to *C. macrocephalum* from short surrounding vegetation (Chapter 2). Plants generally allocate more nitrogen to actively growing sections such as new shoots and apical sections of the plant (Sheoran *et al.*, 2016), making them more palatable to herbivores (Moniruzzaman *et al.*, 2009; Rostami *et al.*, 2012). Plants growing in short vegetation produced more stems and displayed lateral growth which promotes development of new shoots that are relatively rich in nitrogen, thus supporting the establishment and abundance of *L. tractabilis* (Mudau, 2019). The findings of chapter 4 indicate that light intensity significantly influences the performance and establishment of thrips. As a result, mowing or burning the surrounding vegetation before and after the release of thrips could support their establishment and enhance the effectiveness of the biocontrol agent. In conclusion,

integrating fire and herbicide are the two management strategies that may improve the degree of control of *C. macrocephalum*. It is therefore recommended as a management strategy to control the weed.

The national strategy for the management of *Parthenium hysterophorus* L. (Asteraceae), a species related to pompom weed, in South Africa divides the invaded areas into four management zones based on the invasion range of the weed, namely prevention, local eradication, containment and asset protection zones (Nanni *et al.*, 2016). A similar strategy can also be used in a national strategy for the management of *C. macrocephalum* in South Africa. Preventions zones are areas where there are no known populations of the weed with low probability invasions. These are areas where if *C. macrocephalum* is spotted rapid eradication is required, this will require the use of a lethal dose of registered herbicides such as picloram, metsulfuron methyl and fluroxypyr/triclopyr (impala). In areas where *C. macrocephalum* has been eradicated, it is essential to maintain or even enhance the basal cover of herbaceous vegetation, with good grazing management practices or restoration of sites by seeding indigenous grasses (Cowie et al. 2021). Increasing the basal cover to over 19% has been shown to be associated with a lower density of *C. macrocephalum* (Goodall *et al.*, 2011). This approach is particularly important in regions where *L. tractabilis* has not been introduced for biological control

Extirpation (local eradication) zones are areas with invasive weed populations that are approximately 50 km apart, with the populations covering <1 ha (Nanni *et al.*, 2016). Based on the findings of this study (Chapter 2), integrating fire and registered herbicide may be the most suitable management option. Fire reduces the seedbank of *C. macrocephalum* without affecting the rootstocks, meanwhile the application of a lethal dose of herbicide significantly reduces abundance of mature plants when applied as a spot spray. Such areas will also require one or more follow up applications of herbicide for plants that may have missed or poorly sprayed during the previous application of herbicide before *C. macrocephalum* flowers, as flower and subsequent seed production will likely restore the seed back that has been reduced by the fire.

Containment zones are areas with multiple populations of the invasive weed covering over >1 ha and occurring within 50 km from each other (Nanni *et al.*, 2016). The weed must be prevented from expanding to new areas; this can be done by integrating fire and spot spraying of lethal

herbicide dose to manage the spread of the weed. In part of the invaded areas where application herbicide is not feasible, the introduction of biocontrol agents such as *L. tractabilis* and *Cochylis Campuloclinium* is recommended in such areas. However, mowing or burning of the vegetation in August is recommended as that may improve the chances of *L. tractabilis* establishing and improving its performance against pompom weed (Mudau, 2019). If the biocontrol agent has already been released, additional releases are recommended until the weed can no longer spread to other areas and to reduce the extent of existing infestations. Asset protection zones are areas where the weed is abundant, and the key priority will be to maintain and reduce pompom weed invasion levels in specific locations (Nanni *et al.*, 2016). Given the current management strategies of *C. macrocephalum* similar management techniques can be used in asset protection zones to manage the weed.

The post release evaluation of new agents, such as the flower feeder moth, *Cochylis Campuloclinium*, will be required, and such studies should include how the new agent(s) interact with the *L. tractabilis* and the rust fungus. In addition, how to optimize the use of fire, and herbicides, together with the agents in an integrated management approach. Once again specific agents may be more or less effective in mesic or xeric sites (Goodall, 2012), and one should expect variable effectiveness across the ever-increasing range of *C. macrocephalum.* Furthermore, the possibility of using a sub-lethal dose, with the flower feeder moth, should also be explored.

Another challenge in managing *C. macrocephalum* is not only its prolific seed production (McConnachie et al., 2011), but also its perennial rootstocks that can survive fire and regrow during the growing season starting in October (Goodall and Witkowski, 2014). Given the variable impact of *L. tractabilis* on invaded sites, which reduces the weed's flowering ability (Zachariades et al., 2021), finding a biological control agent that targets the roots is crucial. This would prevent regrowth from underground, addressing the continuing expansion of the weed despite current above-ground management methods. Currently, using a lethal dose of registered herbicide is recommended for eradicating *C. macrocephalum*. Additionally, increasing the release of *Cochylis campuloclinium* in other infested areas is advised. In conclusion, integrated management of invasive plants such as the Australian *Acacia* species, *Acacia cyclops* (Impson *et al.*, 2024), *Solanum mauritianum* (bugweed) and *Chromolaena odorata* (L.) King and Robinson

(Goodall and Erasmus, 1996; Zachariades *et al.,* 2011; te Beest *et al.,* 2012) has resulted in positive results which is a promising outcome for the management of *C. macrocephalum*.

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