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Parthenium hysterophorus: understanding the invasion and potential controls

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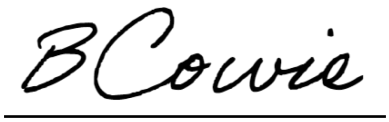
A Dissertation submitted to the Faculty of Science,
University of the Witwatersrand,
in partial fulfilment of the requirements for the degree of
Doctor of Philosophy,
Johannesburg, South Africa
2020

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.



Blair William Cowie

30th day of June 2020 at Johannesburg, South Africa.

Supervisors:

Prof. Marcus J. Byrne (*University of the Witwatersrand*)

Prof. Ed T.F. Witkowski (*University of the Witwatersrand*)

DEDICATION

"What makes life valuable is that it doesn't last forever…
what makes life precious is that it ends"

This PhD thesis is dedicated to my Father, David Alexander Cowie, who was a major influence in my life, but will sadly never get to see this achievement.



To honour the teachings of a tireless Scot:

"You play to win, or you don't play at all…"

ACKNOWLEDGEMENTS

Firstly, I'm indebted to both my supervisors, Prof. Marcus Byrne and Prof. Ed Witkowski, for their guidance, support and patience with me throughout my PhD. To Prof. Witkowski, thank you for your willing to review every aspect of my research and for your humorous and valuable insights into the project. To Prof. Byrne, thank you for constantly striving to get the best out of me and for your 'belt-and-braces' training, it has proved invaluable on many occasions. Above all, thank you both for encouraging my academic curiosity and contributing your respective fields of knowledge, without which this PhD would not have been possible.

I would also like to thank Mr Nic Venter for his friendship and assistance with various aspects of this PhD. Special thanks also to Dr Jeremy Goodall and Ms Lorraine Strathie for all their assistance, insights and roles as valuable advisors to this PhD. In addition, I would also like to express my gratitude to the anonymous journal reviewers who offered helpful comments, which improved the quality of my chapters accepted for publication (Chapters 2, 4, 5 and 6).

To the University of the Witwatersrand, I am grateful for the funding I received through both the PGMA and PV Tobias Award whilst conducting my PhD research. Thanks also to the National Research Foundation (NRF) and the Centre of Excellence for Invasion Biology (CIB) for provision of Doctoral funding and running costs, respectively. To our APES administration staff, particularly Tamelyne Van Tonder and Moffat Mojapelo, thank you for all your "behind the scenes" assistance it was greatly valued.

To my family and friends, you remain the unsung heroes of this PhD, your moral support and assistance is gratefully accepted. To my Mother, Alison Cowie, and girlfriend, Alexandra Turner, thank you for your unwavering love and support, not only throughout my PhD, but all my academic endeavours, your patience and understanding knows no bounds. To my friends Monique Alexander, Nikhail Arumoogum, Dylan Beukes, Anastashia Chetty, Robin Cook, Diana De Sousa, Azraa Ebrahim, Courtney Gardiner, Kirsty Hartman, Brishan Kalyan, Jonathan Levin, Wendy Panaino, Stephanie Payne, Marlanka Prinsloo, Sumeshni Pillay, Christopher Rankin, Chevonne Reynolds, Geethen Singh, Gavin Snow, Alekzandra Szewczuk, Claudia Tocco, Thando Twala, Nic Venter, Marcja Welgus, Joseph White and Melissa Whitecross thank you for all of your support and encouragement, whether it was sharing a beer or simply just checking in on me, it was all greatly appreciated.

ABSTRACT

Native to the neotropics, the noxious annual herb *Parthenium hysterophorus* L. (Asteraceae), has become a troublesome invader in more than 50 countries globally. In South Africa, *P. hysterophorus* remains one of the most damaging invasive weeds, threatening food security, native biodiversity as well as human livelihoods and well-being. Given the ongoing threats posed by *P. hysterophorus*, it has become imperative to assess the invasive ecology of the weed in order to aid management and restoration endeavours.

The extent of the *Parthenium hysterophorus* invasion is believed to be governed largely by the availability of water (soil moisture). Simulating a natural drought in an ambient conditions greenhouse, it was found that the weed is capable of enduring periods of prolonged water stress by employing a drought avoidance strategy. Both the adult and juvenile plants, displayed morphological and physiological adaptations to reduce water stress, suggesting that all but the driest parts of South Africa are susceptible to invasion by *P. hysterophorus*. Within these susceptible regions, *P. hysterophorus* invasions were found to be influenced by the land, more specifically grazer and pasture, management. Long-term monitoring showed that "poorly-managed", particularly disturbed or overgrazed, areas experience exacerbation in both *P. hysterophorus* invasion and impact. Whereas, comparatively "well-managed" areas showed lower levels of *P. hysterophorus* invasion, which gradually decreased overtime, allowing for the recovery of native species (richness and diversity).

In terms of management, South Africa is largely reliant upon biological control to combat *P. hysterophorus* invasions at a national level. However, biocontrol is yet to reach its full potential, with the most promising biocontrol agent, *Zygogramma bicolorata*, failing to deliver any notable impact since its release in 2013. Ecophysiological studies reaffirmed that leaf-feeding by *Z. bicolorata* is highly damaging to *P. hysterophorus*, metabolically impairing leaves and reducing their photosynthetic ability. This suggested that despite an abundance of plants available in the field, *Z. bicolorata* populations are constrained. Thus, investigations into the influence of plant quality on the development and performance of *Z. bicolorata* was undertaken, finding faster development with larger beetles and more fecund females occurred on plants of higher quality.

In regions where biocontrol alone is ineffective or unsuitable, chemical control is often employed as the alternative management strategy. Although herbicides are effective in controlling *P. hysterophorus*, they are costly and unsustainable for large-scale or long-term management. Furthermore, concerns surround the likelihood of herbicide resistance in the future as CO_2 levels rise. To explore this a multi-generational Conviron study was conducted, finding that increases in atmospheric CO_2 are likely to decrease the susceptibility of *P. hysterophorus* to commercially used glyphosate herbicides.

The restoration of landscapes invaded by *P. hysterophorus* remains an understudied but valuable avenue of research for many South African savannas and their rangelands. Using a randomised block design, the feasibility of restoring pastures invaded by *P. hysterophorus* within a savanna reserve was explored. The sowing of native grasses, with and without the clearing of *P. hysterophorus*, was found to promote basal grass cover as well as the establishment of perennial grasses. Moreover, these increases in grass cover enhanced the competitiveness of pastures, gradually suppressing the *P. hysterophorus* invasion within the trial plots over a three year period.

Overall, the research presented in this thesis contributes significantly towards the understanding of *P. hysterophorus* invasion and the controls utilised in South Africa. Although biological control, the use of herbicides, good land management practices and restoration using native grasses all hold promise, these will need to be formally integrated to attain successful control over *P. hysterophorus.* At present, South Africa must work towards enhancing and integrating its current controls and limiting the spread of the weed and its environmental and socio-economic impacts. This will be a challenging and long-term endeavour but one which must be supported by government, research and the public.

Keywords: biological control; ecophysiology; elevated CO_2; Famine weed; glyphosate; herbicide control; land management; invasion ecology; Parthenium weed; restoration; water stress

GLOSSARY OF TERMS & ABBREVIATIONS

A:Ci – Photosynthesis CO_2 response curves, often used to examine the photosynthetic response of C3 and C4 plants to different environmental conditions.

Allelopathy – The production of biochemicals by plants which are used to suppress or inhibit the growth and germination of surrounding plant species.

Biotic release – Term linked to the ERH, that suggests the success of invading species, in novel areas or habitats, is strongly attributed to being "freed" from their native predators, allowing them to invest resources into growth and reproduction.

Chlorophyll fluorescence – A non-destructive measure of plant photosystem activity used to measure various physiology parameters (see *Fv/Fm* and O-JIP).

Ecosystem resilience – The ability of an area or ecosystem to respond to disturbance, such as invasion, by resisting and recovering from the disturbance.

F_v/F_m – A chlorophyll fluorescence measure indicating the relative "health" of a leaf's photosystems (*PSII*), frequently used as a plant stress indicator.

NPQ – 'Non-photochemical quenching', a protective process in plants, which dissipates excess light energy as heat to prevent damage.

O-JIP – A technique based on chlorophyll fluorescence and used to monitor plant stresses through insights into biochemical and physiological function.

Osmotic adjustment – A biochemical mechanism which aids plants in maintaining cellular turgor and function, which is primarily elicited during periods of water stress.

Photosynthetic upregulation – The physiological process by which plants may enhance productivity (photosynthesis), frequently in response to biotic or abiotic stressors.

SPAD – 'Single Photon Avalanche Diode', a handheld device used to quantify plant "greenness", used to estimate the chlorophyll and nitrogen content of leaves.

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CHAPTER 1:

GENERAL INTRODUCTION

Biological invasions

Excluding the destructive activities of mankind, biological invasions exist as the single largest threat to biodiversity and ecosystem services globally (Courchamp et al., 2017; van Wilgen et al., 2020). More specifically, alien plants are understood to be one of the main contributors to the ever increasing problems associated with biological invasions (Pyšek et al., 2008; Downey and Richardson, 2016). Although biological invasions are arguably a natural phenomenon, the frequency and rate at which they occur have been drastically enhanced due to numerous anthropogenic activities, namely globalisation, travel and international trade (Westphal et al., 2008; Faulkner et al., 2020). Global research predicts that 26 000 plant species are likely to be introduced, intentionally or accidentally, and have the potential to become invasive, 10 000 of these as problematic invaders, with less than half of these problematic species having been introduced worldwide (Reichard and White, 2001).

South Africa in particular, is a country with a long-standing history of alien plant invasions, dating back as far as the 16th Century, with estimates of 8000 - 9000 alien plants, of which approximately 10% (~800) are considered invasive (Van Wilgen and Wilson, 2018; Richardson et al., 2020). Many of the plants recognised as invasive maintain the potential for severe negative impacts, both ecologically and socio-economically (Reynolds et al., 2020). Although the ecological implications of these invasions are well-understood, their economic impacts are not, with the current cost of plant invasions estimated at R 6.5 billion per annum (US$ 400 million) (De Lange and van Wilgen, 2010; van Wilgen and Wilson, 2018). In cases where invasive plants are found to be damaging, there is consensus that these invasions should be controlled, with the South African government spending R 1.5 billion annually to manage invasive plants (van Wilgen and Wilson, 2018). Moreover, South Africa will need to increase these control efforts as new invaders are introduced, species previously introduced may emerge as invasive (see ‘invasion debt’) and widespread invasive species, such as *Lantana camara, Opuntia spp.* and *Prosopis* spp. remain uncontrolled (Rouget et al., 2016).

To date, one of most damaging invasive plants that South Africa is facing, is the noxious herb *Parthenium hysterophorus* (Strathie et al., 2011). Threatening national food security,

biodiversity and human health, *P. hysterophorus*, has had numerous controls initiated against it. Despite these various efforts the weed continues to spread, evading management. Regardless of the weed's status and impacts, questions arise as to what the species invasive potential is, what constrains or enables its invasion and how it can be effectively managed.

Parthenium hysterophorus L.

Parthenium hysterophorus L. (Asteraceae), commonly referred to as Famine Weed, Congress Grass or simply Parthenium weed, is a severe noxious weed of global significance (Dhileepan and Strathie, 2009; Dhileepan and McFayden, 2012a). Native to the neotropics, *P. hysterophorus* has invaded more than 50 countries across Asia, Oceania and Africa, being particularly widespread and problematic within India, Pakistan, Australia, Ethiopia, Eswatini (Swaziland) and South Africa (Shabbir et al., 2019). The earliest reports of *P. hysterophorus* introduction to South Africa were in KwaZulu-Natal during the 1880's (Bromilow, 2010), since then, *P. hysterophorus* has spread rapidly, invading South Africa's eastern, higher rainfall regions, namely the North West, Limpopo, Mpumalanga and KwaZulu-Natal Provinces, the latter being most problematic (Strathie and McConnachie, 2019; Figure 1.1). Over the past decade, *P. hysterophorus* has increased its range by ~500% and is now found in 89 quarter degree squares (25 x 25 km) of South Africa, approximately 55 000 km^2 (Henderson, 2017; Henderson and Wilson, 2017; van Wilgen and Wilson, 2018).

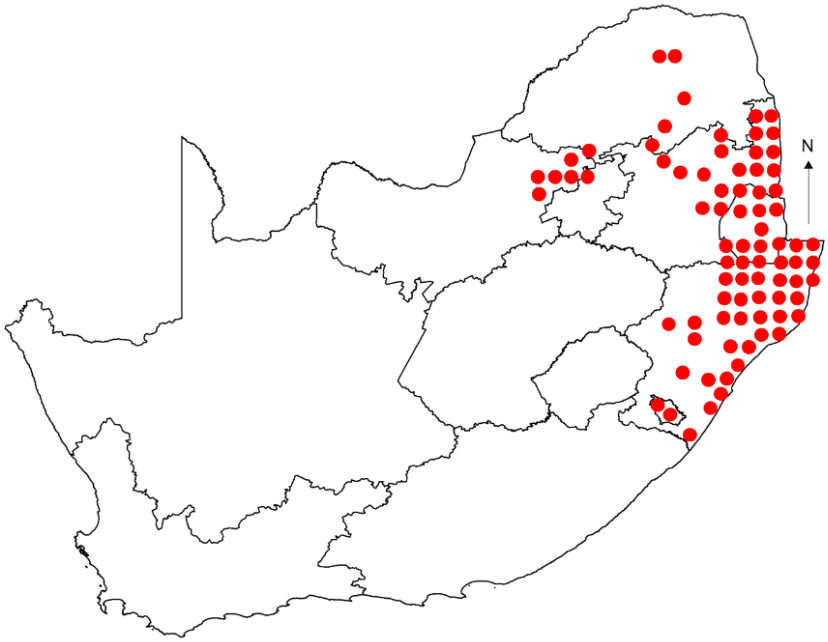

Figure 1.1. Current distribution of the noxious annual weed, *Parthenium hysterophorus* L, in South Africa (adapted from Terblanche et al., 2016; Henderson, 2017).

Within South Africa, *P. hysterophorus* readily colonises a multitude of habitats including road-and-rail sides, wastelands, degraded grasslands, savannas, forest margins, riparian zones and agricultural areas, being most damaging in commercial plantations, grazing lands and communal rangelands (Henderson, 2001; Terblanche et al., 2016; Figure 1.2A-F). The weed has prompted further concern subsequent to its rapid invasions within many of South Africa's conservation and tourism hotspots, including both the iconic Kruger and Hluhluwe National Parks (Strathie et al., 2005; Strathie and McConnachie, 2019).

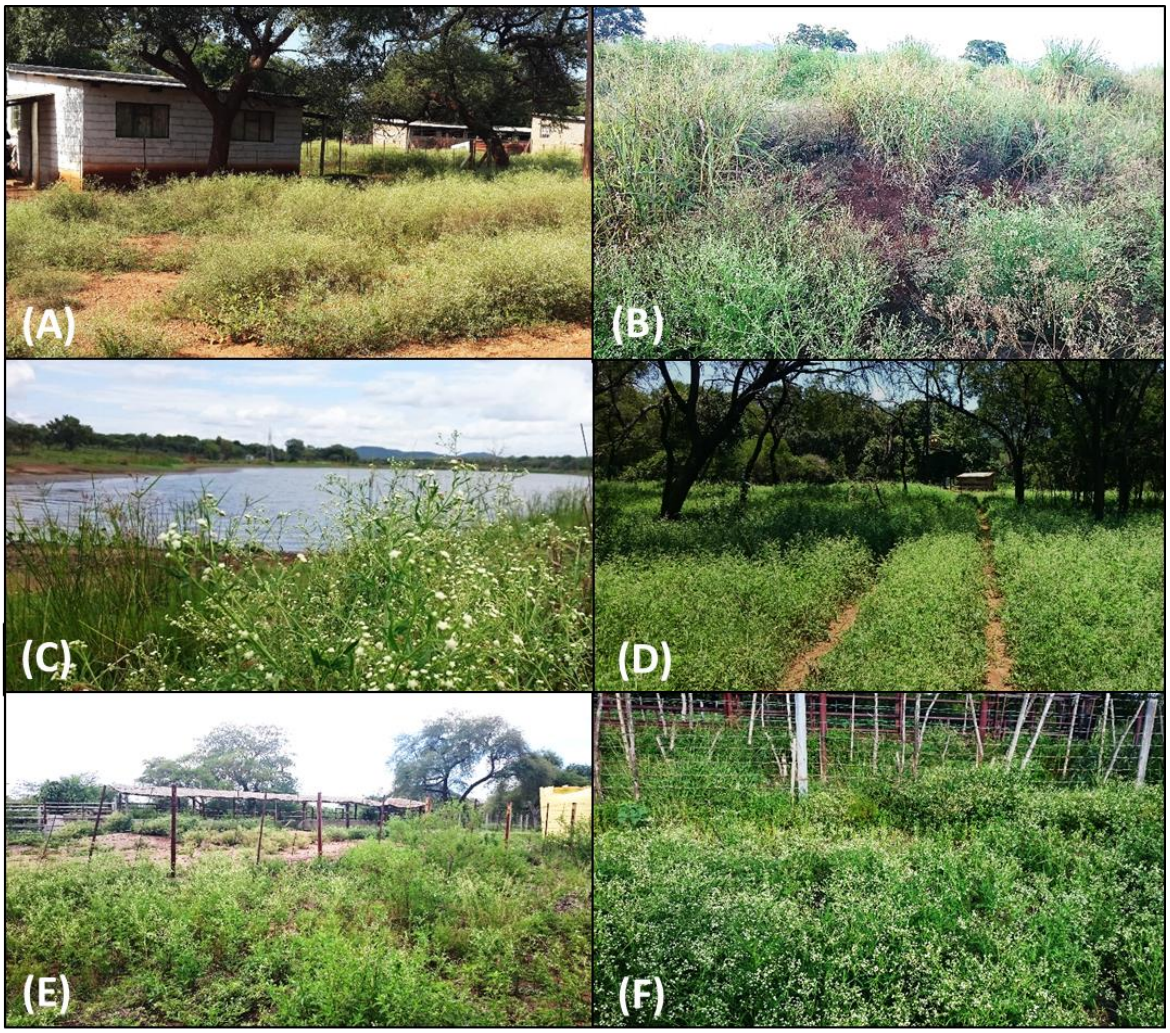

Figure 1.2. Extensive *Parthenium hysterophorus* invasions found: (A) surrounding a rural homestead, (B) within a sugarcane field left fallow, (C) spreading along the banks of a dam, (D) overrunning a dirt road, (E) alongside a livestock holding pen and (F) within a cattle grazing paddock. (Photos: BW Cowie)

Biology and ecology of *Parthenium hysterophorus*

Parthenium hysterophorus is a multi-branched summer annual herb, which grows to around 1-2 m in height (Henderson, 2001; Strathie et al., 2011). The lobed leaves and ribbed stems of this erect herb are covered in small trichomes (hairs), known to be a severe dermal and respiratory irritant (Adkins and Shabbir, 2014). The weed establishes solely from seed, with seedlings germinating in spring and initially forming a basal rosette of leaves (Adkins et al., 2019; Figure 1.3A-B). This rosette stage may be prolonged under unfavourable conditions, seemingly capable of persisting through cold and moisture (water) stress, before maturation (Annapurna and Singh, 2003; Bajwa et al., 2017; Figure 1.3C). Plants may mature as early as four weeks after germination, continually flowering and seeding for several months thereafter. Typically surviving for 7-8 months, mature *P. hysterophorus* produces copious amounts of seed, with as many as 30 000 to 150 000 seeds/plants, in a single growing season (Navie et al., 1996; Dhileepan, 2012; Figure 1.3C-D). Senescence of the weed is strongly linked to temperature and rainfall, with plants dying back during mid to late autumn as rainfall and temperature decrease (Adkins et al., 2019; Figure 1.3E-F).

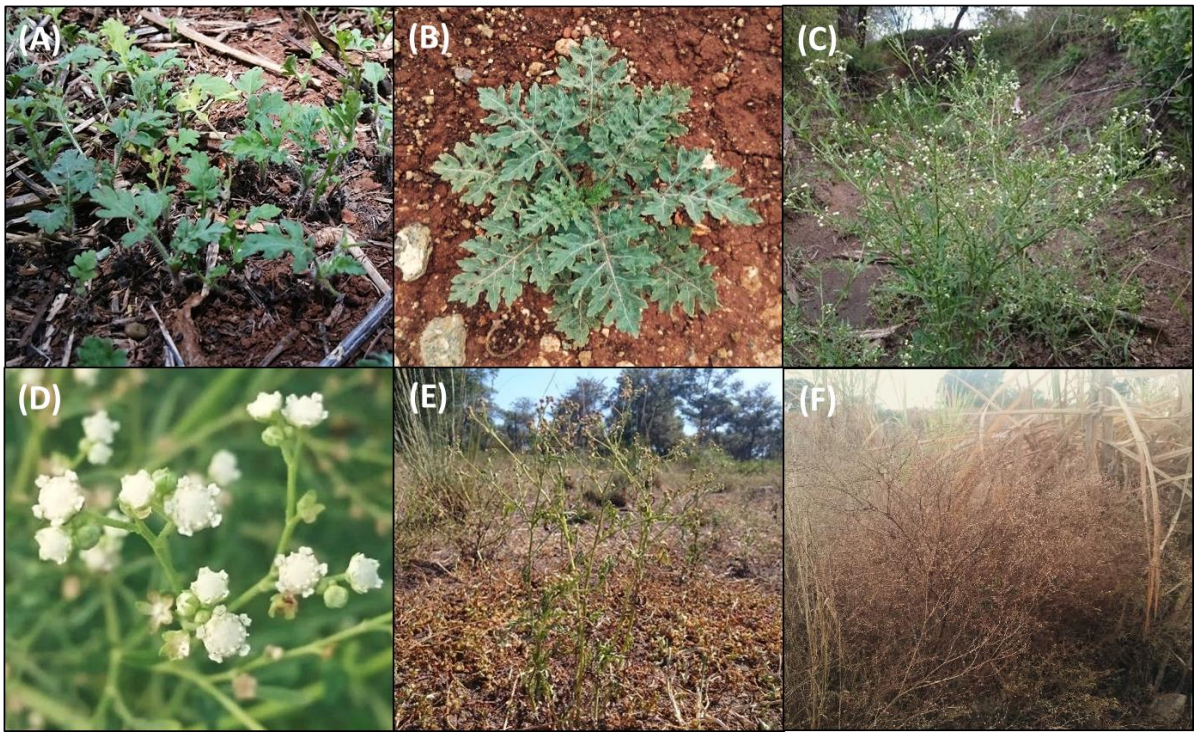

Figure 1.3. *Parthenium hysterophorus* growth stages, namely (A) recently emerged seedlings, (B) an immature leafy basal rosette, (C-D) a mature plant beginning to flower, (E) a senescing plant in autumn and (F) the inconspicuous remnants of a dead plants during early winter. (Photos: BW Cowie)

Parthenium hysterophorus seeds may germinate immediately after seed shed or enter seasonal dormancy and are primarily dispersed by wind and water, however, they are often unintentionally spread via vehicles, agricultural machinery, livestock and as contaminants in fodder and soil (Javaid et al., 2010; Mao et al., 2019). Given their small size (~2 mm), these seeds are easily dispersed long distances and are key to *P. hysterophorus's* invasive potential (Navie et al., 2004), capable of persisting in the soil, as seedbanks, for more than 10 years (Navie et al., 1996, 1998; Tamado et al., 2002). Although *P. hysterophorus* seedbank studies are limited, they highlight that heavily invaded areas are likely to experience the formation of extensive seedbanks, as large as 200 000 seeds/m^2 (Joshi, 1991; Strathie et al., 2011).

The annual nature, rapid growth, prolific seed production and persistent seed banks have all been highlighted as factors contributing to the invasion success of *P. hysterophorus* (Adkins et al., 2019). Concerningly, the weed also maintains a competitive advantage through its allelopathic effects (chemical suppression of one plant by another, also termed the "Novel Weapons Hypothesis") (Callaway and Ridenour, 2004; Van Der Laan et al., 2008). Various studies have highlighted the allelopathic properties of *P. hysterophorus*, mainly through the phytotoxic compound Parthenin and its accompanying phenolic acids (Belz et al., 2007, 2009). This sesquiterpene lactone is synthesized and present throughout the plant's roots, leaves and flowers, and can suppress and prevent the growth and germination of surrounding plants (Kohli and Batish, 1994; Kohli et al., 1996). These compounds enter the environment through root exudation, leaching of the plant and its leaves via rainwater and from decaying plant matter (Gupta, 2000; Batish et al., 2002). However, these sesquiterpene lactones and phenolic acids are short-lived, requiring high *P. hysterophorus* densities to reach and remain biologically active levels in the soil (Van Der Laan et al., 2008; Belz et al., 2009).

Impacts of *Parthenium hysterophorus*

Impacts on the environment

Areas in which *P. hysterophorus* readily invades, forming dense stands, are likely to suffer losses in ecological stability through the weed's impacts on native biodiversity (Adkins and Shabbir, 2014; Bajwa et al., 2019a). These dense stands aggressively invade and outcompete surrounding vegetation, often displacing or completely excluding native flora and fauna (Belz et al., 2009; Dhileepan and Strathie, 2009). Increases in the abundance of *P. hysterophorus*

have been strongly linked with decreases in above-ground species diversity and richness in both natural and rangeland ecosystems (Nigatu et al., 2010; Bajwa et al., 2016; Nguyen et al., 2017a). In addition, the prolific seed production of the weed threatens native diversity through reductions in the regenerative capacity of native propagules within the local seedbank (Navie et al., 2004; Belgeri et al., 2014).

Impacts on agriculture

The detrimental effects of *P. hysterophorus* are not solely limited to the environment and its biodiversity (Nigatu et al., 2010). Adverse impacts on agroecosystems remain one of the primary concerns in relation to *P. hysterophorus*. Invasions of *P. hysterophorus* are common in commercial plantations and subsistence farms, actively degrading and removing arable land, reducing crop yields and ultimately threatening food security (Singh et al., 2003; Adkins and Shabbir, 2014). Crops infested with *P. hysterophorus* may experience reductions in their yields of up to 90% (Safdar et al., 2015; Bajwa et al., 2016). Likewise, *P. hysterophorus* and its seeds are a known contaminant of agricultural grains, feeds and pasture seeds, severely reducing their quality, resulting in the restriction or loss of sales (Tamado et al., 2002; Bajwa et al., 2019a). The weed's invasions also prompt further agricultural concern given its indirect effects. *Parthenium hysterophorus* acts as an alternative host for various crop pests, including numerous insects and nematodes as well as fungal and bacterial pathogens (Dhileepan and McFayden, 2012b; Shabbir, 2014).

Impacts on livestock

Animal production is known to be heavily impacted by *P. hysterophorus* (Dhileepan and McFayden 2012a; Reynolds et al., 2020). Invasions by *P. hysterophorus* severely degrade and remove valuable grazing land, reducing the carrying capacity of pastures and limiting livestock and game production (Nigatu et al., 2010; Figure 1.4A-C). Furthermore, milk and meat products attained from livestock are strongly linked to the condition of pastures, with *P. hysterophorus* invasions found to reduce the quality and quantity of these products (Van Oudtshoorn, 2015; Bajwa et al., 2019a). Most domestic livestock and wild game are susceptible to allergenic reactions from contact with *P. hysterophorus*, suffering rashes and other dermal afflictions to their hides and udders (Tefera, 2002). Consumption of the weed (Figure 1.4D-E) has been demonstrated to cause ulceration of the mouth and taint the meat and milk of livestock exposed to contaminated fodder or pastures, making these products

hepatotoxic, and unfit for human consumption (Tamado et al., 2002; Kumari, 2014). Furthermore, if consumed by cattle for prolonged periods or in high enough amounts, *P. hysterophorus* can be lethal, with animals sustained on contaminated fodder, dying within two to four weeks (Narasimhan, et al., 1977; More et al., 1982).

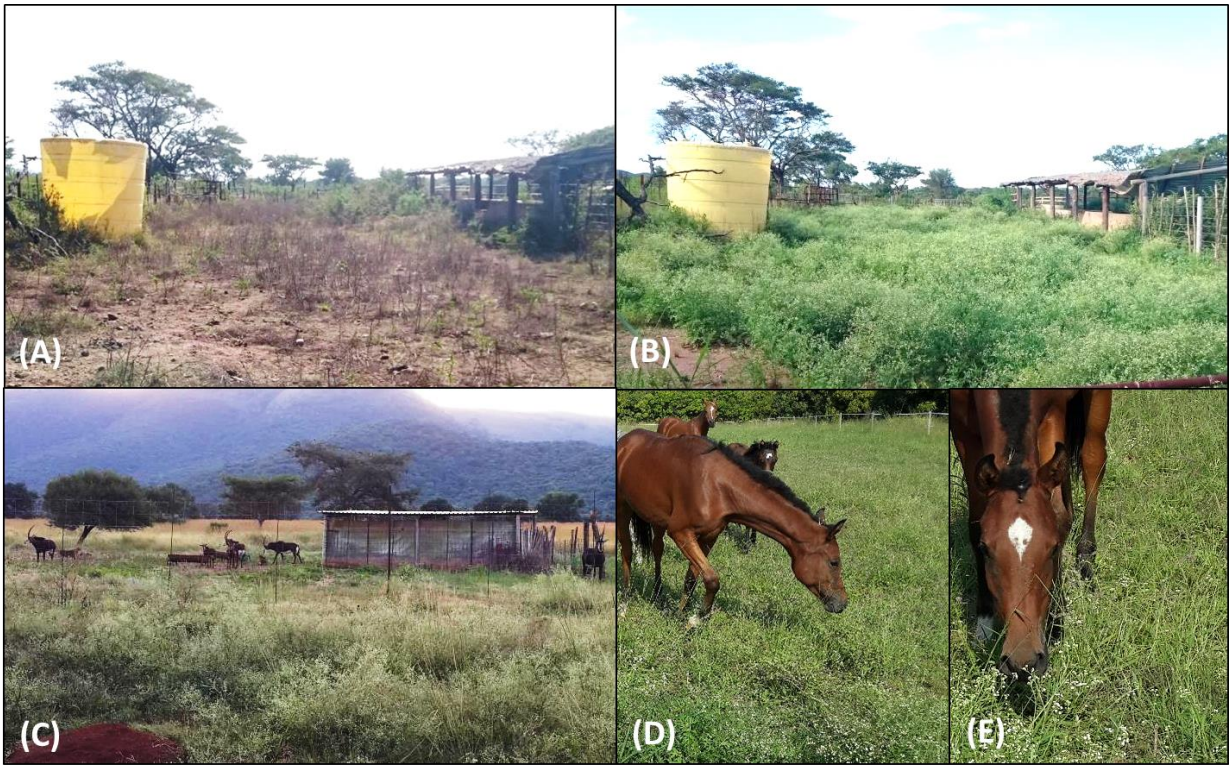

Figure 1.4. Prolific *Parthenium hysterophorus* invasions can be inconspicuous during winter (A) but are often highly damaging during summer (B), in this case forcing the abandonment of a cattle kraal. The presence of *P. hysterophorus* is also a major concern to animal breeders, where antelope (C) and horses (D-E) often consume the weed while grazing in infested pastures / paddocks. (Photos: BW Cowie)

Human impacts

Parthenium hysterophorus is well known for the health risks it poses to humans and is considered a major health concern in all countries it invades (Nadeem et al., 2005). The entire plant is toxic to humans, particularly its leaf trichomes and pollen, which are primarily responsible for the numerous allergenic complications associated with the weed (Bajwa et al., 2016). Contact with *P. hysterophorus* has been documented to cause various dermal conditions including dermatitis, rashes and blistering (Javaid and Anjum, 2005; Allan et al., 2019). Whereas, inhalation of the weed's pollen, may result in respiratory complications,

including asthma, bronchitis and rhinitis (Sharma and Verma, 2012). Furthermore, these afflictions increase in severity with prolonged exposure to *P. hysterophorus* and may result in further secondary ailments including fever and nausea, chronic illness and even death (Allan et al., 2019).

Constraints and drivers of *Parthenium hysterophorus* invasion

Constraints

Parthenium hysterophorus suffers few biotic constraints given the lack of natural predators, termed 'biotic release' (Keane and Crawley, 2002; Schulz et al., 2019). However, in the absence of natural predators' plants are regulated by the prevailing abiotic conditions, particularly temperature and rainfall (McConnachie et al., 2010). The germination of *P. hysterophorus* throughout spring and summer in South Africa, offers the weed ideal growing temperatures (27-30 °C) but increases its dependency on the availability of water (soil moisture). Soil moisture is believed to be one of the few factors constraining the distribution of *P. hysterophorus*, influencing both the flowering phenology and lifespan of the weed (Bajwa et al., 2017; Adkins et al., 2019). Although the weed can complete its life cycle at soil moistures as low as 50% field capacity (Bajwa et al., 2017), the extent of stress tolerance is unknown. Moreover, a comprehensive understanding of *P. hysterophorus* water-relations, within an invasive context, is required but remains limited.

Drivers

Much of Southern and South Africa remains climatically well matched to the weed's native range and is suggested to facilitate invasion (McConnachie et al., 2010). The country's extensive road and rail networks are also likely to provide an avenue to overcoming the geographic barriers (Terblanche et al., 2016). At more local scales, the poor management of various natural and semi-natural landscapes is highlighted as the main factor contributing to the rampant spread and prolific invasion of *P. hysterophorus* across South Africa (Van Oudtshoorn, 2015; O'Connor and van Wilgen, 2020). Several studies highlight the ability of the weed to completely invade areas which are overutilised or heavily disturbed, namely by domestic livestock (Nigatu et al., 2010; Nguyen et al., 2017a; Strathie and McConnachie, 2019).

Additionally, climate change, namely increasing CO_2 levels and temperature, are predicted to enhance *P. hysterophorus* invasions globally in the future (Nguyen et al., 2017b; Bajwa et al., 2019b). Studies suggest that *P. hysterophorus* is likely to become more invasive under elevated CO_2 conditions through increases in growth, productivity and reproduction (Navie et al., 2005; Nguyen et al., 2017b; Bajwa et al., 2019b). This highlights not only the severity of the weed, but the importance in optimising our controls and understanding if, and how, they may be affected in the future.

Controlling *Parthenium hysterophorus*

Physical and mechanical control

Physical control involves the manual removal of *P. hysterophorus* from infested areas, commonly by hand or with the aid of tools (Dhileepan and McFayden, 2012b). Although this technique is only feasible at small scales, it is still heavily utilised by many subsistence farmers and rural communities in South Africa (Strathie et al., 2011; Strathie and McConnachie, 2019). The removal of *P. hysterophorus*, including its roots, can be a time consuming and labour intensive process, only offering short term respite from invasion, and unless followed up regularly can worsen invasions through disturbance (Campbell et al., 2019). Consequently, labourers who frequently clear *P. hysterophorus* are also at risk of developing chronic health problems given their prolonged exposure to the weed and its pollen (Goodall et al., 2010; Bajwa et al., 2016).

Similarly, mechanical controls such as ploughing, slashing and mowing may be used at larger scales to reduce *P. hysterophorus* invasions temporarily, but like manual clearing these remain ineffective as long-term solutions (Dhileepan and Strathie, 2009; Strathie et al., 2011). Ploughing may quickly remove *P. hysterophorus* but often exacerbates the problem by disturbing the soil and stimulating high levels of germination (Adkins and Shabbir, 2014). Whereas mowing and slashing may not be as destructive but only partially remove the weed, allowing for its rapid regrowth and the copious production of seeds shortly afterwards (Campbell et al., 2019). Additionally, machinery and equipment used in the clearing of *P. hysterophorus* are known to spread seeds (Dhileepan and Strathie, 2009). Despite the short-term potential of manual and mechanical clearing efforts, the annual nature of *P.*

hysterophorus and size of its infestations, means these controls are not reliable long-term management approaches (Navie et al., 1996; Adkins and Shabbir, 2014).

Chemical control

Investigation into chemical control highlight their effectiveness, with herbicides designated as a preferred alternative to hazardous manual and mechanical removal of *P. hysterophorus* (Goodall et al., 2010; Strathie et al., 2011). A variety of herbicides are registered for the pre- and post-emergent control of *P. hysterophorus* globally, with optimal results frequently attained using residual pre-emergent herbicides or spraying plants prior to their flowering or seed set (Kumari, 2014; Campbell et al., 2019). In the case of South Africa, several broad spectrum pre-emergent herbicides are available, however, only three post-emergent herbicides are registered, the majority of which are glyphosate based, limiting the potential effectiveness of chemical control.

Overall, herbicides remain costly to obtain and apply over large invaded areas, often restricting their usage to agricultural lands or areas of high commercial value (Campbell et al., 2019). The use of herbicides in grazing lands and natural ecosystems is often unwanted and avoided, owing to the environmental risks associated with repeated applications (Dhileepan and Strathie, 2009). The reliance on herbicides in many agroecosystems has also prompted concerns regarding potential reductions in herbicide efficacy in the future (Bracamonte et al., 2016; Heap and Duke, 2018). Both improper spraying and increasing CO_2 levels have been suggested to reduce the effectiveness of herbicides, particularly glyphosate, (Goodall et a., 2010; Bracamonte et al., 2016) and may allow for the development of less susceptible *P. hysterophorus* populations (Bajwa et al., 2019b), however, this remains to be assessed within the South Africa context.

Biological control

Given the socio-economic impacts of *P. hysterophorus*, biological control, using host-specific phytophagous insects and pathogenic fungi, remains the most sustainable long-term control option (Dhileepan 2001; Dhileepan and McFayden, 2012a). South Africa was the first African country to initiate biological control against *P. hysterophorus* in 2003, when it was noted that herbicide usage, although effective, was costly and unsustainable (Strathie et al., 2011). A total of six agents, were imported into quarantine from Australia, for the biological control of *P. hysterophorus* (Klein, 2011; Strathie et al., 2011). These agents were primarily

selected for their potential efficacy and compatibility with South Africa's warm and drier climate (Dhileepan and Strathie, 2009). To date, four of the imported agents have been released, and include the leaf-rust fungus *Puccinia xanthii* (syn. *Puccinia melampodii*) Dietel and Holway var. *parthenii-hysterophorae*, the stem-and-root-boring weevil *Listronotus setosipennis* Hustache (Coleoptera: Curculionidae), the leaf-feeding chrysomelid *Zygogramma bicolorata* Pallister (Coleoptera: Chrysomelidae) and the seed-feeding weevil *Smicronyx lutulentus* Dietz (Coleoptera: Curculionidae) (Klein, 2011; Strathie et al., 2014).

Largely regarded as one of the most promising candidates, *Z. bicolorata*, was first released in KwaZulu-Natal and Mpumalanga during 2013 (McConnachie et al., 2015; Chidawanyika et al., 2017). Releases of the chrysomelid were intended to limit the vigorous growth and prolific flower and seed production of *P. hysterophorus*, constraining the spread and recruitment of the weed (Strathie et al., 2014; Strathie and McConnachie, 2019). The chrysomelid feeds extensively on the leaves of *P. hysterophorus* resulting in severe defoliation (Dhileepan and Strathie, 2009). Larvae emerge from eggs, laid on leaves, stems or floral buds, feeding voraciously on the leaves as they progress through four instars, prior to pupation (Strathie et al., 2011; Figure 1.5A-B). Pupation occurs within the soil, lasting approximately two weeks, after which adults emerge and begin feeding on *P. hysterophorus* plants (Strathie et al., 2011; Figure 1.5C-D). During autumn (April-May), *Z. bicolorata* adults enter diapause, in the soil, in response to reductions in food availability, rainfall and temperature. This diapause lasts throughout winter, with adult beetles re-emerging during spring, with the arrival of rains and warmer temperatures (Dhileepan and Strathie, 2009).

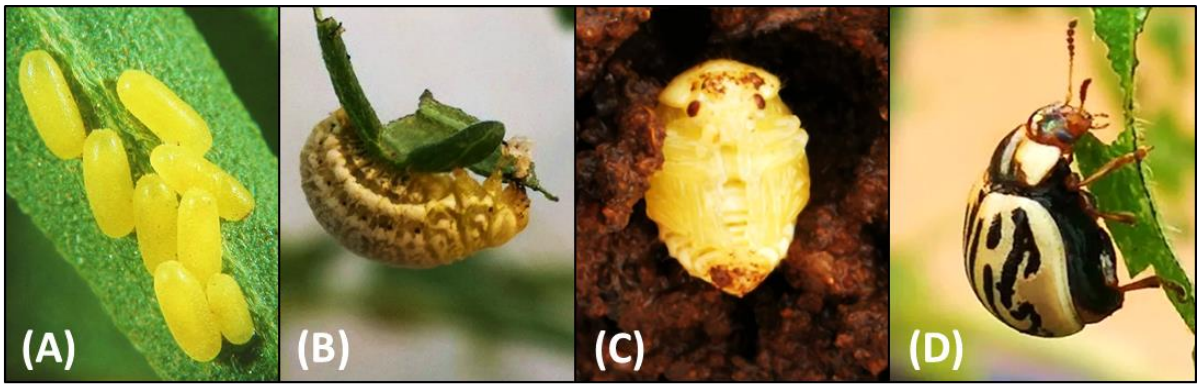

Figure 1.5. Development of *Zygogramma bicolorata,* a biological control agent released against *Parthenium hysterophorus*: (A) eggs, (B) larval instar, (C) pupa and (D) newly emerged adult beetle. (Photos: BW Cowie).

Since its release in 2013, *Z. bicolorata* has established poorly, at less than 13% of its release sites, and only a few localised outbreaks documented causing damage to *P. hysterophorus* (Strathie et al., 2016; Strathie and McConnachie, 2019). This poor establishment and persistence have led to concerns regarding potential abiotic and biotic constraints on the agent. Climate studies by King (2008) suggested that *Z. bicolorata* is well suited to South Africa and would be able to establish, survive and reproduce. Although native generalist predators were suggested and have been found to hinder *Z. bicolorata* populations in the field, they are unlikely to completely prevent establishment and persistence of the beetle (Abels, 2018; Strathie and McConnachie, 2019). Additionally, suggestions that (i) *P. hysterophorus* may be tolerant to herbivory by *Z. bicolorata* and (ii) plant quality may be an underlying constraint to the establishment and persistence of *Z. bicolorata* in the field, have been emphasised but remain untested (Chidawanyika et al., 2017).

Remediation / Restoration

Initial approaches towards remediating *P. hysterophorus* invaded landscapes focused mainly on reducing the disturbances known to facilitate or intensify invasion, particularly overstocking and overgrazing (Bajwa et al., 2019a; Khan et al., 2019). Research has shown that subsequent to the removal of grazers within an invaded grassland, native species may recover and displace *P. hysterophorus* (Nigatu et al., 2010; Belgeri et al., 2014). Although reducing grazing pressure may limit the invasive potential of *P. hysterophorus*, allowing for the recovery of native pastures, this process may take extended periods of time and depending on the severity of invasion may not be feasible for landowners (Khan et al., 2019; O'Connor and van Wilgen et al., 2020; Shabbir et al., 2020).

More recent approaches have attempted to actively suppress *P. hysterophorus* invasions by using competitive plant species (Shabbir et al., 2013; Khan et al., 2013, 2019). The planting or sowing of pasture species holds merit as a management and restoration tool in many *P. hysterophorus* infested rangelands (Van Der Laan et al., 2008). Several grasses and legumes have been shown to effectively compete with *P. hysterophorus*, suppressing the growth, reproduction and density of the weed (Van Der Laan et al., 2008; Khan et al., 2019). However, this remains to be rigorously tested in South Africa, with only one grass transplant study, highlighting the ability of a few perennial grasses to establish and suppress the growth of *P. hysterophorus* under low levels of invasion (van Der Laan et al., 2008).

Rationale

Given the severe threats *P. hysterophorus* poses to agriculture, biodiversity and human health, it is imperative that the weed's invasive ecology be further understood. The risk of further invasions and spread into sub-Saharan countries, including South Africa (McConnachie et al., 2010), prompts investigations into *P. hysterophorus's* abiotic constraints and invasion dynamics. Although biological control is accepted as an integral core towards managing *P. hysterophorus*, it remains in its infancy in South Africa, having not yet attained desirable levels of control (Strathie and McConnachie 2011). This then requires investigations into optimising biocontrol efforts as well as assessments into alternative controls, such as herbicides efficacy and the sowing with native grass seed to supplement other management techniques or to prevent further *P. hysterophorus* invasions in regions where biocontrol alone is ineffective or not feasible.

Study Aim and Objectives

Overall this study aimed to assess the invasive potential of *P. hysterophorus* as well as the effectiveness of South Africa's main and proposed control techniques, namely land management, biological control, herbicide use and the sowing of competitive native grasses. The research objectives of this thesis were as follows:

Objective 1. Identify and assess the morphological and ecophysiological responses of *P. hysterophorus* to progressive water stress at different growing stages, namely juvenile basal rosettes and flowering adult plants.

Objective 2. Determine the influence that land management, particularly grazing, has on the invasive potential of *Parthenium hysterophorus* within savannas.

Objective 3. Assess whether *Z. bicolorata* remains a promising biocontrol agent, through ecophysiological assessments into the beetle's leaf feeding impacts on the weed.

***Objective 4*:** Evaluate the influence of *P. hysterophorus* plant quality on the survival, development and fecundity of the biological control agent *Z. bicolorata.*

Objective 5: Determine the susceptibility of *P. hysterophorus* to glyphosate herbicide under ambient and elevated atmospheric CO_2 levels anticipated in the future.

Objective 6: Investigate the feasibility of sowing of native grasses to suppress *P. hysterophorus* invasion and restore pastures within a sustainably stocked savanna reserve.

Thesis outline*

The first chapter of this thesis consists of a general introduction, including a brief literature review of the biology and impacts of *Parthenium hysterophorus* as well as the control efforts utilised against the weed in South Africa. Chapter Two is a published ecophysiological paper which sought to assess the morphological and physiological response of *P. hysterophorus* to water stress and how this is likely to influence the weed's invasion (Objective 1). Chapter Three, investigated the role that land management maintains on the invasion dynamics of *P. hysterophorus*, both above- and below-ground, in savanna systems (Objective 2). Chapter Four is a published paper which focused on understanding the ecophysiological interactions of *Z. bicolorata* and *P. hysterophorus*, particularly the effects of leaf-feeding on photosynthesis (Objective 3). Chapter Five is a published paper which sought to investigate the role that *P. hysterophorus* plant quality holds in constraining biological control efforts using *Z. bicolorata* (Objective 4). Chapter Six is a published paper which assessed the susceptibility of *P. hysterophorus* to glyphosate herbicide under current and elevated CO_2 levels (Objective 5). Chapter seven examined the feasibility of remediating savanna areas invaded by *P. hysterophorus* through the sowing of native perennial grass seed (Objective 6). The final chapter provides a brief overview of the prospects for controlling *P. hysterophorus*. This chapter also provides recommendations for future research toward understanding the *P. hysterophorus* invasion and its management within a South African context.

** This thesis has been prepared and written in the format of scientific papers. The repetition of information, given this format, is unfortunately unavoidable but has been minimised as much as possible.*

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Parthenium avoids drought: Understanding the morphological and physiological responses of the invasive herb *Parthenium hysterophorus* to progressive water stress

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ABSTRACT

Parthenium hysterophorus L. is a noxious annual invasive herb which threatens biodiversity, food security and human and animal health in various tropical and sub-tropical regions globally. Given that these regions, including South Africa, experience frequent drought events, it is important that the invasive potential of this weed be better understood in the context of water stress. This study aimed to assess the physiological and morphological responses of *P. hysterophorus* to water stress. To elicit these responses, juvenile and adult plants were progressively water stressed over a six-week period. Despite significant reductions in leaf water potential, relative leaf water content and stomatal conductance from severe soil water stress, plants maintained positive net photosynthesis, albeit at a severely reduced rate. Concomitantly, water-use efficiency of *P. hysterophorus* increased by more than 300 %. Morphologically, water-stressed plants exhibited reductions in leaf area and leaf number, with adults accelerating flower production when compared to well-watered plants. Linked to this, were alterations in biomass, with juveniles and adults reducing above-ground biomass by 75 % and 35 %, respectively. Overall, many of the physiological and morphological responses are indicative of a largely drought avoidance strategy, allowing *P. hysterophorus* to persist during periods of water-stress as leafy basal rosettes (juveniles) or as reproductive herbs (adults). This research yielded valuable insights into the underlying water relations of *P. hysterophorus*, and how these may relate to the potential spread, detriment and management of the weed in various sub-tropical environments.

1. Introduction

Native to Central and South America, the herb *Parthenium hysterophorus* L. (Asteraceae: Heliantheae) is a noxious annual invasive plant prevalent in at least 50 tropical and sub-tropical countries across the globe (Dhileepan and Strathie, 2009; Adkins and Shabbir, 2014). In South Africa, *P. hysterophorus* is recognised as one of the country's most threatening invasive plants, with large populations continuing to spread in KwaZulu-Natal, Mpumalanga, North-West and Limpopo provinces (Terblanche et al., 2016). *Parthenium hysterophorus* grows and matures rapidly (4–6 weeks), producing prolific amounts of seed (~30 000 seeds/plant) which form extensive and persistent seedbanks (Strathie et al., 2011). The weed readily invades and colonises a wide variety of environments, including road-verges, rail-sides, cultivated lands, watercourses, pastures as well as natural grassland and savanna habitats (Strathie et al., 2011). Invasions of *P. hysterophorus* impose severe ecological and socio-economic impacts, including reductions to crop yields, displacement of native species, losses to livestock and their productivity as well as threats to human and animal well-being (Adkins and Shabbir, 2014). Further concern surrounds predictions by McConnachie et al. (2010), which suggests that large portions of sub-Saharan Africa are climatically suited and susceptible to further *P. hysterophorus* invasion.

Numerous environmental factors are known to constrain the establishment and proliferation of invasive plants within their introduced ranges (Theoharides and Dukes, 2007; McConnachie et al., 2010). In the absence of natural predators and pathogens (termed 'biotic release'), weed invasions are largely regulated by the prevailing abiotic

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conditions (Melbourne et al., 2007), the most prominent of which is climate (Theoharides and Dukes, 2007). Climatic variables, particularly temperature, rainfall and their interaction (seasonality), often predict the potential distribution and invasiveness of weeds at a broad scale (Sakai et al., 2001). Furthermore, this is also influenced by the ecology and adaptability of the weed itself (Friedman and Rubin, 2015). For annual species, like *P. hysterophorus*, seasonal germination during the warm spring and summer months affords plants' favourable growing temperatures, but simultaneously increases their dependency on the availability of water in the soil (Friedman and Rubin, 2015). Soil moisture availability is largely determined by the amount and frequency of precipitation in sub-tropical regions, meaning that the high variability in annual and seasonal rainfall within these regions of South Africa (MacKellar et al., 2014) is likely to expose short-lived plants to periods of water stress.

Water stress is one of the most important physiological constraints to tropical and sub-tropical invasive weeds, widely accepted to limit their productivity, growth, reproduction and lifespan (Touchette et al., 2007; Oliver et al., 2010). Drought events are characterised by prolonged periods of below-average rainfall, which result in the progressive decline and subsequent shortage of soil water available to plants (Touchette et al., 2007). Periods of water stress may elicit a myriad of morphological and physiological plant responses, which can be generally classified as 'drought escape',' drought avoidant' or 'drought tolerant' (Lawlor, 2012). Drought escape allows plants to complete their life-cycle before the onset of severe water stress (drought stress), typically by accelerating flowering and seed set prior to senescence (Verslues et al., 2014). Whereas drought avoidance serves to minimise plant water loss and/or increase water uptake, and frequently includes increases in root biomass, decreases in leaf area and reductions in stomatal conductance (McCann and Huang, 2008). Drought tolerance however, is largely metabolic seeking to maintain cell turgor and function, mainly through osmotic adjustment, in which plants accumulate solutes to better retain water (Touchette et al., 2007; Lawlor, 2012). Although drought tolerance and avoidance are common responses to ongoing water stress, species-specific studies are required to identify the underlying mechanisms and to what extent these responses are likely to be elicited.

Described as a C_3-C_4 intermediate species (Moore et al., 1987), *P. hysterophorus* is physiologically a highly responsive weed, capable of phenotypically adapting to and overcoming various environmental constraints (Kohli et al., 2006; Cowie et al., 2018). Despite this plasticity, soil moisture remains one of the few abiotic limitations to the annual lifecycle and distribution of the weed (Dhileepan et al., 2000; McConnachie et al., 2010). Although seeds are resilient and can persist in dry soil for many years, their germination is highly dependent upon rainfall, with seedlings typically emerging throughout spring to summer, in areas with summer rainfalls > 500 mm (McConnachie et al., 2010). Young seedlings will often transition through a leafy basal rosette stage before maturing (flowering), however, during periods of water stress young plants may persist as rosettes awaiting favourable rainfall (Annapurna and Singh, 2003). As adults, water availability is linked to the flowering phenology of *P. hysterophorus*, with decreasing soil moisture prompting earlier flowering and seed set (Bajwa et al., 2017). Despite limited understanding of *P. hysterophorus's* water-relations, recent studies have shown that the weed may complete its life-cycle at soil moistures as low as 50 % field capacity (Bajwa et al., 2017; Nguyen et al., 2017). This suggests that *P. hysterophorus* plants possess a strategy to persist and set seed during periods of water stress.

Global climate models predict that increasing temperatures and decreasing rainfall, will result in much of South Africa experiencing more frequent and severe drought events (MacKellar et al., 2014; Chami and Moujabber, 2016). However, it is not fully understood how many of the country's most problematic invasive weeds will respond, nor if these species are physiologically able to tolerate increases in water stress. Given the threat *P. hysterophorus* poses to South African agriculture, natural areas and human and animal health, it is imperative that the invasive potential of the weed be better understood in the context of the weed's ability to cope with water stress. This study aimed to identify and assess the morphological and ecophysiological responses of *P. hysterophorus* to progressive water stress at different growing stages. To better elicit and assess these responses, both juvenile and adult plants were progressively watered stressed, simulating a natural dry period over a six-week period.

2. Methods

Parthenium hysterophorus plants were grown from field-collected seed during September 2017. Plants were grown in 30 cm diameter, 25 cm deep plastic pots, filled with a clay-loam soil mixture (30 % clay: 20 % silt: 50 % sand), under full-sunlight (~1800 μmol^{-1}) in an ambient conditions greenhouse at the University of the Witwatersrand, Johannesburg, South Africa (26°11'28.9"S 28°01'55.4"E). Mean day and night time temperatures experienced within the greenhouse were 28.9 ± 3.4 °C and 17.4 ± 2.4 °C respectively, with relative humidity fluctuating between 45–75% daily. Plants were watered daily and fertilised monthly with 100 ml of Seagrow® organic fertiliser mixed, with water, at a concentration of 5 ml/ℓ. Two batches of *P. hysterophorus* (n = 25/batch) were germinated approximately three weeks apart to attain plants of three and six weeks old, termed 'juveniles' and 'adults' respectively.

2.1. Progressive water stress and soil water content

Twenty juvenile and 20 adult plants were each selected at random, and evenly divided into two groups of well-watered (controls) and water-stressed treatments (n = 10 / treatment). Well-watered plants were watered daily by means of an irrigation system for a total of 12 min/day, receiving ~180 ml of water. Simulating a progressive and natural decline in soil moisture, water-stressed plants had water added or withheld as necessary by hand. Soil water content (SWC) for the water-stressed juvenile and adult plants began under well-watered conditions (~27 % SWC) and had SWC declined at no more than 1.5 % per day.

Soil moisture was measured twice a day, in each of the well-watered and water-stressed pots, using a Hydrosense II meter with a 12 cm moisture probe (Campbell Scientific Inc., Utah). Within each pot, three measures were taken at random positions and the volumetric soil water content (%) (VSWC) was averaged. As per Venter et al. (2017), VSWC values were converted to soil water content (%) (SWC) using the equation: $SWC = 0.0004(VSWC)^3 - 0.0268(VSWC)^2 + 1.3182(VSWC) + 0.2862$; $R^2 = 0.99$. To generate this equation, five 30 cm pots were filled with the same soil, watered to field capacity and allowed to dry down naturally under ambient conditions in the greenhouse. During the dry-down, pots were measured daily for VSWC as well as SWC, which was done gravimetrically, until VSWC reached 0 %.

2.2. Parthenium hysterophorus gas exchange and growth

To determine the effects of progressive water stress on the physiology of *P. hysterophorus*, weekly measures were made on all plants (N = 40) for a period of six weeks. Gas exchange parameters of *P. hysterophorus* were measured using a Li-6400XT portable open-path infrared gas analyser (LICOR Biosciences, Lincoln, Nebraska, USA). Net leaf CO_2 and H_2O exchange were measured on the 4th fully expanded leaf below the apical bud. The CO_2 concentration was set at 400 ppm within the curvette and photosynthetic photon flux density (PPFD) at 1800 μmol^{-1}, using the standard blue-red LED light source. All photosynthetic measurements were made at a leaf temperature of 27 °C, with leaf to air vapour pressure deficit (VPD) kept below 1.2 kPa.

Leaves, on each of the plants, were inserted into the cuvette for initial measurements on day 0, prior to any water stress. In the case that

leaves inserted into the cuvette did not completely fill the 6 cm^2 area, they were photographed and the leaf area within the cuvette was calculated using ImageJ (Ver. 1.50, Wayne Rasband, National Institute of Health, USA). Photosynthesis (*A*), stomatal conductance (*gst*), and intercellular CO_2 concentration (*Ci*) were calculated using the equations of von Caemmerer and Farquhar (1981), incorporating any leaf area corrections. Intrinsic water-use efficiency was calculated as *A/gst*. Gas exchange data were recorded when *A* and *gst* showed less than a ± 2 % change over a 2 min period. All measurements were conducted between the hours of 11:00 and 14:30.

Similarly, the morphology of *P. hysterophorus* was measured weekly and included flower/seed production, plant height, leaf number, and leaf area. For floral and seed counts, only fully formed flowers and mature seeds were included. Plant height was measured from the root crown to the tallest apical shoot (cm). Leaf number was recorded as the total number of fully expanded leaves present on the plant, excluding small leaflets. Leaf area was calculated as per Cowie et al. (2019) using the length and width of each fully expanded leaf present on the plant: Eq. (1).

$$Leaf\ Area\ (cm^2) = \sum (0.4104[L_1 \times W_1]) + (0.4104[L_2 \times W_2]) + \cdots (0.4104[L_n \times W_n]) \quad (1)$$

L = leaf length (cm); W = leaf width (cm); n = total leaves/plant

2.3. Chlorophyll fluorescence and leaf chlorophyll

Following gas exchange measurements, the same leaves were used to measure the maximum quantum yield of Photosystem II (*PSII*) (*Fv/Fm*). Leaves were dark-adapted overnight using Opti-Science leaf clips. To ensure that leaves were fully dark adapted (i.e. complete Q_A oxidation), *Fv/Fm* measures were taken prior to sunrise the following morning. Measurements were taken using an OS30p + fluorometer (Opti-Sciences, Inc. Hudson, New Hampshire, USA), with the modulation and saturation intensities set at 40 % and 3500 µmol respectively. *Fv/Fm* values of 0.8 and above, were considered as 'healthy' (Adams and Demmig-Adams, 2004). Leaf chlorophyll content was measured at two random positions on all fully expanded leaves using a CCM-300 Chlorophyll meter (Opti-Sciences, Inc.), and averaged to attain a single leaf chlorophyll value per plant.

2.4. Leaf water potential and relative leaf water content

Leaf water potential (Ψ_{leaf}) was measured at midday, following chlorophyll fluorescence and leaf chlorophyll measures, using a 1505D pressure chamber (PMS Instrument Company, Albany, Oregon, USA) attached to a nitrogen cylinder. Once a week, the same leaves used for gas exchange and chlorophyll fluorescence were cut at the base of the petiole, weighed (wet weight) to three decimal places, and immediately placed into the pressure chamber. Cut leaves were inserted, via their petioles, into the rubber stopper and then tightly sealed into the pressure chamber with only a small (~1 cm) section of the petiole showing. Pressure in the chamber was gradually increased, at a rate of approximately 0.05 MPa per second, and Ψ_{leaf} (MPa) was recorded when the exposed section of the petiole showed signs of exuding water (bubbling).

Immediately after each water potential measurement, leaves were removed from the pressure chamber, cut along the petiole (longitudinally) under water and placed into a container. Containers were filled with enough water to fully cover the entire leaf petiole and sealed. All 40 leaves within the containers were then left overnight for leaves to become turgid. Leaves were then removed individually, dried off and weighed (turgid weight). Dry weight was obtained from oven drying the leaves at 70 °C for three days (~72 h). Relative leaf water content (RLWC) per leaf was calculated as the percentage of wet weight to turgid weight, whilst accounting for dry weight of the leaf (Eq. (2)).

$$RLWC\ (\%) = \frac{Wet\ weight\ (g) - Dry\ weight\ (g)}{Turgid\ weight\ (g) - Dry\ weight\ (g)} \times 100 \quad (2)$$

2.5. $A:C_i$ curves and limitations to photosynthesis

Net photosynthesis to intercellular CO_2 ($A:C_i$) curves were conducted on four randomly selected plants from each of the well-watered and water-stressed juvenile and adult treatments (N = 16) at week 5. *A:Ci* curves were fitted to the data using the C_3 model of von Caemmerer (2000). Parameters calculated from the $A:C_i$ curves included maximum electron transport rate (*Jmax*), maximum RuBisco carboxylation rate (*Vcmax*), carboxylation efficiency (*CE*) and CO_2 compensation point (Γ). Additionally, stomatal limitations (S_L) to photosynthesis were calculated for juvenile and adult *P. hysterophorus* plants as per Farquhar and Sharkey (1982). Relative stomatal (R_{SL}) and metabolic limitations (R_{ML}) were calculated for juvenile and adult water-stressed plants relative to their well-watered controls at day 35.

2.6. Biomass allocations of Parthenium hysterophorus

At the end of the sixth week, all juvenile and adult plants (N = 40) were harvested for dry biomass. Each of the plants was carefully uprooted and washed to remove all soil and debris, particularly from the roots. Root length was assessed after washing and was measured from the root crown to the base of the longest primary root. Plants were then sectioned into flowers, stems, leaves and roots, oven dried at 60 °C for five days and weighed to three decimal places (0.001 g). Flower, leaf and stem biomass were summed, per plant, to attain above-ground shoot biomass, used for root : shoot ratios.

2.7. Statistical analysis

Student t-tests (unequal variance), were used to determine if the soil water content differed between the juvenile and adult plants in the well-watered and water-stressed treatments, respectively. Multiple repeated measures analysis of variance (ANOVA) with a Tukey HSD *post-hoc* were used to statistically assess the physiological, growth, biochemical and metabolic parameters of well-watered and water-stressed juvenile and well-watered and water-stressed adult *P. hysterophorus* plants over the six-week period. Lastly, one-way ANOVAs with Tukey HSD *post-hocs* were used to assess the selected $A:C_i$ and dry biomass parameters between the well-watered and water-stressed juvenile and adult plants. For the assessment of root : shoot proportions, data were arcsine transformed prior to the ANOVA.

3. Results

3.1. Progressive water stress and soil water content

Given that the watering treatment yielded no differences between the soil water content (SWC) of the well-watered juvenile and adult plants (t_{82} = 0.7; $P > 0.05$), and the water-stressed juvenile and adult plants (t_{82} = 1.2; $P > 0.05$), SWC data were combined per day. Soil moisture for *P. hysterophorus* plants (juvenile and adult) in the well-watered treatment was consistently high, maintaining an average of 26.2 ± 0.7 % (range: 24.6–27.8) throughout the 42-day experiment (Fig. 1). Juvenile and adult plants within the water-stressed treatment experienced a gradual decline in soil water content, ~0.9 ± 0.1 % per day, with SWC dropping below 17 % on day 14. *Parthenium hysterophorus* only showed signs of wilting at day 38, when soil moisture dropped below ~2.2 % (Fig. 1).

3.2. Parthenium hysterophorus gas exchange and growth

Parthenium hysterophorus showed significant declines in net

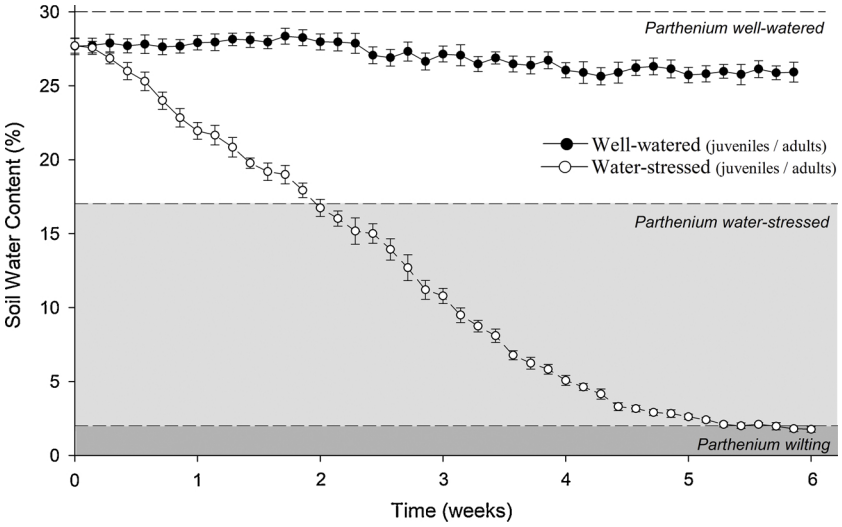

Fig. 1. Mean (± SE) soil water content (SWC) for well-watered (controls) and progressively water-stressed *Parthenium hysterophorus* plants over a 6-week period. Juvenile and adult SWCs were combined per day in each of the well-watered and water-stressed treatments (n = 20 per point). Dashed horizontal lines denote SWCs at which both juvenile and adult *P. hysterophorus* plants exhibited signs of water-stress / wilting.

photosynthesis (*A*) in response to water-stress, with both juvenile ($F_{6,108}$ = *77.3; P < 0.001*) and adult ($F_{6,108}$ = *106.3; P < 0.001*) plants experiencing overall reductions of 69 % and 79 % respectively (Fig. 2A–B). Adult plants, however, were more susceptible as they were significantly affected after one week of water stress (~22 % SWC), whereas juvenile plants sustained *A* similar to that of the controls for a further week (~17 % SWC). Stomatal conductance (g_{st}) showed similar trends to *A* in response to water stress, with both juvenile ($F_{6,108}$ = *11.2; P < 0.001*) and adult ($F_{6,108}$ = *15.2; P < 0.001*) plants experiencing overall reductions of more than 98 % compared to their well-watered controls (Fig. 2C–D). Intercellular CO_2 concentration (C_i) decreased significantly in both juvenile ($F_{6,108}$ = *31.7; P < 0.001*) and adult ($F_{6,108}$ = *13.3; P < 0.001*) plants, becoming significant from week 4 onwards (Fig. 2E–F). Water-use efficiency was drastically enhanced in response to water-stress, with increases of 380 % for juvenile ($F_{6,108}$ = *38.8; P < 0.001*) and 330 % for adult ($F_{6,108}$ = *26.1; P < 0.001*) plants (Fig. 2G–H).

The progressive decline in soil water significantly reduced the height of both juvenile ($F_{6,108}$ = *50.9; P < 0.001*) and adult ($F_{6,108}$ = *4.3; P < 0.01*) *P. hysterophorus* plants after 42 days (Fig. 3A–B), with juveniles experiencing the greatest reduction of ~86 %. Leaf production declined significantly, with the juvenile ($F_{6,108}$ = *180.5; P < 0.001*) and adult ($F_{6,108}$ = *99.2; P < 0.001*) plants showing reductions of 50 % and 41 % respectively (Fig. 3C–D). Similarly, leaf area decreased significantly, from week 3 onwards, for juvenile ($F_{6,108}$ = *166.7; P < 0.001*) and adult ($F_{6,108}$ = *49.3; P < 0.001*) plants (Fig. 3E–F). Flower production of juvenile plants decreased by 80 % ($F_{6,108}$ = *4.2; P < 0.05;* Fig. 3G) but adults displayed a significant increase of 15 % in flower production ($F_{6,108}$ = *6.9; P < 0.001;* Fig. 3H).

3.3. Chlorophyll fluorescence and leaf chlorophyll

Maximum quantum yield of *PSII* showed no difference between the well-watered or water-stressed juvenile plants ($F_{6,108}$ = *0.5; P > 0.05*), with *Fv/Fm* values remaining above 0.8 over the six-week period (Fig. 4A). Although *Fv/Fm* values declined below 0.8, to 0.76, in the water-stressed adult plants, this was not significant ($F_{6,108}$ = *2.3; P > 0.05*) compared to their well-watered controls (Fig. 4B). In terms of leaf chlorophyll content, significant declines were recorded for both the water-stressed juvenile and adult plants (Fig. 4C–D). Relative to their well-watered controls, adult plants suffered the greatest decline in leaf chlorophyll content of 15 % ($F_{6,108}$ = *21.4; P < 0.001*), whereas the juvenile plants suffered a smaller 7 % decline ($F_{6,108}$ = *4.8; P < 0.01*).

3.4. Leaf water potential and relative leaf water content

Water-stressed plants experienced a significant decrease in Ψ_{leaf} compared to their well-watered controls over the six-week period, decreasing from −1.2 to −3.6 MPa in juveniles ($F_{6,108}$ = *145.1; P < 0.001*) and from −1.1 to −3.3 MPa in adults ($F_{6,108}$ = *140.7; P < 0.001*) (Fig. 5A–B). RLWC declined significantly over the six-week period, with juvenile plants experiencing a total reduction of 40 % and adults a 44 % decline (Fig. 5C–D).

3.5. A:C_i curves and limitations to photosynthesis

Maximum electron transport rate (J_{max}) decreased significantly in water-stressed plants, with adult plants suffering a greater reduction of ~33 % compared to juvenile plants ($F_{3,12}$ = 14.5; $P <$ 0.01). Both maximum RuBisco carboxylation rate (Vc_{max}) and carboxylation efficiency (*CE*) declined significantly in response to water-stress in juvenile and adult plants (Table 1). CO_2 compensation point (Γ) remained largely unchanged in all treatments ($F_{3,12}$ = 2.5; *P > 0.05*), but stomatal limitations (S_L) increased by 343 % and 151% in water-stressed juvenile and adult plants respectively. Water-stressed *P. hysterophorus* suffered relative stomatal (R_{SL}) and metabolic limitations (R_{ML}) to photosynthesis, however, these did not differ between juvenile and adult plants (Table 1).

3.6. Biomass allocations of Parthenium hysterophorus

Floral biomass was significantly reduced and delayed in juvenile *P. hysterophorus* plants in response to water stress, but not in adult plants which maintained similar biomass allocations between the well-water and water-stressed treatments ($F_{3,30}$ = 88.2; $P <$ 0.001). Well-watered juvenile and adult plants invested substantially in above-ground biomass, showing similar root to shoot ratios of 1 : 3.2 and 1 : 3.9, respectively (Table 2). However, water-stressed plants, increased the proportion of biomass allocated below-ground (roots), significantly increasing their root to shoot ratios by 54 % in juveniles and 21 % in adults ($F_{3,36}$ = 66.9; $P <$ 0.001). Overall, water-stressed *P. hysterophorus* plants suffered significant reductions in total biomass as both juveniles (65 %) and adults (16.6 %) ($F_{3,36}$ = 145.3; $P <$ 0.001), influencing their biomass allocation patterns (Table 2).

4. Discussion

Parthenium hysterophorus showed an impressive ability to survive and reproduce during unfavourable periods of progressive water stress

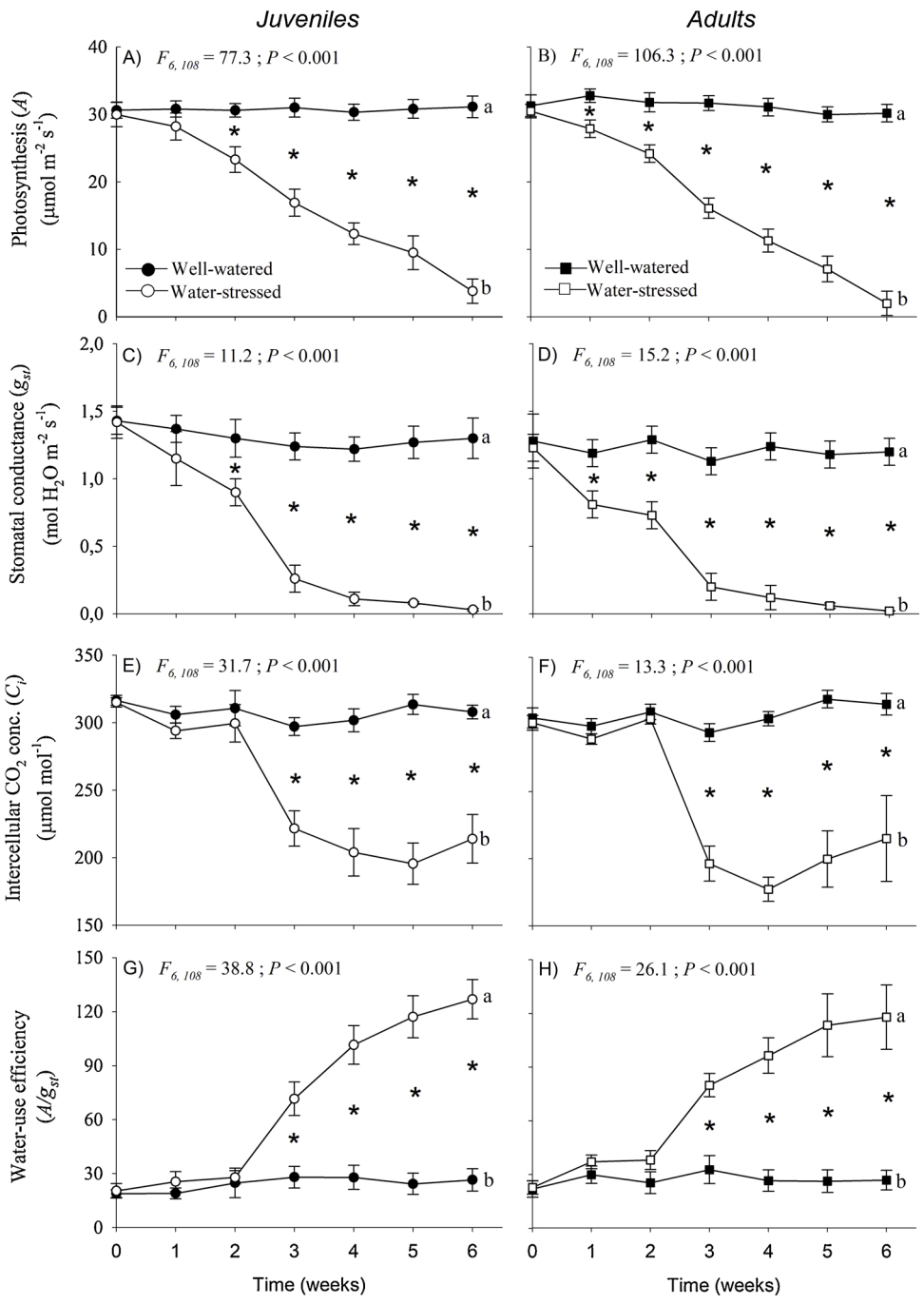

Fig. 2. Mean (± SE) (A–B) photosynthesis, (C–D) stomatal conductance, (E–F) intercellular CO_2 concentration and (G–H) water-use efficiency for well-watered and water-stressed juvenile and adult *Parthenium hysterophorus* plants. *Fstat* and *P-values* indicate overall difference in *P. hysterophorus* gas exchange between treatments using a repeated measures ANOVA. Different lowercase letters indicate significant differences between treatments overall and asterisks (*) represent significant differences between well-watered and water-stressed plants on corresponding weeks ($P < 0.05$).

as both juvenile and mature adult plants. This ability to persist was attained by employing a combination of largely drought avoidance and to a lesser extent, drought tolerance responses (see McCann and Huang, 2008; Lawlor 2013). Little to no change in productivity or growth was recorded over the first two weeks, which supports the preference of *P. hysterophorus* to grow optimally in relatively moist soils (Bajwa et al., 2017; Nguyen et al., 2017), which are not waterlogged (i.e. SWC of 18–28%). The notable physiological responses suggest that *P. hysterophorus* is highly sensitive to declines in soil moisture and that stress responses are triggered at SWCs < 20 %, although this will be dependent on the soil type and climatic conditions present (Annapurna and Singh, 2003; Bajwa et al., 2017). The age at which water-stress was experienced did not affect the physiological strategy that the plants adopted, but rather the growth type that plants invested in, with juveniles investing in vegetative growth and persistence and adults towards prolonging their reproductive phase.

Decreasing SWC strongly influenced the physiology of *P. hysterophorus*, most notably the plant's photosynthesis (productivity). This gradual reduction in photosynthesis can be attributed to the decreased stomatal conductance, which prevents excessive water loss by reducing evapotranspiration, but limits the plants CO_2 uptake (Chaves et al., 2002; Osakabe et al., 2014). Limitation to CO_2 uptake by the stomata of *P. hysterophorus* resulted in declines in intercellular CO_2 (C_i) of both juvenile and adult plants, which consequently reduced the

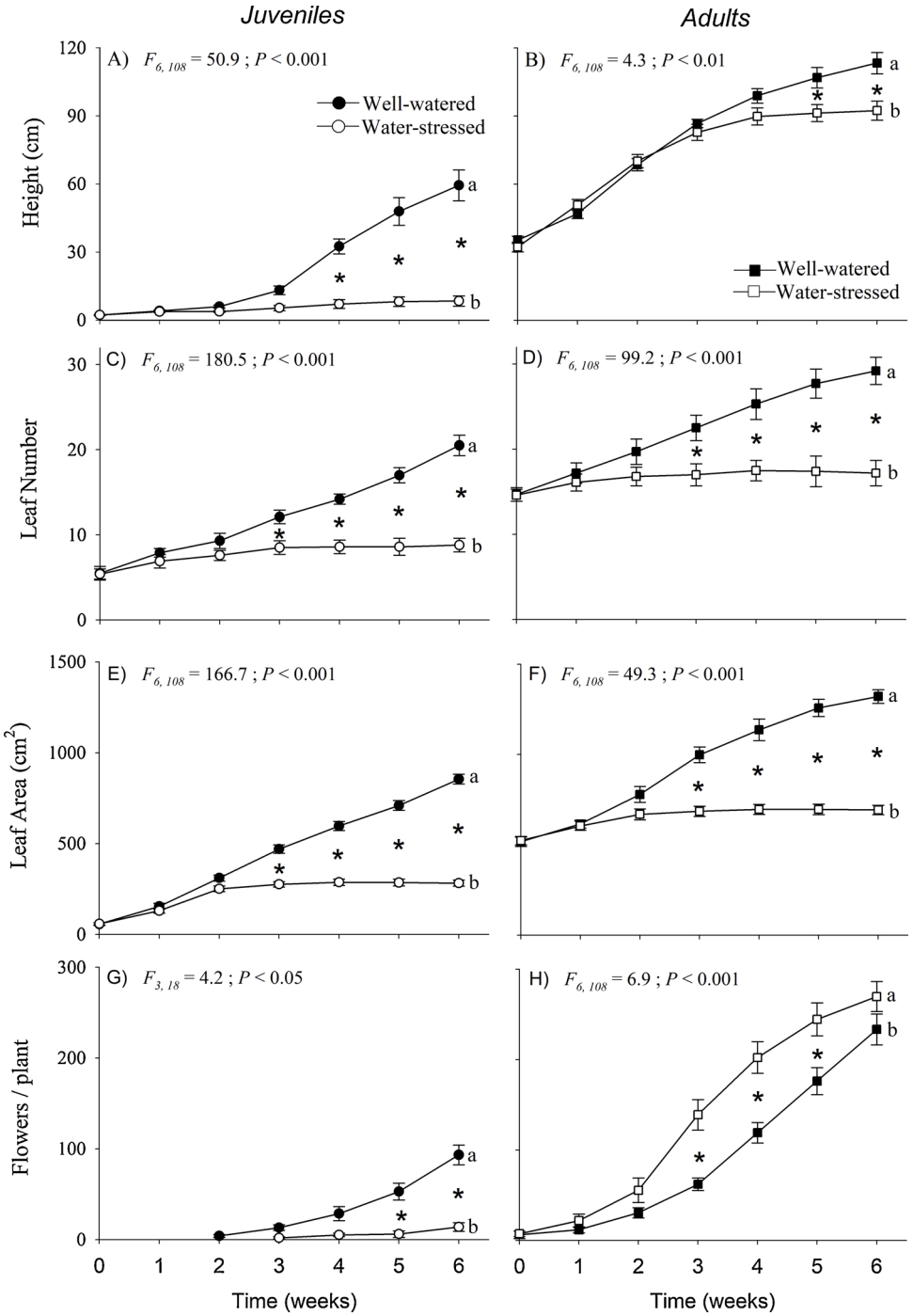

Fig. 3. Mean (± SE) (A–B) height, (C–D) number of leaves, (E–F) leaf area and (G–H) flower production for well-watered and water-stressed juvenile and adult *Parthenium hysterophorus* plants over a 6-week period. *Fstat* and *P-values* indicate overall difference in *P. hysterophorus* parameters between treatments using a repeated measures ANOVA. Different lowercase letters indicate significant differences between treatments overall and asterisks (*) represent significant differences between well-watered and water-stressed plants on corresponding weeks ($P < 0.05$).

photosynthetic maximum plants could attain. However, these reductions in stomatal conductance during water stress periods serve to decrease the amount of water lost in relation to the water used in photosynthetic processes, beneficially making plants more water-use efficient (Verslues et al. 2014). Interestingly, the increase in intrinsic water-use efficiency by *P. hysterophorus* may be linked to *P. hysterophorus's* underlying C_3-C_4 intermediate physiology, which may confer improved water-stress response when compared to typical C_3 plants (Moore et al., 1987; Vogan and Sage, 2011).

Co-occurring with *P. hysterophorus's* physiological responses to water-stress, were morphological adaptions to further conserve or limit water loss. The most prominent of these responses in juvenile and adult *P. hysterophorus* plants, were the reductions in leaf area and leaf production of water-stressed plants. By abscising older and larger leaves and producing smaller leaves, plants may decrease their overall leaf area, greatly reducing their above-ground water demands (Chaves et al., 2002; Oliver et al., 2010). In addition to the production of smaller leaves, water-stressed *P. hysterophorus* plants produced leaves which were more dissected, further reducing leaf area. The lack of floral production and height in water-stressed juvenile *P. hysterophorus* plants

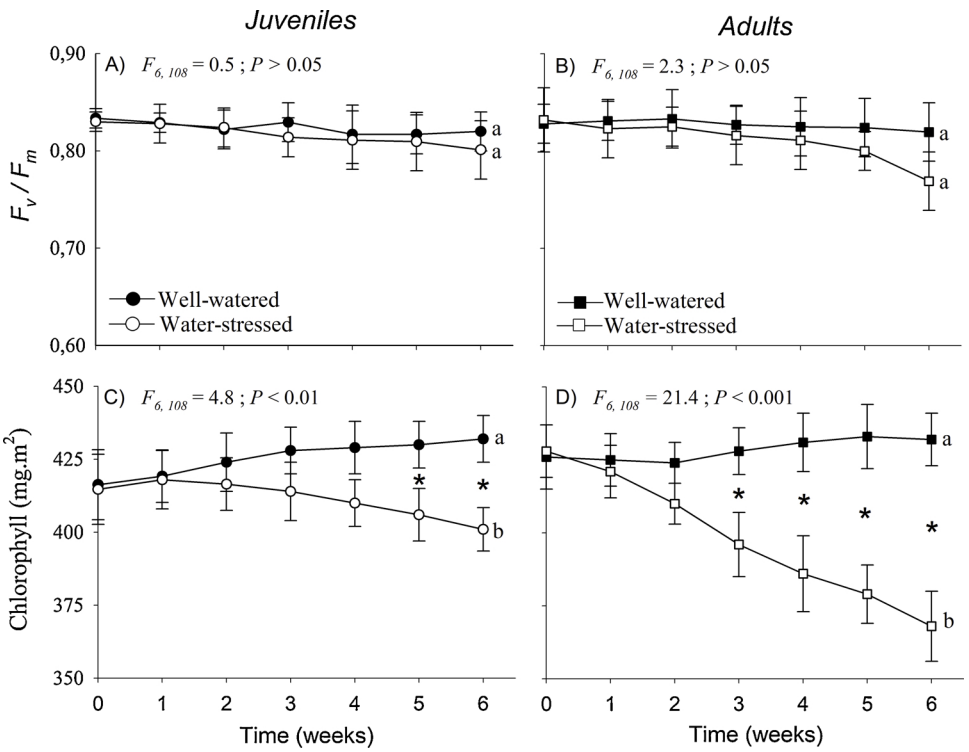

Fig. 4. Mean (± SE) (A–B) maximum quantum yield of *PSII* (*Fv/Fm*) and (C–D) total chlorophyll for well-watered and water-stressed juvenile and adult *Parthenium hysterophorus* plants. *Fstat* and *P-values* indicate overall difference in *P. hysterophorus* treatments using a repeated measures ANOVA. Different lowercase letters indicate significant differences between treatments overall and asterisks (*) represent significant differences between well-watered and water-stressed plants on corresponding weeks ($P < 0.05$).

was expected, given these plants initially invest in vegetative rather than reproductive growth. As immature (non-flowering) plants, *P. hysterophorus* opts to persist as basal rosettes during unfavourable periods of water stress (Annapurna and Singh, 2003; Kohli et al., 2006). Likewise, adult *P. hysterophorus* plants expectedly increased their floral production in response to increasing water stress. Accelerated floral and seed production, with a shortened lifespan has been documented in *P. hysterophorus* in response to decreased soil moisture (Bajwa et al., 2017; Nguyen et al., 2017), offering a level of reproductive assurance under stress conditions.

The metabolic and biochemical responses to water stress are more subtle, but equally as important for the continuation of photosynthetic activity (Touchette et al., 2007; Verslues, 2010). Although *P. hysterophorus* showed a gradual decline in RLWC as water stress increased, both juvenile and adult plants maintained adequate levels of leaf functionality. This may be explained by the significant increases in leaf water potential (Ψ_{leaf}), a drought tolerance mechanism, brought about by osmotic adjustment in the leaves. As shown by Bajwa et al. (2017),

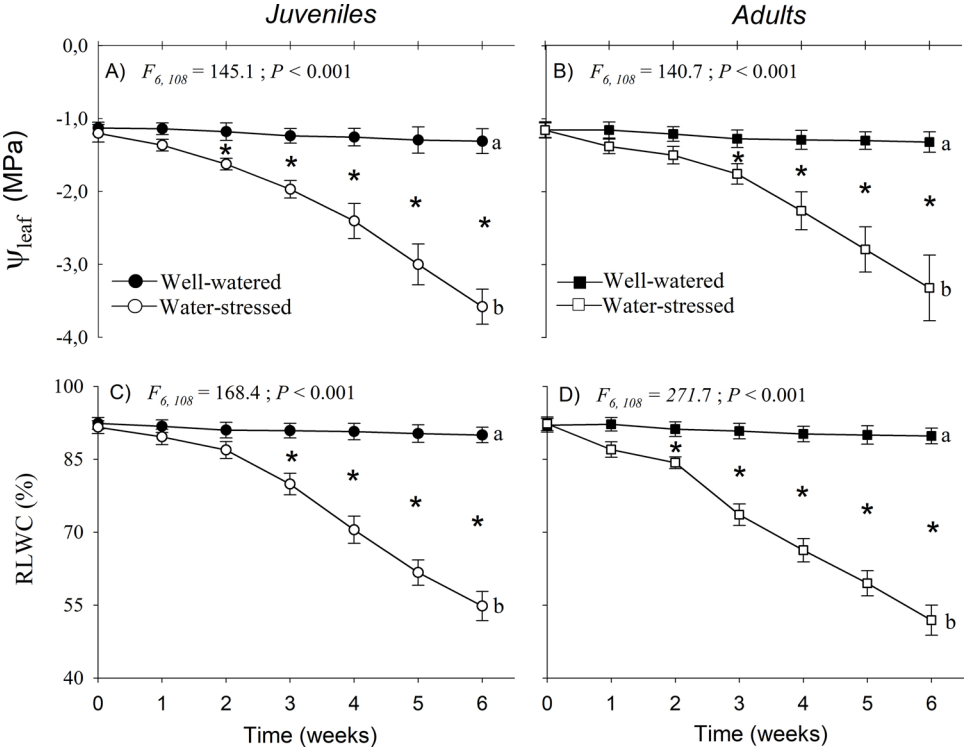

Fig. 5. Mean (± SE) (A–B) leaf water potential (Ψ_{leaf}) and (C–D) relative leaf water content (RLWC) for well-watered and water-stressed juvenile and adult *Parthenium hysterophorus* plants. *Fstat* and *P-values* indicate overall difference in *P. hysterophorus* treatments using a repeated measures ANOVA. Different lowercase letters indicate significant differences between treatments overall and asterisks (*) represent significant differences between well-watered and water-stressed plants on corresponding weeks ($P < 0.05$).

Table 1
Mean (± SE) of selected $A:C_i$ parameters generated using the C_3 models of von Caemmerer (2000) on well-watered and water-stressed juvenile and adult *Parthenium hysterophorus* plants. Different lowercase letters indicate significant differences between treatments ($P < 0.05$).

$A:C_i$ parameters	Juveniles		Adults		F/t_{stat}; P-value
	Well-watered	Water-stressed	Well-watered	Water-stressed	
J_{max} (μmol m^{-2} s^{-1})	223 ± 5.8^a	191 ± 4.9^b	231 ± 10.3^a	155 ± 12.7^c	$F_{3,12}$ = 14.5; P < 0.01
Vc_{max} (μmol m^{-2} s^{-1})	91.2 ± 2.6^a	55.4 ± 1.3^b	84.4 ± 4.1^a	59.9 ± 5.8^b	$F_{3,12}$ = 20.7; P < 0.001
CE (μmol m^{-2} s^{-1})	0.22 ± 0.01^a	0.14 ± 0.01^b	0.20 ± 0.01^a	0.13 ± 0.01^b	$F_{3,12}$ = 22.1; P < 0.001
Γ (μmol m^{-2} s^{-1})	11.2 ± 1.1^a	16.8 ± 2.8^a	12.6 ± 0.5^a	17.5 ± 2.4^a	$F_{3,12}$ = 2.5; P > 0.05
S_L (%)	9.3 ± 1.2^c	41.2 ± 1.4^a	14.8 ± 1.1^b	37.2 ± 4.2^a	$F_{3,12}$ = 45.9; P < 0.001
R_{SL} (%)	–	28.8 ± 4.8^a	–	29.9 ± 1.7^a	t_6 = 0.20; P > 0.05
R_{ML} (%)	–	27.7 ± 3.1^a	–	36.9 ± 5.8^a	t_6 = 1.58; P > 0.05

P. hysterophorus readily increases its leaf solute and free proline content in response to water stress, aiding the plant in maintaining leaf turgor and cellular function. Strongly linked to drought recovery, the increases in proline may have also indirectly accounted for the lack of notable damage to the photosystems of *P. hysterophorus*, particularly *PSII*, as shown by the unchanged *Fv/Fm* values (Adams and Demmig-Adams, 2004). Decreasing leaf chlorophyll however, is unlikely to be explained by changes in leaf water content or potential, but rather by the presence of detrimental reactive oxygen species (ROS) created in the chloroplasts. The uncontrolled production of ROS in the chloroplasts is a frequent occurrence in water-stressed plants, often causing over-excitation and oxidative damage to chloroplasts and other associated photosynthetic machinery / processes (Allen, 1995; Oliver et al., 2010; Foyer, 2018).

Decreases in photosynthetic processes and activity can be attributed to the stomatal and metabolic limitations experienced by *P. hysterophorus*. Maximum electron transport rate (J_{max}), maximum RuBisco carboxylation rate (Vc_{max}) and carboxylation efficiency (CE) all declined in response to prolonged water stress (drought stress), suggesting metabolic impairment or down-regulation (Venter et al., 2017). Adults also suffered proportionally more metabolic than stomatal limitation when compared to juveniles, likely due to the greater declines in leaf chlorophyll as water-stress progressed. Despite these limitations, *P. hysterophorus* continued photosynthesising, albeit at a low rate, when leaves where partially wilted. This may be associated with *P. hysterophorus's* C_3-C_4 physiology and low CO_2 compensation point (Γ), which allows plants to re-fix photorespired CO_2 in bundle sheath cells and photosynthesise whilst stomata are effectively 'closed' (Moore et al., 1987; Vogan and Sage, 2011). Although relative stomatal and metabolic limitations accounted for most of the photosynthetic decline, the shuttling of resources and reallocation of biomass may have exacerbated this decline (Venter et al., 2017).

The reallocation of resources is a common response to moderate or prolonged water stress (drought stress) (Verslues et al., 2014; Eziz et al., 2017). Furthermore, associated changes in biomass allocation patterns, in response to water stress, is well documented amongst many similar invasive annuals within the Asteraceae, including *Argemone mexicana* L., *Ageratum conyzoides* L., *Bidens pilosa* L., *Tithonia* spp. and *Xanthium strumarium* L. (Mortensen and Coble, 1989; Kohli et al., 2006; Wang et al., 2007; Muoghalu, 2008; Eziz et al., 2017). The increased root to shoot ratios of the water-stressed juvenile and adult plants, is an additional drought avoidance strategy, which allows plants to persist and operate by obtaining water from deeper within progressively drying soils (Annapurna and Singh, 2003; McCann and Huang, 2008). However, the developmental stage at which the water stress was experienced influenced the biomass allocation of *P. hysterophorus*. Juvenile plants invested primarily in root and leaf biomass, allowing them to form and persist as leafy basal rosettes, with a relatively well-developed root network. As for adult plants, increased investment in floral biomass is an escape response to water stress in mature annuals, allowing for substantial reproduction effort prior to potential senescence (Friedman and Rubin, 2015). The allocation to root biomass and length by adult plants, is likely a strategy to prolong persistence and maximise the time spent producing seed (Friedman and Rubin, 2015; Eziz et al., 2017).

Ultimately, these findings have implications for the invasive potential, spread and management of *P. hysterophorus* in drier regions and areas which present sporadic or seasonal water-stress. The ability of these plants to persist and produce substantial amounts of seed during water deficits is concerning, as many native species, particularly grasses, cease to remain competitive under similar conditions, opting to persist and producing little to no seed (see Venter, 2015). In addition, increased solute (phenolics) production under water stress is suggested to enhance the allelopathic and competitive effects of *P. hysterophorus*, furthering the weeds invasive ability (Bajwa et al., 2016, 2017). Although this adaptability is inherently difficult to incorporate into distribution models such as McConnachie et al. (2010), it may allow *P. hysterophorus* to invade and persist in drier areas previously thought unsuitable. Lastly, the responses of *P. hysterophorus* to water stress are likely to hinder ongoing management efforts, namely herbicide and biocontrol programmes. Severe reductions in herbicide efficacy are known when applied to water- or drought-stressed plants (Varanasi et al., 2016), meaning that whilst water-stressed, *P. hysterophorus* is unlikely to be affected by pre- or post-emergent registered herbicides. Likewise, water-stressed *P. hysterophorus* plants show decreased RLWC,

Table 2
Mean ± SE dry biomass (allocation %) for sectioned well-watered and water-stressed juvenile and adult *Parthenium hysterophorus* plants. Different lowercase letters indicate significant differences between treatments (ANOVA; $P < 0.05$).

	Juveniles		Adults		F_{stat}; P-value
	Well-watered	Water-stressed	Well-watered	Water-stressed	
Floral weight (g)	0.77 ± 0.1^b (6.4%)	0.27 ± 0.03^c (6.5%)	2.88 ± 0.16^a (12.7%)	3.19 ± 0.18^a (16.7%)	$F_{3,30}$ = 88.2; P < 0.001
Stem weight (g)	4.06 ± 0.4^c (33.9%)	0.87 ± 0.2^d (21.1%)	10.37 ± 0.4^a (45.5%)	6.94 ± 0.5^b (36.5%)	$F_{3,36}$ = 101.6; P < 0.001
Leaf weight (g)	4.42 ± 0.3^a (35.6%)	1.18 ± 0.06^c (28.5%)	4.85 ± 0.3^a (21.4%)	2.79 ± 0.2^b (14.6%)	$F_{3,36}$ = 54.8; P < 0.001
Root weight (g)	2.87 ± 0.2^c (24.1%)	1.82 ± 0.13^d (43.9%)	4.65 ± 0.2^b (20.4%)	6.11 ± 0.36^a (32.2%)	$F_{3,36}$ = 69.7; P < 0.001
Root : Shoot	1 : 3.2 (0.33)^c	1 : 1.2 (0.87)^a	1 : 3.9 (0.26)^d	1 : 2.1 (0.47)^b	$F_{3,36}$ = 66.9; P < 0.001
Root Length (cm)	10.6 ± 0.4^d	14.6 ± 0.9^c	21.1 ± 2.1^b	26.1 ± 1.3^a	$F_{3,36}$ = 77.9; P < 0.001
Total biomass (g)	11.93 ± 0.6^c	4.14 ± 0.3^d	22.76 ± 0.7^a	19.03 ± 0.96^b	$F_{3,36}$ = 145.3; P < 0.001

nutrients (chlorophyll) and growth, making it harder to sustain large and effectively damaging populations of biocontrol agents in the field (Dhileepan et al., 2000; Cowie et al., 2019). Ectophages and leaf-affecting agents such as the leaf-feeding beetle *Zygogramma bicolorata* (Coleoptera: Chrysomelidae) or the *Puccinia* fungal pathogens may be particularly sensitive to water-stressed *P. hysterophorus* plants, more so than endophagous biocontrol agents.

In conclusion, understanding the water relations of *P. hysterophorus* provides valuable insights into the biology and invasive potential of the weed during periods of water-stress, which are predicted to increase in Southern Africa. By persisting as a hardy rosette or a reproductive herb, *P. hysterophorus* may remain productive and continue to invade even under severe water-stress (drought stress). Additionally, many of the water-stress responses serve to make *P. hysterophorus* more water-use efficient, competitive and reproductive, increasing its invasive capabilities, potential range and impact. The influence of water-stress on management efforts warrants further investigation, but hindrances are likely to be experienced when attempting to control *P. hysterophorus* under water-stressed conditions through chemical and biological means.

Author statement

Blair Cowie: Conceptualization, Methodology, Investigation, Visualisation, Writing - original draft, Data curation, Formal analysis.

Marcus Byrne: Conceptualization, Methodology, Supervision, Resources, Writing - review & editing.

Ed Witkowski: Conceptualization, Methodology, Supervision, Resources, Writing - review & editing.

Lorraine Strathie: Conceptualization, Methodology, Supervision, Resources, Writing - review & editing.

Jeremy Goodall: Conceptualization, Methodology, Supervision, Resources, Writing - review & editing.

Nic Venter: Conceptualization, Visualisation Methodology, Supervision, Resources, Writing - review & editing.

Declaration of Competing Interest

None.

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CHAPTER 3:

The influence of land management on the above- and below-ground invasion dynamics of *Parthenium hysterophorus* in a South African savanna?

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Abstract

Parthenium hysterophorus L. (Asteraceae) is a notorious noxious, annual invasive herb prevalent in more than 50 countries worldwide. In South Africa, the weed is a highly damaging invasive species, particularly in savanna regions, threatening food security, native biodiversity, livelihoods and human health. Given the multitude of threats posed by *P. hysterophorus*, this study aimed to determine the influence of land management on the invasion of *P. hysterophorus* and its associated seedbank dynamics. To assess this, two neighbouring savanna reserves, with differing land management (i.e. poorly- and well-managed), were selected and assessed seasonally over four years. Poor land management, namely overgrazing, exacerbated the invasion of *P. hysterophorus*, with greater densities, larger plants and increased seed production. This enhanced invasion was also reflected in changes to the site's above-ground plant community, with significantly lower species richness, evenness and diversity. *Parthenium hysterophorus* comprised more than 80% of the poorly-managed reserve's seedbank, and was responsible for continued declines in the richness, evenness and diversity of species below-ground. Whereas *P. hysterophorus* at the well-managed reserve, occupied a smaller proportion (~70%) of the seedbank, with richness, evenness and diversity all showing signs of recovery over the four year period. Overall, this research highlighted the impacts of *P. hysterophorus* in South Africa, as well as the potential role land management has in limiting or facilitating *P. hysterophorus* invasions and their impacts within savanna reserves.

Keywords: Biological invasions; Parthenium weed; Savanna assessment; Soil seedbanks; Species diversity; Weed management

1. Introduction

Native to the neotropics, *Parthenium hysterophorus* L. (Asteraceae: Heliantheae) is a globally recognised invasive weed, prevalent in more than 50 countries across America, Asia, Oceania and Africa (Dhileepan and Strathie, 2009; Shabbir et al., 2019). On the African continent, *P. hysterophorus* is a troublesome invader found in several countries including Ethiopia, Somalia, Eritrea, Kenya, Uganda, Tanzania, Madagascar, Mozambique, Zimbabwe, Mauritius, Eswatini and South Africa (Bajwa et al., 2016; Shabbir et al., 2019). Furthermore, McConnachie et al. (2010) suggest that much of Southern, particularly South Africa, remains climatically well-suited and susceptible to further invasion by *P. hysterophorus*. South Africa first documented *P. hysterophorus* during the 1880s, in the KwaZulu-Natal province, however, it was not until the mid-1980s, after cyclone Demoina, that the weed became a prominent invader (Strathie et al., 2011; Strathie and McConnachie, 2019). Since then, *P. hysterophorus* infestations have rapidly intensified within KwaZulu-Natal and spread northward, invading the Mpumalanga, North-West, and Limpopo provinces (Terblanche et al., 2016), with the most recent assessments suggesting the weed has increased its range by ~500% in the last decade (Van Wilgen and Wilson, 2018).

Parthenium hysterophorus is an annual herb with a multi-branched growth form, which typically germinates during early spring (September), with the onset of the first rains, and senesces during mid- to late-autumn (Nigatu et al., 2010; Strathie et al., 2011). The plants are characterised by their rapid germination and growth rates, flowering and producing seed as early as six-weeks after germination (Dhileepan, 2012). These small seeds are easily dispersed by wind, water, vehicles, animals and human activities, enabling *P. hysterophorus* to invade a wide variety of habitats, including rail-and road-sides, riparian areas, crop fields, wastelands, commercial pastures, grasslands, savannas and communal rangelands (Henderson, 2001; Bajwa et al., 2016; Mao et al., 2019). Although *P. hysterophorus* invades an array of environments, the weed flourishes in disturbed, degraded or over-utilised areas, frequently resulting in the formation of recurring, dense monospecific stands (Adkins and Shabbir, 2014; Tanveer et al., 2015). These stands are problematic as they can readily outcompete and chemically exclude, through allelopathy, surrounding vegetation and are unpalatable / toxic to any wild or domestic herbivores (Van Der Laan et al., 2008; Dhileepan and Strathie, 2009). Additionally, the weed's copious seed production leads to the rapid build-up of long-lasting and extensive *P. hysterophorus* seedbanks, with heavily invaded areas reporting up to 100 000 seeds/m^2 (Strathie et al., 2011).

The annual nature, rapid growth rate, allelopathicity, prolific seed production and persistent seedbanks of *P. hysterophorus* have together been highlighted as the major factors contributing to the invasive success of the weed in South Africa (Dhileepan and Strathie, 2009). Moreover, the lack of adequate land management in many natural savanna and rangeland settings, given the country's high levels of poverty, has promoted *P. hysterophorus* invasion and exacerbated management challenges (Terblanche et al., 2016; Reynolds et al., 2020). Various management options have been implemented against *P. hysterophorus*, most notably biological control, however these are yet to attain the desired levels of control (Cowie et al., 2018, 2019). Thus, there is an ongoing need for managerial approaches to help slow the

spread and impact of *P. hysterophorus* (Tanveer et al., 2015; Nguyen et al., 2017). In grasslands, savannas and rangelands, weed infestations often reflect the land use of an area, with overutilized or poorly-managed areas frequently suffering reductions in the richness, abundance and cover of native vegetation, ultimately degrading the land and increasing its susceptibility to invasion (DiTomaso, 2000; Goodall et al., 2011). Numerous studies highlight the rapid colonisation and prolific invasion of *P. hysterophorus* within degraded areas, suggesting that good land management practices hold promise in reducing the invasibility and overall impact of the weed (Dhileepan and Strathie, 2009; Nigatu et al., 2010; Tanveer et al., 2015; Khan et al., 2019).

Weed management through land-use involves conserving an area's diversity and processes, maintaining its ecological resilience and inherently limiting the abundance, spread and impact of invasive weeds (DiTomaso, 2000; Radosevich et al., 2007). In the case of *P. hysterophorus*, maintaining the competitive ability of native vegetation, particularly grasses (Poaceae), has the potential to suppress the growth, reproduction and invasion of the weed (Van der Laan et al., 2008; Belgeri et al., 2014; Khan et al., 2019). However, the management of pastures in many of South Africa's natural savanna and rangeland settings is confounded by their misuse or absence of fire as well as the high demands for grazing and livestock (O'Connor and van Wilgen, 2020). These grass communities are highly responsive to disturbance, with overgrazing often resulting in the loss of valuable perennial grasses and their replacement with various annual grass and weed species (Navie et al., 2004; Van Oudtshoorn, 2012, 2015). In addition, the diversity and abundance of native species within an area represents its ecological resilience, whilst the number of species present below-ground (i.e within the seedbank) are indicative of its potential regenerative capacity from invasion (Navie and Rodgers, 1997; Navie et al., 2004). Ultimately, the temporal and spatial variability of savanna plant communities in response to invasion suggest that long-term small-scale assessments be used, given their ecological insights into the invasion dynamics and impact (Belgeri et al., 2014).

Given the severe socio-economic and ecological threats posed by *P. hysterophorus* to agriculture, native biodiversity, as well as animal and human well-being, it is imperative that the invasion of the weed and any additional management options be fully assessed. Furthermore, the understanding of *P. hysterophorus* invasive ecology and seedbank dynamics within a South African context remains limited. Therefore, this study aimed to determine the influence of land management practices on the invasive potential of *P. hysterophorus* and its associated seedbank dynamics. To investigate this, two neighbouring game reserves with differing land management approaches, were selected and 100 m^2 plots were assessed seasonally for *P. hysterophorus* density and seedbank size over a four year period.

2. Methods and Materials

2.1 Study localities

This study was conducted at two sites located outside the farming town of Malalane (Malelane) in the Ehlanzeni District of Mpumalanga, South Africa. The altitude of the study region ranged between 277 and 291 m a.s.l. The region is classified as Granite Lowveld (Savanna), composed of deep sandy soils which originate from Archaean granite (Mucina and Rutherford, 2006). Mean annual temperature in Malelane is 22.2 °C, with average summer (min | max) temperatures of 19.1 | 30.2 °C and average winter (min| max) temperatures of 9.9 | 25.4 °C (Figure 1A). The region experiences rainfall of ~560-600 mm per annum, with the majority (80%) occurring during the spring and summer months of October to March (Figure 1B).

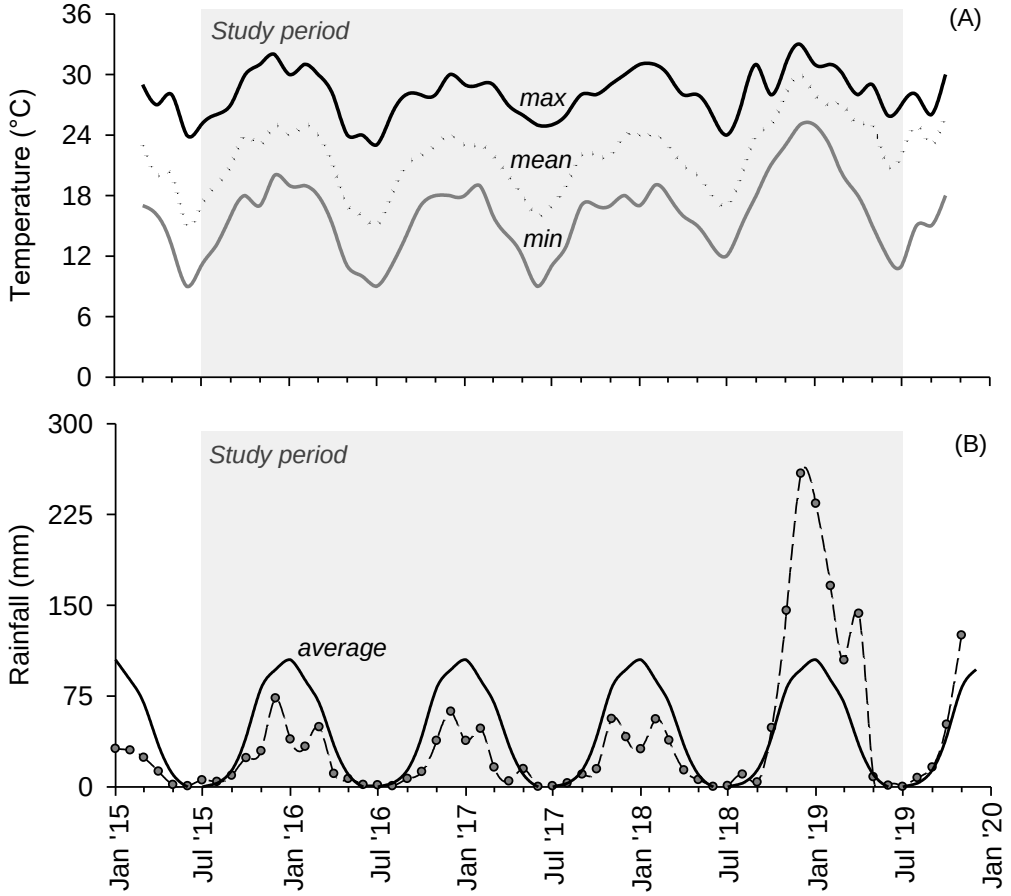

Figure 1. Monthly (A) minimum, maximum and mean temperatures and (B) rainfall (mm), long-term average and actual, for the Malelane region of Mpumalanga over a four year period ranging from January 2015 to January 2020. (Data from WWO, 2020).

Two neighbouring game reserve properties, with differing land management approaches, were initially surveyed and selected for long-term *P. hysterophorus* assessments during March to June 2015. The first site was a relatively ‘poorly managed site’ (25° 32’48.401” S,

31° 37’5.139” E) located 13 km south-east of Malalane. Currently used as a game ranch / reserve, this 6 600 ha property is situated in the savanna biome with a number of savanna tree and grass species present, a mostly flat terrain and predominantly sandy soils with moderate to low levels of grass cover. The reserve is stocked with a high abundance of wild game (< 8 ha/LSU), predominantly grazers, Buffalo (*Syncerus caffer*), Zebra (*Equus quagga*) and Blue Wildebeest (*Connochaetes taurinus*), Sable antelope (*Hippotragus niger*), Blesbok (*Damaliscus pygargus phillipsi*), with mixed feeders, Impala (*Aepyceros melampus*), and browsers, Kudu (*Tragelaphus strepsiceros*) and Bushbuck (*Tragelaphus scriptus*). The site’s veld condition is rated as moderate to poor, displaying signs of overstocking / overgrazing, erosion and invasion by various non-indigenous species including *P. hysterophorus, Chromolaena odorata* and various invasive Solanaceae. Although no management plans are in place for invasive species, including *P. hysterophorus*, current management activities include burning to remove moribund grass and create fire breaks.

The second and comparatively ‘well-managed site’ (25° 33’16.84” S, 31° 37’26.58” E), was located 15 km south-east of Malelane. Previously used as a cattle farm until 2012, the 1 400 ha savanna reserve has a flat terrain with largely sandy soils and predominantly savanna vegetation. Unlike the poorly-managed site, this site presents higher levels of grass cover, with veld condition ranked as moderate. The reserve also contains a variety of small to medium antelope, stocked at much lower abundances (> 25 ha/LSU) and includes predominantly grazers, Blue Wildebeest, Impala, Zebra and Blesbok along with a few cattle. Ongoing management activities, started in late 2014, aim to restore the vegetation, particularly grass communities, to support and stock large game in the future. Managerial activities on the reserve include prescribed burning to reduce moribund grass, limit bush encroachment and promote healthy regrowth as well as the creation of grazing lawns. The creation of various grazing lawns aims to draw game away from overutilized areas of the reserve, preventing overgrazing and allowing the recovery of native vegetation, particularly grasses, which indirectly limits unwanted alien plant invasions.

2.2 Parthenium hysterophorus and grass assessments

Parthenium hysterophorus and grass were assessed in both sites using monitoring plots. One plot per reserve was established, 100 m^2 (10 m x 10 m), during winter 2015 (July) representing similar savanna areas with high *P. hysterophorus* invasion (> 20 plants/m^2). Within these plots, *P. hysterophorus* density, height and flower / seed production as well as grass basal cover and biomass were assessed seasonally (winter, spring, summer and autumn) over a four year period. *Parthenium hysterophorus* density was assessed as the number of live plants per m^2, using a 1 m^2 (1 m x 1 m) quadrat in all 100 m^2 of each (10 x 10 m) plot. Along with the *P. hysterophorus* counts, these plants had their height (cm) measured, excluding quadrats presenting more than 10 *P. hysterophorus* plants, in which the five tallest and five shortest plants were measured and averaged per quadrat (m^2). All flowers on *P. hysterophorus* plants were counted when present, averaged per quadrat, and multiplied by five to gain an estimate of seed production.

Grass basal cover (%) was generated per plot each season, following the protocols and equation of Hardy and Tainton (1993; Equation 1), in which 100 paired grass measurements were taken (1 pair per m^2) from the centre of each quadrat to the nearest living tuft as well as the basal diameter of the respective tuft. Grass biomass was assessed per m^2 using a standard disc pasture meter (DPM) for all 100 m^2 quadrats within each of the plots. DPM measures were taken from the ground to the resting height (nearest 0.5 cm) of the disc after being dropped (see Zambatis et al., 2006). For quadrats that presented highly non-uniform (uneven) grass patches, two DPM measures were taken and averaged. All DPM readings (cm) were then averaged per plot each season and converted into biomass (tons/ha) as per the low grass height (< 26 cm) equation from Zambatis et al. (2006; Equation 2).

$$(Eq.1) \quad Basal\ cover\ (\%) = 19.8 + 0.39(D) - 11.87(log_e\ D) + 0.64(d) + 2.93\ (log_e\ d)$$

D- mean distance from centre to nearest living tuft (cm) d – mean basal diameter of nearest tuft (cm)

$$(Eq.2) \quad Biomass\ (kg.ha) = \left[31.7176 \left(0.3218 \text{ x} \frac{1}{DPM} \right) \text{x}\ DPM^{0.2834} \right]^2$$

DPM - mean DPM (cm) per plot

2.3 Above-ground plant community and vegetation indices

To best assess the above-ground plant community response to *P. hysterophorus* at each of the reserves, all plant species (trees/shrubs, grasses, forbs and herbs) were identified within the 100 m^2 plots once a year during summer (mid-January). For species that could not be readily identified in the field, a sample of the plant was clipped, pressed and used to key out the species at a later stage. Morphospecies groups were used for plants that could be not be identified to species level. Following Nguygen et al. (2017), these data were then used to calculate vegetation, namely biodiversity indices, which included species richness, evenness and diversity. Species richness was used to gain insight into the richness of the plots and was taken as the total number of species present within each of the plots per year (species with only one individual were not included). Species 'Pielou' evenness (*J*) was used to assess how homogenous the plant community within and between plots were. Lastly, species diversity was used as a proxy for 'overall ecosystem resilience', accounting for both the richness and evenness of species in the plots and was calculated according to the Shannon-Wiener index (*H'*).

2.4 Seedbank assessments and below-ground plant community

To assess the seedbank abundance and diversity, soil samples were collected subsequent to the above-ground assessments in both reserves. Soil samples were collected in all four seasons (winter, spring, summer autumn), across the four year study period. For each collection, a single randomly collected, using a cylindrical soil core (7 cm in diameter and 6 cm deep), within each of the 100 m^2 plots, totalling 100 cores per plot at each reserve. Samples were then transported to the Wits Insectary glasshouse and placed into shallow

plastic germination trays (30 cm width x 55 cm length x 6 cm depth) filled with ~2.5 cm of sterilised (autoclaved) potting soil. Soil samples were mixed per site and spread thinly across the germination trays (~2.5 cm) and experienced temperatures of 24 ± 2.3 °C, with relative humidity maintained above 60%. Three control trays containing only sterilised soil were setup after each collection to monitor for any seedling emergence originating from the potting soil. Germination trays were watered weekly, or as required, to field capacity, and all emerging seedlings were identified using the relevant literature (Henderson, 2001; Schmidt et al., 2002; Van Oudtshoorn, 2012), counted and removed every two weeks. Emergent seedlings that were not readily identifiable, were counted, marked, transplanted and left to grow until they could be identified. Every two months, all trays had the soil disturbed to promote germination as per Nigatu et al. (2010) and were assessed for a period of six months so as to draw out all species (and to get a very high proportion of the seeds/individuals present) within the seedbank, including those with longer seed dormancies. Identified and counted species were grouped as either *Parthenium hysterophorus*, grass or other (all remaining growth forms) each season to provide overall insights into seedbank composition and changes therein. Lastly, all identified species were used to generate the biodiversity indices of species richness, evenness and diversity (as per section 2.3).

2.5 Data Analysis

Parthenium hysterophorus density, height and estimated seed production were all assessed between sites over time using one-way repeated measures analysis of variance (ANOVA) with Tukey HSD *post-hoc*. Likewise, seasonal grass basal cover and biomass at each of the reserves was assessed and compared using repeated measures ANOVA with Tukey HSD *post-hoc*. The proportion of perennial to annual grasses present above ground was calculated each year, per site, and compared between the sites using a paired t-test. Yearly above-ground vegetation indices, namely species richness, evenness (*J*) and diversity (*H'*) were calculated from summer identifications for the well- and poorly-managed sites using the 'vegan' package within R (R Core Team, 2018). These above-ground vegetation indices were then compared between sites using a paired samples t-test, grouped by year, for the four years of data. Seedbank proportions were calculated and expressed as percentage of total seedbank per season, per site and were arcsine transformed prior to analysis using paired t-tests for the *P. hysterophorus* and grass seedbank data respectively. Below-ground vegetation indices were calculated using R and assessed using a one-way repeated measures ANOVA along with a Tukey HSD *post hoc*.

3. Results

3.1 Parthenium hysterophorus and grass assessments

Overall, the different land management strategies had contrasting impacts on the invasion levels of *P. hysterophorus*, with the poorly-managed reserve suffering significantly higher densities of *P. hysterophorus* per m^2 ($F_{1,16} = 24.8$; $P < 0.001$). The poorly-managed reserve had the highest density of *P. hysterophorus* throughout the year(s), although the weed's

seasonal peaks differed to the well-managed reserve (Figure 2A). The poorly-managed site experienced its peak *P. hysterophorus* density during the spring with an average of 45 ± 3 plants/m^2, whereas the well-managed reserve peak occurred in summer with an average of 17 ± 2 plants/m^2. *Parthenium hysterophorus* plants also grew 23% taller ($F_{1,16}$ = 15.6; $P < 0.01$) at the poorly-managed site (136 ± 5 cm), compared to plants invading and growing at the well-managed reserve (107 ± 4 cm) (Figure 2B). Similarly, *P. hysterophorus* plants at the poorly-managed reserve produced on average 40% more flowers and subsequently seed ($F_{1,16}$ = 13.3; $P < 0.01$) than the well-managed reserve (Figure 2C).

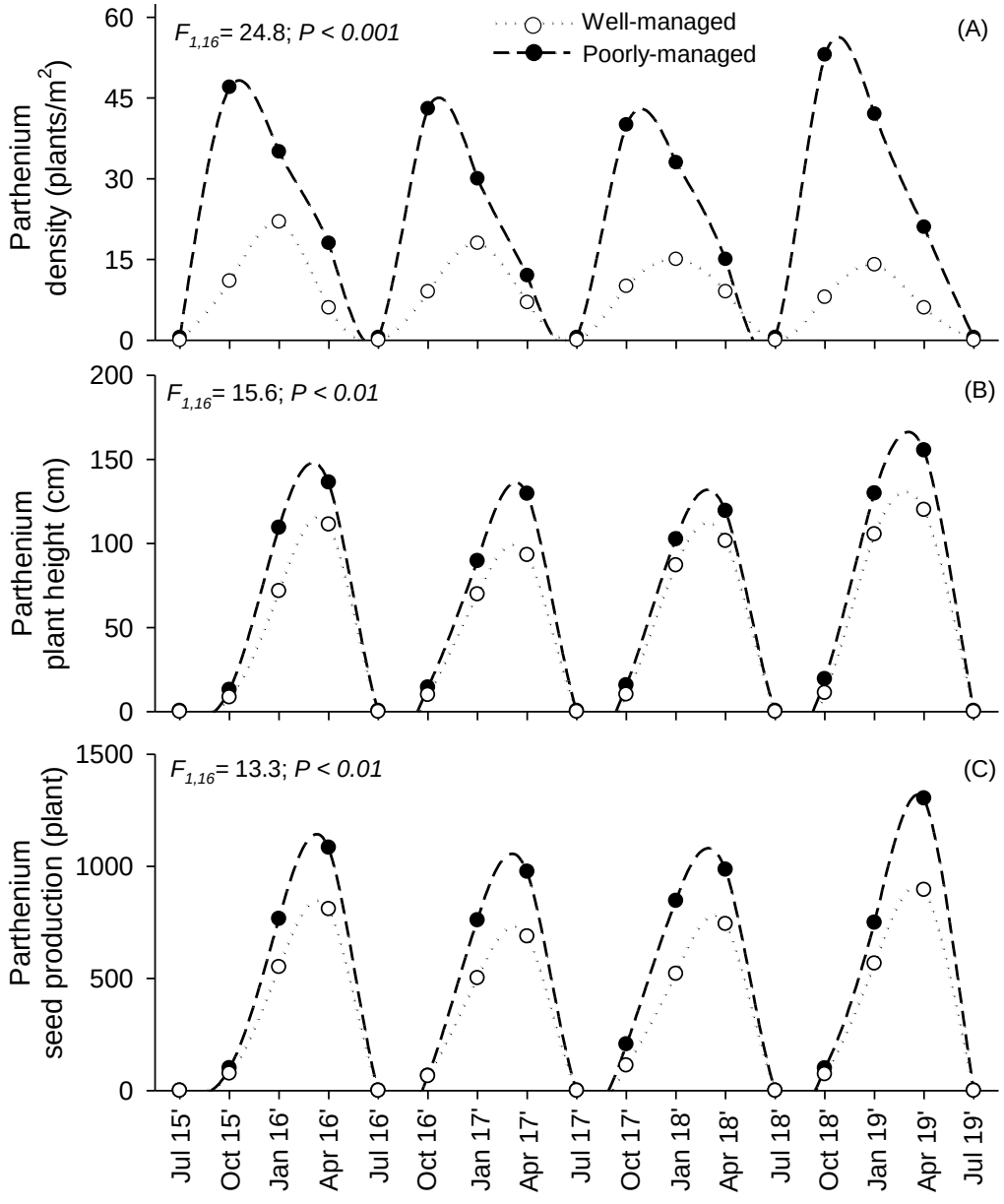

Figure 2. Mean *Parthenium hysterophorus* (A) density, (B) height and (C) seed production recorded seasonally (winter, spring, summer, autumn) from a well-managed and neighbouring poorly-managed reserve in South Africa, over a four-year period. *Fstat* and *P-values* indicate overall difference in *P. hysterophorus* parameters between reserves using a repeated measures ANOVA ($P < 0.05$).

Grass basal cover was strongly influenced by land management, with the well-managed reserve gradually increasing its basal cover from 7 to 10% over the 4 years (Figure 3A). Whereas the poorly-managed reserve had significantly less basal cover over the same period, with an average of 5.6 ± 0.3% ($F_{1,16}$ = 20.9; P < 0.001). Grass biomass followed a similar trend, with the well-managed reserve producing significantly more biomass per season ($F_{1,16}$ = 20.9; P < 0.001), on average ~900 kg/ha more than the poorly-managed reserve (Figure 3B).

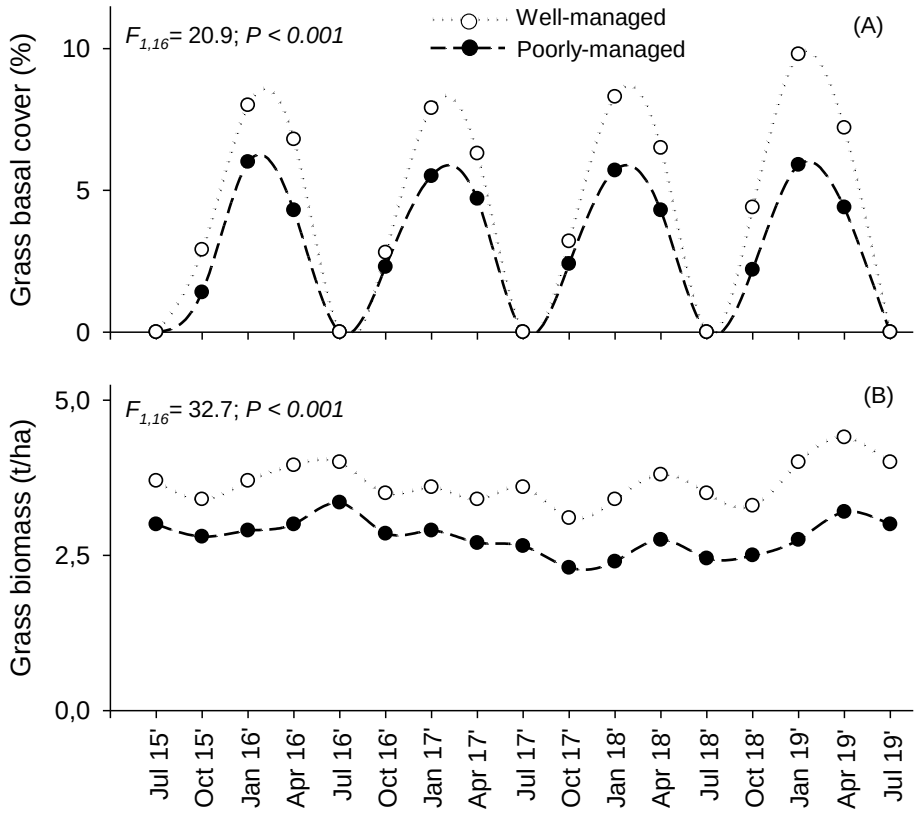

Figure 3. Mean (A) grass basal cover and (B) biomass recorded seasonally within a 100 m^2 plot from a well-managed and neighbouring poorly-managed reserve in South Africa, over a four-year period. *Fstat* and *P-values* indicate overall difference in grass parameters between reserves using a repeated measures ANOVA (P < 0.05).

3.2 Above-ground plant community and vegetation indices

Yearly (summer) species assessments highlighted notable changes with regards to the above-ground plant community at the well- and poorly-managed reserves invaded by *P. hysterophorus*. Both reserves had *P. hysterophorus* recorded as the most abundant species, followed by various grass species. Although the reserves shared several grass species, the grass types differed (t_3 = 4; P < 0.05), with a majority of grasses at the well-managed site being perennials (52%), [most commonly] *Panicum maximum*, *Eragrostis curvula*, *Digitaria eriantha* and *Eragrostis superba*. Whereas the poorly-managed site, had a greater proportion

of annual grasses (63%), which included *Dactyloctenium spp.*, *Eleusine coracana*, *Aristida congesta*, *Digitaria longiflora* and *Sorghum bicolor*.

In terms of species richness, the well-managed reserve was consistently higher in richness (t_3 = 4; P < 0.05), gradually increasing to 33 species/site whilst the poorly managed reserve lost ~13% of its species richness over time (Figure 4A). Although both sites had relatively low species evenness indices (Figure 4B), the well-managed reserve maintained a significantly higher average evenness of 0.31 when compared to the poorly-managed reserve at 0.26 (t_3 = 3.4; P < 0.05). Similarly, average species diversity at the well-managed reserve (3.36) was significantly higher (t_3 = 4.2; P < 0.05) than that of the poorly managed reserve (2.81) across the four years (Figure 4C).

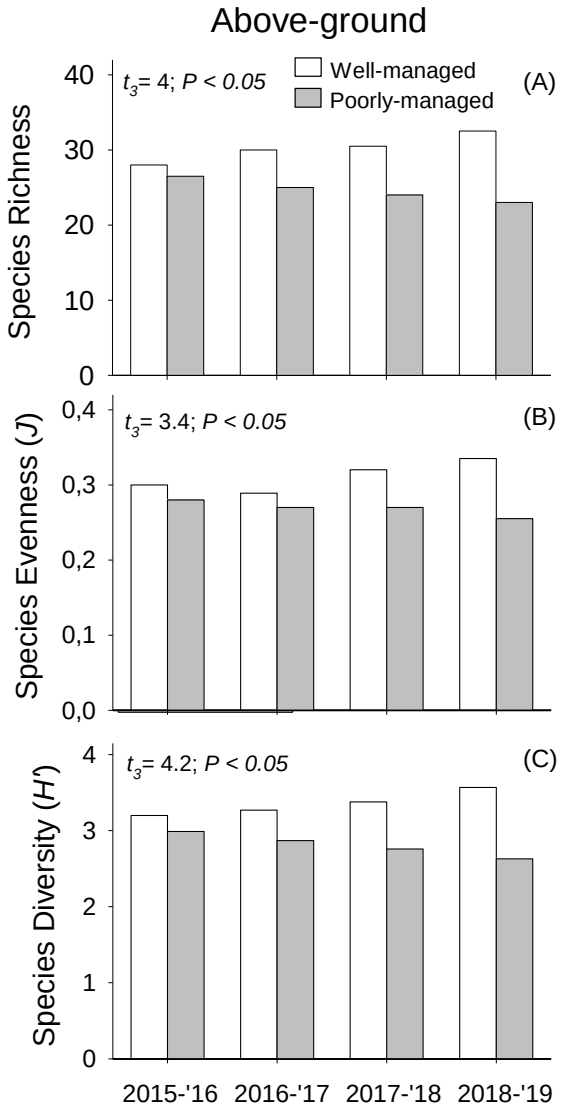

Figure 4. Species (A) richness, (B) evenness and (C) diversity of the above-ground plant community assessed within a 100 m^2 plot in a well-managed and neighbouring poorly managed reserve in South Africa, over a four-year period (2016-2019). *t-stat* and *P-values* indicate overall difference in grass parameters between reserves using a paired t-test (P < 0.05).

3.3 Seedbank assessments and below-ground plant community

Parthenium hysterophorus was the most abundant species within the seedbank at both the well- and poorly-managed reserves, averaging 17 158 ± 457 and 28 558 ± 589 seeds per m^2, respectively. The high abundance of *P. hysterophorus* seeds was also reflected in the seedbank proportions, with the weed occupying 71 ± 3% of the seedbank in the well-managed reserve and a significantly higher 82 ± 2% of the seedbank in the poorly-managed reserve (t_{16} = 6.8; P < 0.001; Figure 5A-B). At both reserves the remainder of the seedbank comprised grasses, followed by shrubs, forbs and trees (Other spp.). However, the well-managed reserve maintained a significantly greater proportion (t_{16} = 7.7; P < 0.001) of grass species within its seedbank, which increased by ~7% across the four year period (Figure 5B).

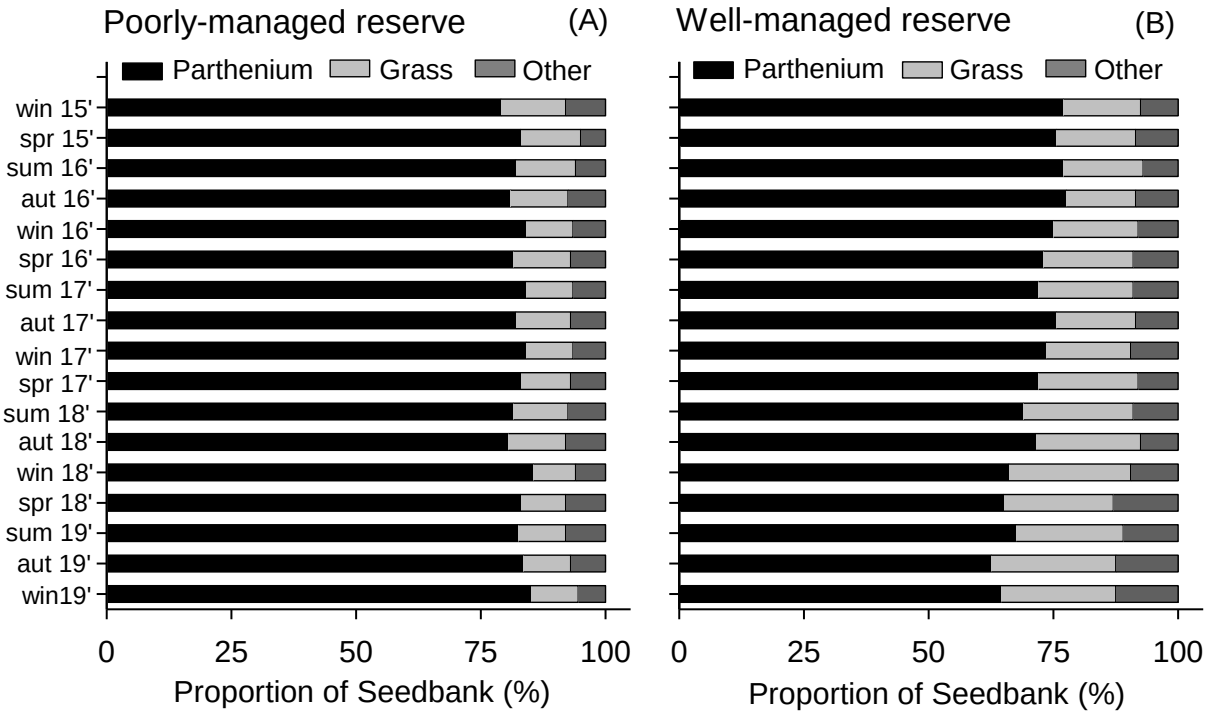

Figure 5. Seasonal seedbank proportions of *Parthenium hysterophorus*, grass spp. and all other spp. (i.e. trees, forbs, shrubs) recorded from germinated soil samples collected from a (A) poorly-managed and (B) well-managed reserve in South Africa, over a four-year period.

The well-managed reserve had significantly more species present ($F_{1,15}$ = 38.2; P < 0.001) within its seedbank than the poorly-managed reserve, which on average had 16% (9 species) less (Figure 6A). Species evenness indices were relatively low (> 0.3) for seedbanks of both the well- and poorly-managed reserves, however, the poorly managed reserve had the lowest average of 0.22, significantly lower ($F_{1,15}$ = 31.4; P < 0.001) than that of the well-managed reserve at 0.26 (Figure 6B). Following the losses in richness and decreases in evenness,

species diversity at the poorly-managed reserve continually declined over time, with significantly lower average values ($F_{1,15}$ = 71.7; P < 0.001) than the well-managed reserve (Figure 6C). The well-managed reserve also showed changes in species diversity across the four year period, gradually increasing from 3 to 3.5 (Figure 6C).

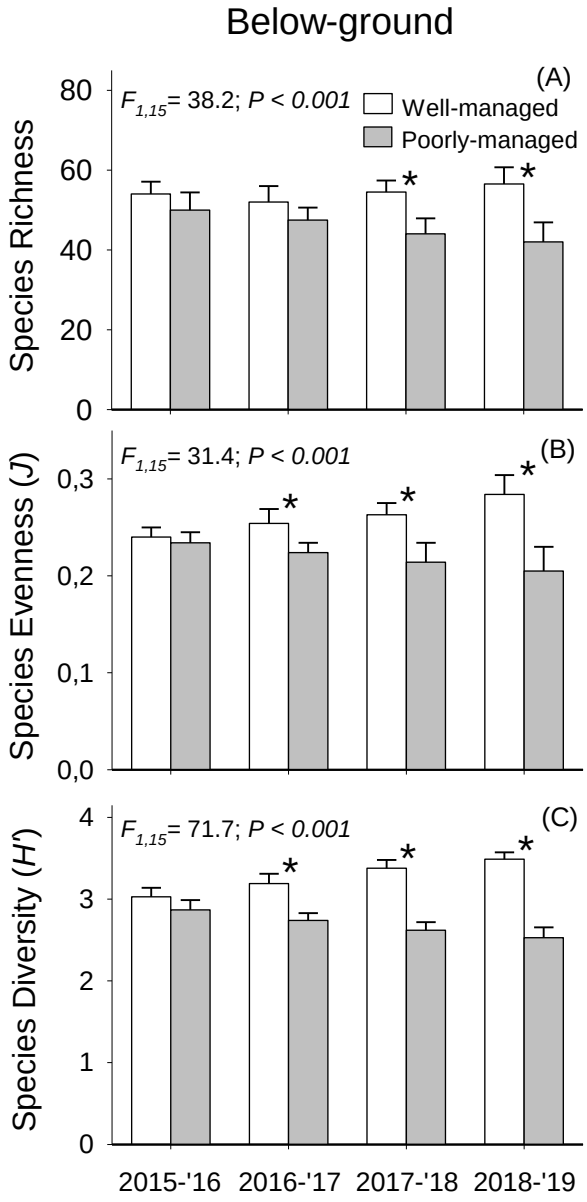

Figure 6. Mean species (A) richness, (B) evenness and (C) diversity of the below-ground plant community (seedbank) assessed seasonally within a 100 m^2 plot in a well-managed and poorly-managed reserve in South Africa, over a four-year period (2016-2019). *Fstat* and *P-values* indicate overall difference in indices between reserves using a repeated measures ANOVA (P < 0.05). Asterisks (*) represent significant differences between well-managed and poorly-managed reserves at corresponding time periods (years).

4. Discussion

The invasion of *P. hysterophorus* and its subsequent impacts were found to be strongly influenced by the land management approaches adopted in a South African savanna. Numerous studies highlight that poorly or unmanaged areas facilitate the invasion of various annual weeds, including *P. hysterophorus* (Nigatu et al., 2010; Goodall et al., 2012; Van Oudtshoorn, 2015). Thus, the high densities of *P. hysterophorus* (~46 plants/m^2) found at the poorly-managed reserve are likely a reflection of poor pasture management, particularly the continued overstocking of various grazer species (see DiTomaso, 2000). Stocking grazers in high abundances, leads to more frequent and intense levels of disturbance and more problematically overgrazing (Van Oudtshoorn, 2012, 2015). Overgrazing severely limits the growth, competitive ability and recruitment of various pasture species, creating ideal conditions for the establishment, invasion and spread of *P. hysterophorus* (Nigatu et al., 2010; Terblanche et al., 2016; Khan et al., 2019). Whereas the lower levels of *P. hysterophorus* invasion documented within the well-managed site, are largely attributed to the reserve's recent managerial approach towards reducing grazing herbivore numbers which promote and aid in pasture recovery (Strathie and McConnachie, 2019). These differing land management strategies also affect the competitive ability of pastures and their species (Riginos, 2009; Frankow-Lindberg, 2012; Van Oudtshoorn, 2012), likely accounting for the differences documented in *P. hysterophorus* invasions at the well- and poorly-managed sites.

Substantially lower *P. hysterophorus* density, plant size and reproductive output observed at the well-managed reserve are all indicative of greater competitive interactions between *P. hysterophorus* and the surrounding species, particularly grasses. Many studies highlight the ability of various grasses to compete with and suppress the growth and invasion of *P. hysterophorus*, under moderate to low grazing pressure, both in South Africa (Van Der Laan et al., 2008) and internationally (Nigatu et al., 2010; Shabbir et al., 2013; Khan et al., 2019). Moreover, the increased levels of grass basal cover and biomass present at the well-managed site, arising from the greater proportion and abundance of large perennial grass species, effectively limits the area available for *P. hysterophorus* to establish and invade (Fargione and Tilman, 2005; Van Der Laan et al., 2008). In addition, the *P. hysterophorus* spring peak density at the poorly-managed site, further suggests that seedlings experienced minimal competition from the surrounding grasses, and that self-limitation (self-thinning) was largely responsible for the weeds decline from spring to summer unlike the well-managed site (Brown et al., 1998; Van Oudtshoorn, 2015). These weed-grass interactions are particularly useful in the context of savannas, however, broader ecological insights into the *P. hysterophorus* invasion are gained when assessing the impacts of the weed with regard to the surrounding plant community (Nigatu et al., 2010; Belgeri et al., 2014).

Parthenium hysterophorus's ability to reduce biodiversity is positively correlated with its above-ground density (Nigatu et al., 2010; Nguyen et al., 2017), accounting for the findings at the poorly-managed site. Ongoing losses in species richness at the poorly-managed site were due to the weed's ability to suppress and displace surrounding species, given its highly competitive nature and allelopathic effects (Belz et al., 2007; Adkins and Shabbir, 2014). Whereas *P. hysterophorus's* dominance was reflected by the low evenness values, which

highlight the tendency of the weed to progressively invade, forming dense monospecific stands in overgrazed areas (Strathie and McConnachie, 2019; Bajwa et al., 2016). These losses in richness and evenness also reduced the poorly-managed reserve's diversity and therefore its ecological resilience (Levine, 2000), suggesting that poor management exacerbates the susceptibility of land to invasion by *P. hysterophorus* (Terblanche et al., 2016; Khan et al., 2019). Contrastingly, the slight recovery of species richness, evenness and diversity at the well-managed reserve was not entirely unexpected, given the reserve's low grazing pressure. Similar research conducted by Belgeri et al. (2014) showed that subsequent to the removal of grazers in *P. hysterophorus* invaded grasslands, native grasses began recovering, competing and displacing weed within two years. Although these findings highlight the differing impacts that *P. hysterophorus* invasions have on biodiversity in savannas, they were not solely restricted to the above-ground plant community.

Various changes noted in the above-ground plant community were reflected within the local seedbanks of the well- and poorly-managed reserves, particularly the dominance of *P. hysterophorus*. The high abundance of *P. hysterophorus* within the reserves' seedbanks is due to the weed's prolific seed production and subsequent seed rain, rapidly forming extensive and persistent seedbanks (Navie et al., 2004; Kohli et al., 2006). However, the comparatively low recruitment of *P. hysterophorus* seedlings at the well-managed site, despite a relatively large seedbank, was interesting. This likely originates from the reserve's lower levels of disturbance and grazing pressure, in which fewer grazers i) limit the frequency of soil disturbance events which promote the germination of *P. hysterophorus*, and ii) permit grasses to grow, forming large tufts which occupy space and compete for resources limiting *P. hysterophorus* recruitment. Overall, these large *P. hysterophorus* seedbanks are indicative of mismanagement, namely overgrazing, at the poorly-managed reserve and historical overuse as a cattle farm at the well-managed site (Navie and Rogers, 1997). Regardless of size of these *P. hysterophorus* seedbanks, the longevity of the weed within the soil will have long-term impacts on the below-ground community at both reserves (Navie et al., 2004).

Over time, *P. hysterophorus* dominated seedbanks often displace native species within the seedbank (Adkins and Shabbir et al., 2014; Nguyen et al., 2017), explaining the continued losses in species richness and evenness below-ground at the poorly-managed reserve. Likewise, losses in species diversity suggest that the regenerative capacity of the poorly-managed reserve is deteriorating as a result of continued *P. hysterophorus* invasion (Navie and Rogers, 1997; Navie et al., 2004), creating an 'invasion trap'. Whereas, findings from the well-managed reserve support the notion that seedbank communities can begin recovering from *P. hysterophorus* invasion following the implementation of and adherence to good land management practices (Strathie and McConnachie, 2019; Khan et al., 2019). Increases in species richness and evenness, at the well-managed reserve, indicate the recruitment of species within the local seedbank and a corresponding loss in dominance by *P. hysterophorus* (Nigatu et al., 2010; Belgeri et al., 2014). The continued influx of seed from native species, particularly grasses, contributed towards the recovery of the well-managed site's seedbank diversity, offering a greater regenerative capacity and resistance to *P. hysterophorus* invasion (Navie and Rogers, 1997; Navie et al., 2004).

Although the small-scale nature of this study offered a focused understanding of *P. hysterophorus* invasion, employing a broader and more spatially independent design in the future is likely to yield insights at a landscape / regional scale. Nevertheless, this study highlights valuable considerations regarding land management and *P. hysterophorus* invasion within South African savannas. The first being climate, the invasion of *P. hysterophorus* as well as savanna grass communities are strongly linked with climatic factors, particularly rainfall, which should be considered prior to management decisions regarding pastures (Cowie et al., 2020; Van Outshoorn, 2012). Although the removal or reduction of grazers is likely to allow the recovery of grass communities, this should be monitored carefully to ensure that pastures / rangelands are not underutilised, which can be equally undesirable in terms of productivity (Belgeri, 2014; Van Outshoorn et al., 2015). Furthermore, the use and reliance on unfenced communal rangelands is a common practice across many savanna regions of South Africa (Reynolds et al., 2020), making the potential removal, reduction or rotation of livestock / game an economically and/or logistically challenging endeavour (Strathie et al. 2011; Terblanche et al., 2016). Overall, the declines in *P. hysterophorus* and its seedbank, under well-managed conditions shows promise in limiting the invasion of *P. hysterophorus*. However, land management alone is unlikely to completely control the weed, advocating for the integration of good management practices with other control programmes such as biological (Strathie et al., 2011; Cowie et al., 2019) and/or chemical controls (Belgeri et al., 2014; Cowie et al., 2020).

In conclusion, this research reaffirmed the ecological detriments of *P. hysterophorus* invasions in South Africa. However, the invasion of *P. hysterophorus*, like many other invasive weeds, may be limited through good land management practices. In the case of *P. hysterophorus* invasions within savannas / rangelands, approaches that maintain the diversity and abundance of native vegetation, particularly perennial grasses, will aid in limiting the establishment and spread of the weed. These approaches also serve to mitigate the detrimental impacts *P. hysterophorus* maintains on both the diversity of above-ground and seedbank communities. Broadly, this highlights the responsibility of landowners and the role their land management decisions hold in limiting invasions by noxious annual weeds, such as *P. hysterophorus*. Thus, the presence or absence of good land, more specifically pasture and livestock / game, management is likely a major factor in limiting or facilitating *P. hysterophorus* invasions within many savanna reserves.

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Implications of elevated carbon dioxide on the susceptibility of the globally invasive weed, *Parthenium hysterophorus*, to glyphosate herbicide

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Abstract

BACKGROUND: The noxious annual herb, *Parthenium hysterophorus* L. (Asteraceae), is an invasive weed of global significance, threatening food security, biodiversity and human health. In South Africa, chemical control is frequently used to manage *P. hysterophorus*, however, concern surrounds increasing atmospheric CO_2 levels, which may reduce the efficacy of glyphosate against the weed. Therefore, this study aimed to determine the susceptibility of *P. hysterophorus* to glyphosate (1L/ha: recommended) after being grown for five generations in Conrons under ambient (400 ppm) and elevated (600 and 800 ppm) CO_2.

RESULTS: Glyphosate efficacy decreased with increasing CO_2, with mortalities of 100, 83 and 75% recorded at 400, 600 and 800 ppm, respectively. *Parthenium hysterophorus* experienced enhanced growth and reproduction under elevated CO_2, however, glyphosate application was highly damaging, reducing the growth and flowering of plants across all CO_2 treatments. Physiologically, glyphosate-treated plants, in all CO_2 treatments, suffered severe declines of >90% in chlorophyll content, maximum quantum efficiency (F_v/F_m), photon absorption (*ABS/RC*), electron transport (ET_0/RC) and performance index (PI_{ABS}), albeit at slower rates for plants grown under elevated CO_2. Low levels of recovery from glyphosate were documented only for plants grown under elevated CO_2 and was attributed to their increased biomass.

CONCLUSION: These results suggest that increasing CO_2 levels may hinder chemical control efforts used against *P. hysterophorus* in the future, advocating for further investigation using multigenerational CO_2 studies and the maintenance of effective spraying programs at present.
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Keywords: chemical control; chlorophyll-*a* fluorescence; global climate change; herbicide efficacy; JIP test; Parthenium weed

1 INTRODUCTION

Over the course of the last century, carbon dioxide (CO_2) levels in the atmosphere have steadily risen, mainly due to anthropogenic activities, and are predicted to reach approximately 800 ppm by the end of the 21st century.[1,2] As CO_2 is the only carbon source for plants, increases in atmospheric CO_2 will enhance their photosynthetic output, translating into increased growth, biomass and yield, particularly species utilising the C_3 photosynthetic pathway.[3,4] These increases in plant biomass and yield are well studied and predicted to be highly beneficial for many C_3 crop plants in the agricultural sector.[5,6] However, weeds represent a major constraint to agricultural activities and are similarly expected to benefit from increases in CO_2.[4,7] Increases in the growth and productivity of C_3 weeds, serves to enhance their competitiveness, with studies suggesting that weeds will increasingly outcompete crop plants in the future.[7,8] Therefore, it is important to understand how elevated CO_2 levels, anticipated in the future, are likely to influence weed management in natural and agrosystems,[9] particularly regarding damaging species such as *Parthenium hysterophorus* L. (Asteraceae).[10]

Native to the neo-tropics, *P. hysterophorus* is a globally significant noxious annual herb, prevalent in more than 40 countries as a troublesome invasive.[11] In South Africa, *P. hysterophorus* is widely recognised as one of the country's most threatening invasive plants, encompassing an array of negative ecological and socio-economic impacts.[12] The weed readily invades a wide variety of habitats, but is most problematic in agricultural settings, including croplands, grazing pastures, rangelands and fields left fallow.[10] Typically germinating with the onset of spring rains, *P. hysterophorus* flowers rapidly (~6 weeks), producing 15 000–150 000 seeds per plant, prior to its annual die-back in late autumn.[12,13] Rapid development and prolific seed production are key to *P. hysterophorus's* invasive potential, allowing the

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formation of dense monospecific stands, which aggressively outcompete and exclude surrounding vegetation.[10,14] Prolific seed production often results in the formation of extensive and long-lasting seedbanks, which can exceed 100 000 seeds/m^2 and remain viable for more than 5 years, making management challenging and costly.[12,15] Although biological control is viewed as the long-term solution in South Africa,[12,16,17] many agricultural and commercially valued areas remain focused and reliant upon herbicidal controls to effectively manage *P. hysterophorus* invasions nationally and internationally.

Chemical control of *P. hysterophorus* in South Africa, as in many countries, is done predominantly with the use of post emergent, non-selective and systemic herbicides, typically glyphosate-based [N-(phosphonomethyl) glycine].[10,18] In the case of *P. hysterophorus*, glyphosate herbicides are applied as foliar sprays, typically to young, non-flowering, plants, which actively absorb the herbicide while growing.[19] Once absorbed through the foliage, glyphosate is highly mobile within the plant, being readily translocated to the developing shoots, roots and leaves.[20,21] Glyphosate acts by inhibiting chloroplast enzymatic activity within the shikimate pathway, leading to an accumulation of shikimate (acid) and a subsequent reduction in the biosynthesis of amino acids, plant proteins and hormones essential for growth.[18,22] The resulting phytotoxic effects include leaf wilting, yellowing, necrosis, and plant death, which typically occur within 1 to 3 weeks of application.[23] Although glyphosate efficacy is highly dependent on the dosage applied, there are concerns that elevated CO_2 will reduce the susceptibility of *P. hysterophorus*, to herbicides in the future.[8,19,24]

Numerous abiotic and biotic factors are known to influence the effectiveness of glyphosate herbicide.[8] Moreover, recent studies suggest that reductions in herbicide susceptibility are to be expected for various annual weed species grown under elevated CO_2 environments.[4,7] In the case of *P. hysterophorus*, the weed frequently accumulates larger amounts of starch and subsequently biomass when grown under elevated CO_2, which may allow for potential reductions in herbicide efficacy through a 'dilution effect'.[7,25] Additionally, increases in leaf thickness and trichome density, anticipated for weeds like *P. hysterophorus*, may serve to limit the uptake and translocation of herbicide through the plant's foliage, further reducing herbicide efficacy.[4,26] Although increased CO_2 enhances the growth and biomass of *P. hysterophorus*,[27] a recent study by Bajwa *et al.*[19] suggested that elevated CO_2 levels are unlikely to lower the efficacy of glyphosate herbicide against this species. However, the findings of short-term unigenerational CO_2 studies,[19] should be used with caution, given the likelihood for unacclimated or premature plant CO_2 responses.[28] Therefore, assessing the susceptibility of *P. hysterophorus* to glyphosate herbicide, subsequent to the growth of multiple generations to elevated CO_2, is likely to further this research by eliciting more representative plant CO_2 responses, offering greater insights.[28,29]

Assessing herbicide susceptibility is traditionally done using growth and survival trials along with lethal or effective dosages as seen for *P. hysterophorus*,[19] however, in recent years, assessments have focused on incorporating leaf chlorophyll fluorescence.[30,31] Chlorophyll fluorescence is a common technique, offering non-destructive and repeatable measures of a plant's physiological 'health' and function, particularly its chloroplasts.[32] Within these chloroplasts, photosystems namely Photosystem II (*PSII*), are highly sensitive to environmental and chemical stresses.[33,34] Reductions in the function and performance of *PSII* is assessed using chlorophyll-*a* fluorescence, with maximum quantum yield (*Fv/Fm*) and related parameters used to monitor herbicide associated stresses.[30] Although glyphosate does not directly inhibit the functioning of *PSII*, its indirect effects on chlorophyll means that fluorometry may be used to assess the physiological status of herbicide exposed plants over time.[31] Overall, reductions in chlorophyll fluorescence allow for the physiological and biochemical effects of herbicides as well as their magnitude to be quantified and understood.[30–32]

The ongoing invasion of *P. hysterophorus* threatens biodiversity, food security, animal health and human well-being, meaning investigations into the potential constraints to its chemical controls in the future are crucial. Ongoing research has highlighted decreases in the efficacy of glyphosate herbicides, and troublingly, these decreases may be precursors to the development of herbicide resistance as documented in many *P. hysterophorus* populations across the world.[18,23] Therefore, this study aimed to determine the susceptibility of the annual invasive herb *Parthenium hysterophorus* to a commercial glyphosate herbicide when first grown for five generations under ambient and two elevated atmospheric CO_2 levels. The study's objectives were (i) to determine the effects of elevated CO_2 on the survival, growth, leaf chlorophyll and chlorophyll-*a* fluorescence of *P. hysterophorus*; and (ii) to evaluate the potential implications of elevated CO_2 the susceptibility of *P. hysterophorus* to glyphosate. Following on from Bajwa *et al.*,[19] it was hypothesised that the elevated CO_2 would decrease the susceptibility of *P. hysterophorus* to glyphosate.

2 MATERIALS AND METHODS

2.1 *Parthenium hysterophorus* plant material and growth chamber conditions

Parthenium hysterophorus plants were initially grown from field collected seed, from a population with no prior glyphosate exposure, during July 2017. Seeds were germinated in plastic pots (20 cm diameter) filled with a loam soil mixture (15% clay: 25% silt: 60% sand) within three identical walk-in Conviron growth chambers (Conviron model PGV40, Winnipeg, Canada). Plants were fertilized once, 1 week after germination, using 5 mL/L of Seagrow® organic fertilizer (N – 53 g kg^{-1}, P – 7 g kg^{-1}, K – 17 g kg^{-1}), to promote healthy growth and were watered on alternating days with 200 mL of tap water applied directly to the soil. To allow plants used in experiments to acclimatize to selected CO_2 levels, *P. hysterophorus* were grown for five consecutive generations, over a year-long period, within three new, calibrated Conviron chambers, prior to experimentation. Twenty-four 5th generation *P. hysterophorus* seeds were germinated per chamber and randomly split into 12 control (unsprayed) and 12 herbicide plants once plants had reached 4 weeks of age.

Within each of the three Conviron chambers, *P. hysterophorus* all plants experienced the same growing conditions, namely temperature, light and relative humidity (RH), excluding atmospheric CO_2 levels, which were set at 400, 600 and 800 ppm (<10 ppm S.E.). To ensure that conditions recorded and displayed by the Convirons chambers was accurate, temperature, light, RH and CO_2 concentration were checked regularly using a Li-6400XT portable open-path infrared gas analyzer (LICOR Biosciences, Lincoln, Nebraska, USA). Daily chamber temperatures were programmed to simulate the average conditions experienced by *P. hysterophorus* plants during mid-summer, with a morning temperature of 20 °C increasing to the maximum temperature of 30 ± 1.3 °C experienced during 11:00 to 16:30, thereafter decreasing (1 °C h^{-1}) to 20 ± 0.8 °C, which was the evening / night temperature. Similarly,

chambers followed summer light patterns, with sunrise at 05:30 increasing to full intensity (~1500 μmol mol^{-1}) between the hours of 10:00–16:30, thereafter gradually declining until 'lights-out' (0 μmol mol^{-1}) at 18:30. Light within each of the chambers was supplied by an adjustable overhead light bank comprising several fluorescent tubes with numerous interspersed incandescent bulbs. To minimize any light gradient effects, plants were randomly shuffled weekly and light banks were adjusted to provide similar light levels (1400–1500 μmol mol^{-1}) based on average plant height per chamber. Lastly, RH within each of the chambers, was set and maintained at a constant 60 ± 3.8%, similar to that experienced by *P. hysterophorus* during summer in the field.

2.2 Glyphosate herbicide application

The herbicide selected for this study was 'Touchdown Forte HiTech'® (Syngenta SA, Pty Ltd.) a widely used commercial glyphosate herbicide registered (L7305: South Africa) for the control of annual and perennial broadleaf weeds, including *P. hysterophorus*, in agricultural and domestic settings. The herbicide is a non-selective and systemic soluble concentrate containing 500 g/L of glyphosate (glycine), which is mixed with water and applied as a foliar spray. Legal guidelines for spraying *P. hysterophorus* recommend that young (non-flowering) plants with less than 12 leaves be targeted, with herbicide applied at a dosage of 0.8–1.0 L/ha.

Glyphosate application was carried out once all plants had reached four-weeks of age (6–8 leaves). To best simulate field management procedures, plants were briefly removed from the Convirons and a full-cover spray technique was used, with the 36 herbicide plants divided equally among three rows (i.e., 12 plants × 3 rows) outdoors. Rows were set 3 m apart to limit any effects of herbicide drift, and plants within rows were randomly assigned and set approximately 30 cm apart. Glyphosate was mixed and applied at the maximum recommended dosage, equivalent to 1 L/ha, using a knapsack sprayer, fitted with flat spray tip nozzle (TeeJet), between the hours of 07:30 and 08:00 am. Knapsack operating pressure was maintained at 190 KPa and spray width at 50 cm above ground was 0.49 ± 0.02 m with a walking pace of 1 m s^{-1}. Once all plants were sprayed, they were returned to their respective Conviron chambers.

2.3 *Parthenium hysterophorus* glyphosate survival and growth

After herbicide application, *P. hysterophorus* plants in each of the CO_2 chambers were assessed daily for survival. Plants were initially considered 'dead' when no green leaf, stem and shoot material was present above ground, however, this was only subsequently confirmed when no active / healthy root growth, was observed. In terms of growth, all *P. hysterophorus* plants were assessed weekly for height, leaf production, leaf area and flower output over a 4-week period, starting on day 0, immediately prior to the application of herbicide. Plant height was measured (cm) from the root crown, located at the base of the stem, to the tallest growing tip for each of the 12 control and 12 herbicide sprayed plants per chamber. Leaf production was taken as the number of fully expanded leaves, excluding any secondary leaflets, present on the plant. Leaf area was calculated (cm^2) as per Cowie *et al.*[17] in which the length and width of all fully expanded leaves were measured and used to gain total leaf area per plant. Flower production was assessed weekly as the number of flowers and flower buds present on each of the plants.

2.4 Chlorophyll content and chlorophyll-*a* fluorescence assessments

Chlorophyll contents were measured ($mg.m^2$) non-destructively using a CCM-300 chlorophyll content metre (Opti-Sciences, Inc. Hudson, New Hampshire, USA). On each of the plants, two spots per leaf were selected randomly for all leaves (excluding leaf mid-ribs) and averaged per plant. One set of measures were taken immediately prior to herbicide application (Day 0) and recorded every 3 days thereafter, for a period of 30 days. In the case of necrosing leaves, measures were made on living material (*i.e.* excluding necrotic leaf tips). Chlorophyll-*a* fluorescence was measured using the '*JIP-protocol*' on an OS30p + handheld fluorometer (Opti-Sciences), with modulation intensity set at 40% and light saturation intensity set at 3500 μmol. To ensure complete Q_A oxidation, all plants (control and herbicide) were dark-adapted overnight (±12 h), using Opti-science leaf clips, and measured prior to 'sunrise' in each of the CO_2 chambers. Each plant had the first and second newest fully expanded leaves clipped, measured and averaged per plant. Like chlorophyll content, one set of fluorescence measures were taken immediately prior to herbicide application (i.e., day 0), when plants were aged 4 weeks. Thereafter, plants were sprayed with glyphosate and measured every 3 days for a period of 30 days on all living plants across all three CO_2 treatments.

The parameters measured included minimum fluorescence (F_O) to maximum fluorescence (F_M), with variable fluorescence (F_v) calculated as = F_m - F_O. In addition, maximum quantum efficiency of *PSII* (F_v/F_m) was also recorded and used to infer the physiological health of photosystem II (*PSII*), with values of ≥0.8 considered as 'healthy'.[35,36] The 'JIP protocol' records chlorophyll fluorescence as transients (Kautsky curves), in which values are collected during the rise from minimal (F_O) to maximum fluorescence (F_m) and represent the various stages of the curve. These stages include: O = fluorescence at 20 μs, J = fluorescence at 2 ms, I = fluorescence at 30 ms and P = maximum fluorescence (F_m).[37] From these curves, various parameters insights into the quantum efficiency of *PSII*, the energy fluxes occurring within reaction centres, the density of reaction centres and their performance were selected. These parameters were selected to gain various insights into the biochemical and physiological function of *P. hysterophorus* in response to chemical stress and were calculated using the OJIP curves as per Strasser *et al.*[38] (Table 1).

2.5 *Parthenium hysterophorus* glyphosate recovery

In terms of recovery, all plants surviving glyphosate application were monitored further than 30 days to record if they were able to maintain their invasive potential (*i.e.* produce viable seed). Once plants were observed to set seed, 25 mature seeds were collected at random, per plant, for viability assessment from all control and surviving *P. hysterophorus* plants across the CO_2 chambers. Collected seed batches, per plant, were then germinated in Petri dishes within an incubator set at 25 °C | 20 °C with a 13 h light: 11 h dark daily cycle. All Petri dishes were watered with 3 mL of sterilized (autoclaved) water every 5 days (or as necessary) over a 2 week period. Seeds that failed to germinate after the 2 week period were placed into a 2% tetrazolium (2,3,5-triphenyl tetrazolium chloride) solution for 36 h and assessed for viability based on staining of the embryo (red/pink = viable and white/clear = dead). Seed viability (%) was calculated as the number of germinated and viable (tetrazolium) seeds per dish / total seeds per dish × 100.

Table 1. Selected chlorophyll-*a* fluorescence parameters derived and calculated from initial O-JIP data measured on 5th generation *Parthenium hysterophorus* plants exposed and unexposed to glyphosate herbicide at CO_2 levels of 400, 600 and 800 ppm

Chlorophyll fluorescence parameters		*Calculation*
Quantum efficiencies of photosystem II		
F_v/F_m	Maximum quantum efficiency	$= F_m - (F_O / F_m)$
Energy fluxes per reaction center		
ABS/RC	Rate of photon absorption	$= (TR_0/RC) / [(F_M - F_O)/F_m]$
ET_0/RC	Rate of electron transport beyond Q_A	$= (TR_0/RC)$ x $(1 - V_J)$
Density of reaction centers		
RC/CS	Functional reaction centers per cross section of leaf area	$= (TR_0/RC)$ x (TR_0/ABS)
Performance index on absorption		
PI_{ABS}	Function of light energy absorption, efficacy of Q_A reduction and conversion of excitation energy to electron transport	$= (ABS/RC)$ x $[(TR_0/ABS) / (F_O/F_m)]$ x $[(ET_0/TR_0) / V_J]$

Descriptions and calculations adapted from Strasser *et al.*[37] and Ripley *et al.*[32] TR_0, trapping flux at the onset of fluorescence; RC, reaction center; V_J, heat dissipation rate; ABS, absorption flux; ET_0, electron transport flux at the onset of fluorescence.

2.6 *Parthenium hysterophorus* biomass assessment

At 10 weeks after germination (i.e., 6 weeks after glyphosate application), when all control (unsprayed) plants had begun producing seed, *P. hysterophorus* plants were collected for biomass assessment. All plants, excluding those which had survived herbicide application in the 600 ($n = 2$) and 800 ppm ($n = 3$) CO_2 treatments, were removed from the Conviron chambers for assessment. Each of the plants was then carefully uprooted and washed to remove all soil and debris, particularly from the roots. Plants were then bagged, and oven dried at 70 °C for a week (7 days) and weighed to three decimal places (0.001 g) to attain overall plant biomass.

2.7 Statistical analysis

Parthenium hysterophorus herbicide exposure survival (%) was calculated as the total number of plants surviving / total number sprayed with herbicide, after 30 days, in each of the three CO_2 treatments. Mean time to death (days) from herbicide application was assessed using a one-way analysis of variance (ANOVA) with a Tukey HSD *post-hoc*.

Survival probability for plants exposed to herbicide, within each of the CO_2 treatments, was generated using a generalized linear model (GLM) with family set as binomial and link as logit. Growth parameters, including height, leaf number, leaf area and flower production were assessed using linear mixed effects models (LMER) with a Tukey *post-hoc* using the 'lme4', 'nlme', and 'multcomp' packages within R.[39] To best account for the unreplicated experimental design arising from the use of Conviron chambers, growth parameters were set as the response variables with CO_2 and herbicide treatment set as the fixed effects, time as a continuous predictor and plant number nested within chamber set as the random effect. LMER outputs were presented as F_{stat} with *P*-values, generated using the 'car' package in R. Plant recovery was assessed for all CO_2 and herbicide treatments, excluding 400 ppm with glyphosate which had 100% mortality, using a one-way ANOVA with a Tukey HSD *post-hoc* for the time (days) taken to seed production as well as mean seed viability (%).

To investigate the rate of change in *P. hysterophorus*'s chlorophyll content and chlorophyll-*a* fluorescence in response to glyphosate over its effective period (~25 days), linear regressions were fitted to mean chlorophyll content, F_v/F_m, ABS/RC, ET_0/RC, PI_{ABS} and RC/CS over time for the herbicide sprayed plants in all CO_2 treatments. A standardized major axis test (β_{SMA}) with time (days after treatment) set as a continuous predictor, was then performed using the 'smatr' package, in R,[39] to detect for changes amongst fitted slopes between CO_2 treatments. Lastly, *P. hysterophorus* biomass was assessed for all plants, excluding those surviving glyphosate application, using a LMER with biomass set as the response variable, CO_2 and herbicide treatment set as the fixed effects and plant number nested within chamber set as the random effect.

3 RESULTS

3.1 *Parthenium hysterophorus* glyphosate survival and growth

Overall, glyphosate herbicide significantly reduced the survival of *P. hysterophorus* across all three CO_2 treatments 30 days after

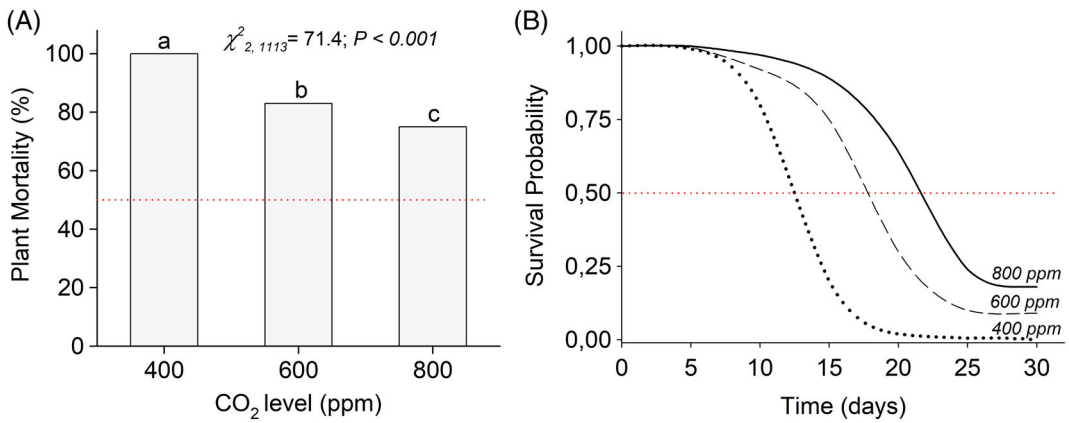

Figure 1. *Parthenium hysterophorus* (a) plant mortality (%) and probability of survival (b) for 5th generation plants exposed to glyphosate herbicide after 30 days and grown under atmospheric CO_2 levels of 400, 600 and 800 ppm.

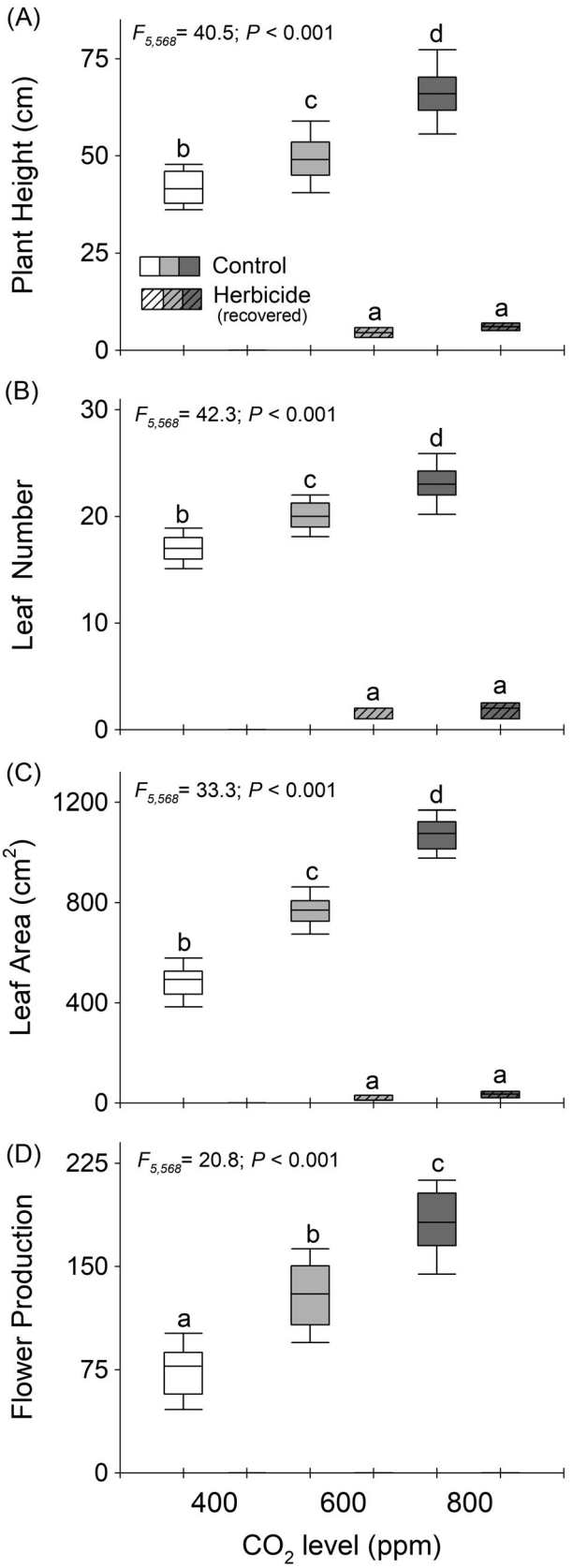

Figure 2. Mean *Parthenium hysterophorus* (a) height, (b) number of leaves, (c) leaf area and (d) seed production of 5th generation plants exposed (recovered) and unexposed (controls) to glyphosate herbicide after 30 days and grown under atmospheric CO_2 levels of 400, 600 and

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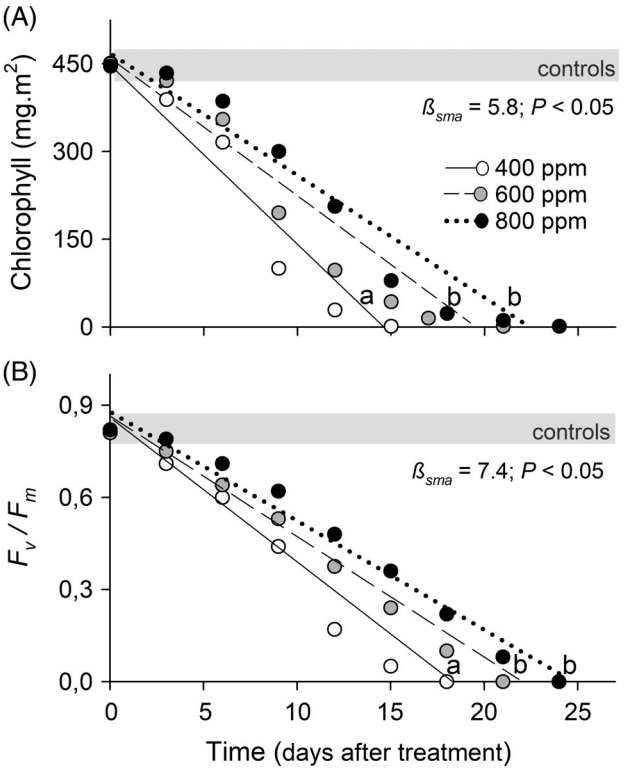

Figure 3. Mean (a) chlorophyll content (mg.m^2) and (b) maximum quantum efficiency of *PSII* (F_v/F_m) for 5th generation *Parthenium hysterophorus* plants exposed to glyphosate herbicide and grown under atmospheric CO_2 levels of 400, 600 and 800 ppm. Different lowercase letters indicate significant differences (P < 0.05) between slopes of different CO_2 treatments using a standardized major axis test (β_{SMA}). Range of all control values are shown as approximations for comparison.

application, with 100%, 83% and 75% mortality recorded at 400, 600 and 800 ppm, respectively ($\chi^2_{\ 21\ 113}$ = 71.4; Fig. 1(a)). Plant mortality was influenced significantly by CO_2, with mean time to death occurring quickest at 17 ± 0.9 days for 400 ppm, followed by 21 ± 0.7 days at 600 ppm and lastly by 23 ± 0.7 days at 800 ppm ($F_{2,33}$ = 23.5; P < 0.001). Additionally, the probability of *P. hysterophorus* surviving glyphosate treatment increased with increasing atmospheric CO_2 concentrations (Fig. 1(b)). The model suggested no survival for *P. hysterophorus* under current CO_2 levels (400 ppm), however, plants reared and sprayed under 600 and 800 ppm CO_2 were predicted to survive, albeit at low levels of approximately 10% and approximately 20% (Fig. 1(b)).

Parthenium hysterophorus exposed to the herbicide suffered significant reductions in height, with glyphosate effectively stunting the growth of plants, in 400, 600 and 800 ppm treatments, over the 30 day period ($F_{5,568}$ = 40.5; P < 0.001; Fig. 2(a)). Leaf production increased significantly with CO_2 but was greatly reduced through the application of glyphosate ($F_{5,568}$ = 42.3; P < 0.001; Fig. 1(b)). Only the plants surviving in the 600 ppm (n = 2) and

(Figure legend continued from previous page.)
800 ppm. F_{stat} and *P-values* indicate overall difference in *P. hysterophorus* parameters between treatments using linear mixed effects models (LMERs). Boxplots show median, 10th, 25th, 75th and 90th percentiles, and differing lowercase letters indicate significant differences between treatments (P < 0.05).

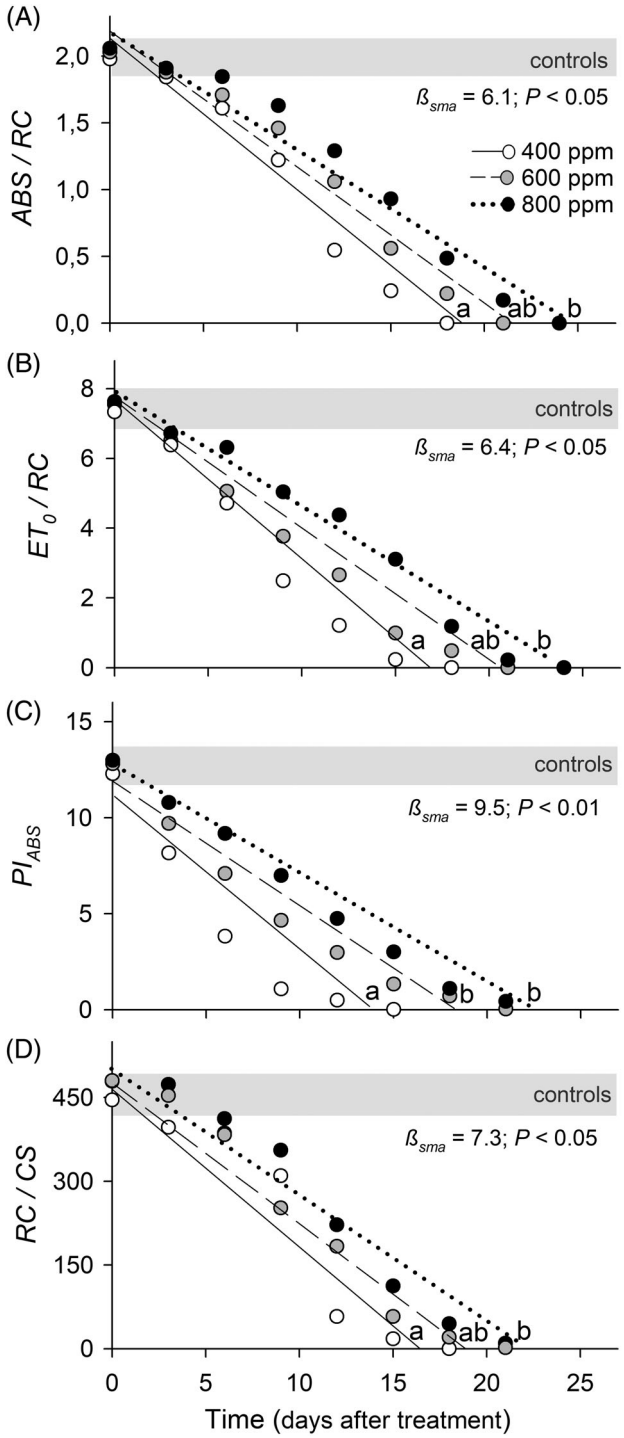

Figure 4. Mean chlorophyll-*a* fluorescence parameters: mean (a) photon absorption rate (*ABS/RC*), (b) electron transport rate (ET_0/RC), (c) performance index (PI_{ABS}) and (d) density of functional reaction centers per cross section of leaf area (*RC/CS*) for 5th generation *Parthenium hysterophorus* plants exposed to glyphosate herbicide and grown under atmospheric CO_2 levels of 400, 600 and 800 ppm. Different lowercase letters indicate significant differences ($P < 0.05$) between slopes of different CO_2 treatments using a standardized major axis test (β_{SMA}). Range of all control values are shown as approximations for comparison.

800 ppm ($n = 3$) treatments produced new leaves approximately 26–27 days after exposure to glyphosate. Similarly, all unsprayed plants accumulated greater leaf areas as CO_2 increased, but when

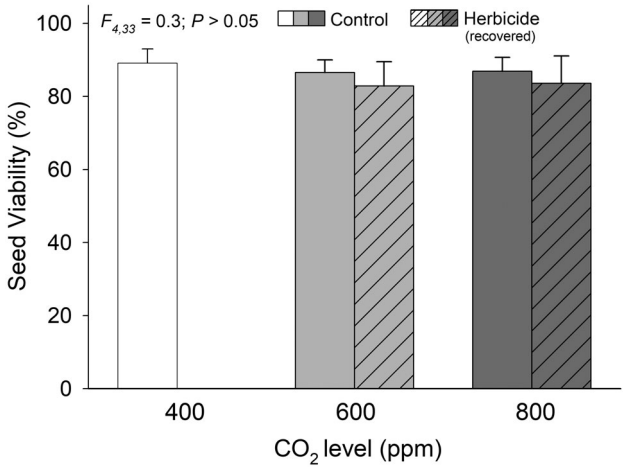

Figure 5. Mean (±SE) seed viability for 5th generation *Parthenium hysterophorus* plants grown under atmospheric CO_2 levels of 400, 600 and 800 ppm and exposed and unexposed (controls) to glyphosate herbicide. F_{stat} and *P-value* indicate no difference in *P. hysterophorus* seed viability between treatments using an ANOVA.

exposed to glyphosate experienced significant reductions of 100% in the 400 ppm treatment and approximately 98% in both the 600 and 800 ppm treatments ($F_{5,568} = 33.3$; $P < 0.001$; Fig. 2 (c)). *Parthenium hysterophorus* flower production increased significantly in response to CO_2, with controls in the 600 ppm and 800 ppm CO_2 treatments producing on average 128 ± 5 and 188 ± 8 flowers/plant, respectively ($F_{5,568} = 25.1$; $P < 0.001$). Whereas all plants sprayed with glyphosate produced no flowers, nor set any seed, over the 30 day period in any of the three CO_2 treatments (Fig. 2(d)).

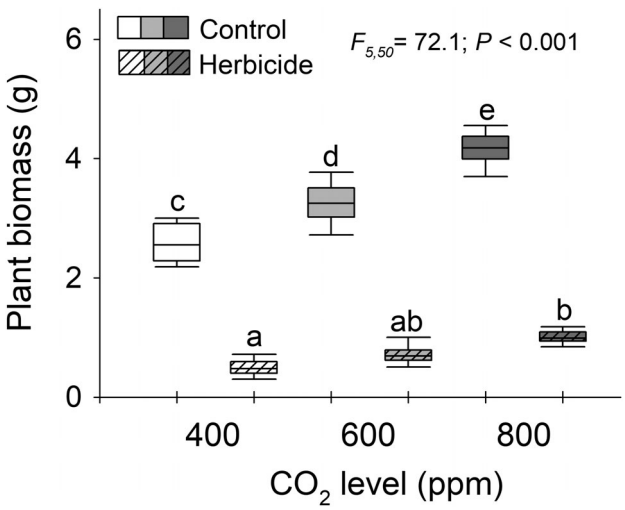

Figure 6. Mean *Parthenium hysterophorus* dry biomass for 5th generation plants grown under atmospheric CO_2 levels of 400, 600 and 800 ppm and exposed and unexposed (controls) to glyphosate herbicide after 6 weeks. Biomass data presented for herbicide exposed plants excludes plants which survived glyphosate application. F_{stat} and *P-values* indicate overall difference in *P. hysterophorus* biomass between treatments using a linear mixed effects models (LMER). Boxplots show median, 10th, 25th, 75th and 90th percentiles, and differing lowercase letters indicate significant differences between treatments ($P < 0.05$).

3.2 Chlorophyll content and chlorophyll-a fluorescence assessments

The application of glyphosate significantly reduced the leaf chlorophyll content of *P. hysterophorus* across all three CO_2 treatments (Fig. 3(a)). However, the rate at which chlorophyll declined was linked to CO_2 (ppm), with significantly slower declines of 40% and 60% occurring at 600 and 800 ppm ($\beta_{SMA} = 5.1$; $P < 0.05$). Maximum *PSII* quantum efficiency (F_v/F_m) declined significantly in response to glyphosate application, with all sprayed plants rapidly dropping below the 'healthy' threshold of 0.8 by day 3 (Fig. 3 (b)). The quickest decline in F_v/F_m however, occurred at 400 ppm reaching 0 on day 18 followed by the 600 and 800 ppm treatments respectively, which reached 0 on days 21 and 24 ($\beta_{SMA} = 7.4$; $P < 0.05$).

The photon absorption rates of *P. hysterophorus* declined significantly when exposed to glyphosate herbicide but remained unchanged for controls across all CO_2 levels (Fig. 4(a)). The rate at which photon absorption declined was linked to CO_2 level, occurring quickest at 400 ppm and slowest at 800 ppm ($\beta_{SMA} = 6.1$; $P < 0.05$). Similarly, the decline in electron transport rates, beyond Q_A, was influenced by CO_2, with the plants grown under 800 ppm experiencing a 34% slower decline when compared to plants grown under ambient $CO_2 = 400$ ppm ($\beta_{SMA} = 6.4$; $P < 0.05$; Fig. 4 (b)). *Parthenium hysterophorus* plants also suffered significant reductions in performance index (PI_{ABS}) with the application of glyphosate, which was most severe under 400 ppm and least severe under 800 ppm CO_2 ($\beta_{SMA} = 9.5$; $P < 0.01$; Fig. 4(c)). Lastly, the density of functional reaction centers, per cross section of leaf area (*RC/CS*), declined significantly in response to glyphosate application ($\beta_{SMA} = 7.3$; $P < 0.05$), with the quickest reduction occurring at 400 ppm and the slowest at 800 ppm (Fig. 4(d)).

3.3 *Parthenium hysterophorus* glyphosate recovery

Only *P. hysterophorus* plants grown under elevated CO_2 survived the glyphosate application, with ($n = 2$) in 600 and ($n = 3$) in 800 ppm. These plants were all able to fully recover, subsequently bolting and producing seed at 77 ± 5 and 69 ± 4 days after herbicide application, respectively. The surviving plants did however, take significantly longer to produce seed compared to their respective controls, which produced seed on average 57 days sooner ($F_{4,35} = 223.7$; $P < 0.001$). Despite the prolonged time to produce seed, seeds collected from the plants surviving glyphosate application in 600 and 800 ppm were equally as viable as seeds collected from their unsprayed (control) plants with average viabilities of approximately 85% ($F_{4,33} = 0.3$; $P > 0.05$; Fig. 5).

3.4 *Parthenium hysterophorus* biomass assessment

Parthenium hysterophorus experienced significant increases in growth, namely in plant biomass as atmospheric CO_2 concentrations increased ($F_{5,50} = 72.1$; $P < 0.001$; Fig. 6). Compared to *P. hysterophorus* under ambient CO_2 (400 ppm), plants grown under 600 ppm accumulated 23.4% more biomass, whilst those grown under 800 ppm experienced a 54.5% increase. The application of glyphosate however, significantly reduced plant biomass across all three CO_2 treatments, reducing biomass by 81%, 78% and 76% in the 400, 600 and 800 ppm treatments, respectively (Fig. 6).

4 DISCUSSION

Parthenium hysterophorus is a highly damaging invasive weed, and over recent years has been managed in agrosystems using glyphosate herbicides.[10,23,40] Recent research by Bajwa *et al.*[19] suggested that increases in atmospheric CO_2 are unlikely to affect the efficacy of glyphosate utilized against *P. hysterophorus*. In contrast, findings shown here suggest that increases in CO_2, assessed after multiple generations, may diminish the efficacy of such chemical controls used against this weed. The survival and recovery (seed set) of glyphosate-treated *P. hysterophorus* plants grown under elevated CO_2, albeit at low rates, may be attributed to increases in growth (biomass), rather than any glyphosate resistance mechanism/s. Substantial increases in *P. hysterophorus* biomass, as shown here, are well-documented under elevated CO_2,[19,27] and can serve to reduce the efficacy of glyphosate, through a 'dilution effect'.[3,8,9] Increases in biomass, leaf area and root: shoot ratios are suggested to dilute glyphosate concentrations across the plant, slowing translocation, potentially limiting the phytotoxic accumulation and overall effectiveness.[4,7,19] Moreover, increases in leaf thickness and trichome density of C_3 annual plants grown under elevated CO_2, will likely limit the absorption of glyphosate through the foliage, further reducing glyphosate efficacy.[4,26] Glyphosate application, however, elicited an array of plant stresses, which were reflected predominately in the growth and chlorophyll fluorescence of *P. hysterophorus*.

As expected, *P. hysterophorus* experienced increases in growth and biomass when grown under elevated CO_2[27] and suffered substantial reductions to these at both ambient and elevated CO_2 as a result of glyphosate application (recommended dosage). Glyphosate indirectly affects a plants' photosynthetic ability, resulting in the rapid cessation of CO_2 uptake as well as the biosynthesis of amino acids/proteins.[41,42] Furthermore, the inhibition of the shikimate pathway by glyphosate limits the production of the phytohormones including Auxin, which is primarily involved in cell elongation and stem/shoot growth.[41] The reduction in leaf number and leaf area are likely to be a result of herbicide absorption and translocation, resulting in the accumulation of shikimate in the leaves of *P. hysterophorus*. The accumulation of glyphosate is phytotoxic in actively growing tissues, particularly leaves and leaf tips in the case of *P. hysterophorus*, causing substantial damage and accounting for the halted production, wilting, necrosis and subsequent abscission of leaves.[23,43]

Significant increases in flower and seed production are well-documented for numerous weed species when grown under elevated CO_2,[8,9] and although *P. hysterophorus* experienced increased flower / seed production at elevated CO_2, the weed's flower production was substantially reduced after exposure to glyphosate. The phytotoxic accumulation of glyphosate in *P. hysterophorus* meristem shoot tips inhibits the production of new shoots, limiting the growth of inflorescences (flowers) and subsequent seed set.[43,44] Although this initial reduction in *P. hysterophorus* flowering is promising towards control efforts, the ability of plants to eventually recover and produce viable seed under elevated CO_2 remains problematic.[23] The production of high levels of viable seed, even after herbicide exposure, is supported by recent studies[19,27] and offers *P. hysterophorus* the potential to establish less susceptible populations in the field. Ultimately, these reductions in the reproductive output of *P. hysterophorus*, across all CO_2 treatments, may be attributed to many of the underlying biochemical and physiological stresses associated with exposure to glyphosate.[19]

Glyphosate is accepted to decrease chlorophyll content, although, the causal mechanisms underlying this decline remain unclear as to whether disruptions in chlorophyll metabolism leads to reduced chlorophyll synthesis or if the build-up of reactive

oxygen species (ROS) causes oxidative damage, degrading chlorophyll.[42,45] Regardless of the potential mechanisms involved, the resulting losses in chlorophyll impaired the functionality of *P. hysterophorus*'s chloroplasts, particularly photosystem II (*PSII*), as indicated by rapid declines in maximum quantum efficiency of *PSII* (F_v/F_m).[33,46] Decreases in *P. hysterophorus* F_v/F_m were slowed under elevated CO_2, indicating slower rates of biochemical/physiological impairment, particularly to CO_2 uptake. Limitations to CO_2 assimilation are frequently coupled with decreases in electron transport (ET_0/RC) and photon absorption rates (*ABS/RC*), as observed in all of the glyphosate-treated *P. hysterophorus* plants. Decreases in ET_0/RC were likely due to the inhibition of sink processes and impairment of *PSII* photochemistry/photosynthetic apparatus resulting from glyphosate accumulation.[41,46] Whereas reductions in the *ABS/RC*, are a result of the rapid decline in the density of functional reaction centers (*RC/CS*), which are associated with declines in chlorophyll concentration.[33,34] This rapid decline in *RC/CS* is linked to disruptions in a suite of downstream biochemical processes, evidenced by the severe declines in PI_{ABS}, which is a product of energy flux and reaction center processes.[34,37]

Broadly, these findings offer preliminary implications linked to the chemical control of *P. hysterophorus* under elevated and ambient CO_2 over multiple generations, warranting further investigation. This research provides valuable biological insights into the potential response of *P. hysterophorus* to glyphosate in the future; however, caution should be exercised when up-scaling these findings. Although increasing glyphosate concentration is likely to increase the efficacy, this may serve as a selection pressure, promoting the survival of less susceptible plants and potentially glyphosate-resistant populations, as documented for *P. hysterophorus* in numerous other countries.[10,18,23] Substantial increases in the growth and reproduction of *P. hysterophorus* under elevated CO_2, could also intensify the demands of spraying programs, requiring more frequent, rigorous and costly follow-ups.[19,47] Additionally, the climatic changes expected with increasing CO_2 may further reduce glyphosate efficacy by limiting herbicide uptake,[48] effective spraying times and spraying conditions.[24] Mitigations to these potential problems might be attained through the rotation of herbicide types, however, South Africa currently only has one non-glyphosate herbicide (Picloram) registered for the control of *P. hysterophorus*, which may limit this approach at present. Likewise, new or altered formulations of glyphosate may enhance *P. hysterophorus* mortality, offering short-term solutions,[49] but are unlikely to maintain optimal efficacy in the future, as CO_2 levels rise. Thus, ongoing monitoring of herbicide efficacy as well as the development and adherence to effective spraying regimes, including herbicide rotations, not just in South Africa but globally, are advised.

5 CONCLUSIONS

In conclusion, these findings offer insights towards the understanding and control of the noxious invasive herb, *P. hysterophorus*, when using glyphosate-based herbicides. Although the decreases in susceptibly of *P. hysterophorus* are unlikely to impact the herbicidal control under present CO_2 levels (~400 ppm), they suggest that increasing atmospheric CO_2 may hinder chemical control programs, in natural and agroecosystems, in the future. Increased growth experienced by *P. hysterophorus* under elevated CO_2 may allow plants to be more tolerant of glyphosate by diluting the effective dosage absorbed across the plants' biomass. In addition, the slowed declines in chlorophyll and associated biochemical parameters experienced under elevated CO_2 may also allow *P. hysterophorus* to recover from spraying, facilitating the development of more tolerant and potentially resistant populations. More generally, this research advocates for the usage of a multigenerational approach prior to assessments of weed control techniques, such as herbicide susceptibility, particularly in the context of elevated CO_2.

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CHAPTER 5:

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Physiological response of *Parthenium hysterophorus* to defoliation by the leaf-feeding beetle *Zygogramma bicolorata*

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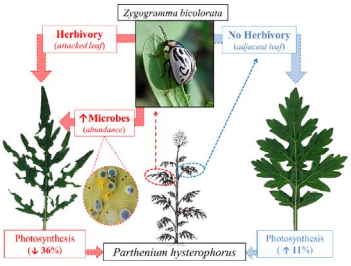
GRAPHICAL ABSTRACT



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ABSTRACT

The annual shrub, *Parthenium hysterophorus* L. (Asteraceae), is a severe noxious weed of global significance, having been the target of control programmes in several affected countries. Biological control efforts in South Africa have seen a total of four agents released to date, one of the most promising of which is the leaf-feeding beetle, *Zygogramma bicolorata* Pallister (Coleoptera: Chrysomelidae), first released in 2013. Detailed study of the ecophysiological interactions of insect herbivores and their target weeds are under-represented in the biological control literature. Extensive leaf-feeding by *Z. bicolorata* adults metabolically impaired *P. hysterophorus* leaves, resulting in a ~36% reduction to photosynthesis in remaining leaf tissue. However, in response to *Z. bicolorata* herbivory, adjacent undamaged leaves showed an up-regulation in photosynthesis of ~11%. Photosynthetic reduction was attributed to the removal of leaf tissue, physiological impairment and increased microbial abundance resulting from *Z. bicolorata*. *Zygogramma bicolorata* was found to increase the number of bacterial and fungal microbes present on *P. hysterophorus* leaves. Despite the ability to photosynthetically up-regulate in response to herbivory, *P. hysterophorus* is unlikely to overcome the physiological implications associated with continued *Z. bicolorata* feeding and the increase in microbes. Given the potential effectiveness of *Z. bicolorata* as a biological control agent, efforts should be made to maximise incidence of the beetle in the field.

1. Introduction

Parthenium hysterophorus L. (Asteraceae: Heliantheae) is an annual herbaceous shrub that has become a severe noxious weed of global significance (Adkins and Shabbir, 2014). Native to Central and South America as well as the Caribbean, *P. hysterophorus* has invaded numerous countries across America, Asia, Oceania and Africa (Dhileepan and Strathie, 2009; McConnachie et al., 2010). South Africa, first

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documented *P. hysterophorus* in KwaZulu-Natal during the 1880's (Hilliard, 1977), but it was not until the 1980's that the weed became prominent, subsequently invading the KwaZulu-Natal, North West, Limpopo and Mpumalanga provinces (Terblanche et al., 2016). *Parthenium hysterophorus* is capable of growing in a multitude of habitats, maturing rapidly to form dense stands and extensive seedbanks, which aggressively outcompete and chemically inhibit native flora (Dhileepan and McFayden, 2012). The weed further prompts concern given its detrimental effects to agriculture (crop yield), animal husbandry and human health (Adkins and Shabbir, 2014). The annual nature (ephemeral) and extensive seedling recruitment of *P. hysterophorus*, has meant that mechanical and chemical clearing procedures are largely not feasible, ineffective and/or unsustainable. Thus, following prior successes in Australia, biological control efforts were initiated in South Africa during 2003, as one of the long-term solutions to managing *P. hysterophorus* invasion (Strathie et al., 2005, 2011).

Considered one of the more promising biological control agents, the leaf-feeding beetle *Zygogramma bicolorata* Pallister (Coleoptera: Chrysomelidae) (Strathie et al., 2005), was released at various densely invaded sites throughout KwaZulu-Natal and Mpumalanga from 2013 onwards (Strathie et al., 2014; McConnachie, 2015). Both adult beetles and their larvae feed extensively on *P. hysterophorus* leaves, displaying a preference for those which are recently developed. Feeding by *Z. bicolorata* occurs continually from spring to autumn, resulting in severe, if not complete, defoliation of attacked leaves and the subsequent skeletonisation of whole plants (Dhileepan et al., 2000; McConnachie, 2015). These beetles hold great promise given their high fecundity (~45 eggs) rapid development (egg to adult in ~6 weeks) and longevity of up to 2 years (Dhileepan et al., 2000; Chidawanyika et al., 2017). During late autumn (April-May) *Z. bicolorata* enters a facultative soil diapause in response to decreasing temperatures, rainfall and photoperiod, re-emerging with the onset of favourable conditions during spring (October) (Strathie et al., 2011). The relatively recent release of *Z. bicolorata* in South Africa has meant the agent's efficacy remains under investigation, but ongoing studies highlight successful establishment, with variable incidence (Strathie et al., 2016). Despite the relatively recent establishment of *Z. bicolorata* in South Africa, extensively damaging outbreaks have been recorded (Chidawanyika et al., 2017).

Folivory (feeding on leaf tissue) directly reduces leaf area, disrupting leaf function and ultimately altering the photosynthetic capacity (Zangerl et al., 2002). However, the photosynthetic impact of folivory cannot be predicted based on the amount of tissue removed, with studies showing increased, decreased or unchanged photosynthetic rates, all in response to folivory (Turnbull et al., 2007; Delaney, 2008; Kant et al., 2015). Although it is widely accepted that plants are well adapted to tolerate partial or complete defoliation (Peterson et al., 2004), recent studies suggest that photosynthetic reduction may be more common than previously thought (Delaney and Higley, 2006; Nabity et al., 2009). The indirect consequences of folivory, rather than the removal of leaf area, are believed to be responsible for the declines in photosynthesis (Ripley et al., 2008; Nabity et al., 2009). Insect herbivore studies frequently incorporate artificial herbivory to gain insight towards the underlying effects and implications of folivores, whilst accounting for the direct effects of tissue removal (Lehtilä and Boalt, 2004; Schooler et al., 2006). Indirect effects include the severing of leaf vasculature, impairment to nutrient- and water-transport systems and increases in the susceptibility of leaves to infection by micro-organisms (Venter et al., 2013; Humphrey et al., 2014). These implications alter the energetic demands of damaged leaves and are frequently reflected in changes to leaf physiology and related gaseous exchange parameters (Peterson et al., 2004; Aldea et al., 2005).

A myriad of plant responses are elicited in response to herbivory and are generally classified as defensive or compensatory (Nabity et al., 2009). Defensive responses are typically structural or chemical, seeking to pre-emptively minimise or prevent herbivory (Kant et al., 2015). In the case of *P. hysterophorus* these are the presence of numerous leaf trichomes (Dale, 1981) and secondary compounds, namely the sesquiterpene lactone Parthenin (Picman et al., 1981). Whereas compensatory responses are induced in an attempt to counteract or offset the losses incurred during and subsequent to herbivory (Schwachtje and Baldwin, 2008; Kant et al., 2015). Defoliation elicits a variety of complex physiological responses, with the most prominent including adjustments to leaf water relations, nutrient status and photosynthetic rate (Thomson et al., 2003; Turnbull et al., 2007). Subsequent to folivory, plants may compensate by enhancing (up-regulating) photosynthesis in the remaining leaf tissues, particularly in neighbouring or developing undamaged leaves (Turnbull et al., 2007; Delaney, 2008). Although this up-regulation is usually performed under favourable conditions, it is temporary and heavily reliant on resources available to the plant (stored reserves) (Thomson et al., 2003; Suwa and Maherali, 2008). Studies also suggest that in certain cases, photosynthetic up-regulation may not be substantial enough to overcome severe or repeat defoliation events (Delaney et al., 2008; Nabity et al., 2009; Kant et al., 2015).

Although studies have demonstrated the effects of insect herbivory, few have focused on annual weed species and their co-evolved herbivores, particularly at an ecophysiological level. This study aimed to assess the physiological, namely photosynthetic response, of the invasive weed *Parthenium hysterophorus* to leaf-feeding by its biological control agent *Zygogramma bicolorata*. The hypothesis that adjacent leaves, directly upstream from defoliated leaves, would display an up-regulation in photosynthesis was tested. Secondarily, increases in the abundance of bacterial and fungal microbes resulting from *Z. bicolorata* beetle herbivory, was investigated at a broad scale.

2. Materials and methods

Zygogramma bicolorata used in these experiments were sourced from a culture, currently maintained at the University of the Witwatersrand, Johannesburg (Wits Insectary). The beetle culture originated from the ARC-PPRI research facility in Hilton, KwaZulu-Natal. Fifty *P. hysterophorus* plants were grown from seed collected in the field during October 2015. Plants were grown to approximately 1 m in height under full-sun conditions in an outdoor nursery at the University. Plants were watered daily and fertilised weekly, using Seagro® organic fertiliser (5 ml/ℓ), to promote healthy leaf growth. Minimum and maximum temperatures experienced during the course of the experiment were 18 °C and 27 °C respectively.

2.1. Herbivory treatments and leaf defoliation

Herbivory experiments were conducted in the outdoor nursery, in which 21 *P. hysterophorus* plants were divided equally amongst three treatments: (i) Beetle herbivory (using *Z. bicolorata*), (ii) Simulated herbivory and (iii) Control. Each of the plants had the third and fourth youngest fully expanded leaves, below the apical bud, classified as the 'adjacent' and 'attacked' leaves respectively. These leaves were individually bagged using a sterilised fine mesh bag for a period of five days. 'Attacked' leaves in the beetle treatment were enclosed with two adult *Z. bicolorata* beetles, selected at random from the rearing culture. Defoliation of 'attacked' leaves was assessed twice a day, at 12 h intervals, by means of non-destructive photographic analyses using ImageJ imaging software (Ver. 1.50, Wayne Rasband, National Institute of Health, USA).

Simulated herbivory plants were each assigned a corresponding beetle herbivory plant to 'mimic'. Defoliation of the simulated herbivory plants occurred on the assigned 'attacked' (fourth fully expanded) leaf, having the same percentage of leaf area removed as the corresponding 'attacked' leaf on beetle herbivory plants, every 12 h for five days. Herbivory was simulated using a pair of sterilised dissecting scissors in similar fashion to that of the beetles' feeding patterns. In the case of control plants, the 'adjacent' and 'attacked' leaves were similarly

bagged but were neither cut nor exposed to *Z. bicolorata*.

2.2. Gas exchange and leaf chlorophyll / nitrogen (SPAD)

Photosynthetic and associated gas exchange parameters were measured using a Li-6400XT portable open-path infrared gas analyser (LI-COR Biosciences, Lincoln, Nebraska, USA). Net leaf CO_2 and H_2O exchange on the 'adjacent' and 'attacked' leaves below the apical bud were measured. photosynthetic photon flux density (PPFD) within the cuvette was set at 1500 μmol mol^{-1}, using a blue–red LED light source. For all photosynthetic measurements, leaf temperature was maintained at 25 °C, leaf to air vapour pressure deficit (VPD) was kept below 1.2 kPa and CO_2 concentration was supplied at 400 ppm.

The 'adjacent' and 'attacked' leaves, on each of the plants, were inserted into the cuvette for initial measurements on Day 0, prior to any herbivory (pre-herbivory). Subsequent to herbivory (post-herbivory), in which at least 80% of total leaf area was removed after 5 days, the 'adjacent' and 'attacked' leaves for all herbivory treatments were measured. In the case that *P. hysterophorus* leaves inserted into the cuvette did not fill the 6 cm^2 area for both pre- and post-herbivory measures, leaf area was calculated using photographic analysis (ImageJ) and incorporated into all gas exchange equations. Photosynthesis (A), stomatal conductance (g_{st}), and intercellular CO_2 concentration (C_i) were all calculated using the equations of von Caemmerer and Farquhar (1981) corrected for leaf area. For post-herbivory measurements on the 'attacked' leaves within the beetle treatment, eggs or frass were removed, immediately prior to gas exchange measurements, to prevent any respiration interference. Intrinsic water-use efficiency (A/g_{st}) was calculated as A/g_{st}. Data were recorded when A and g_{st} showed less than a ± 2% change over a 3 min period. All gas exchange measurements were conducted between the hours of 11:00 and 15:00.

Leaf greenness was measured using a single photon avalanche diode (SPAD) 502 Plus Chlorophyll Meter (Minolta, Osaka 542, Japan) on the same section of leaf area used for gas exchange assessment. Three random spots, within the leaf area used for gas exchange, were measured and averaged per leaf, both prior and subsequent to herbivory. Numerous authors report strong relationships of SPAD values to leaf chlorophyll/nitrogen content in various plant families and tribes, including the Asteraceae and Heliantheae (Pepo and Novak, 2016).

2.3. Microbial assessments

Microbial assessments carried out on *Z. bicolorata* were adapted from Venter et al. (2013). Adult beetles, larvae, eggs and frass were all sampled from the culture currently maintained at the Wits Insectary. Five adult beetles and five fourth instar larvae were randomly selected from several rearing cages and individually placed in sterile 1.5 ml Eppendorf tubes for assessment. Rearing cages each contained five *P. hysterophorus* plants and approximately 45 to 50 adult beetles. Five additional adult beetles were randomly selected and had their mouthparts (labial palp, mandible, labium, maxillary palp and maxilla) dissected out and placed in sterile Eppendorf tubes. For egg and frass samples, five leaves (fourth fully expanded leaf from apical bud) were selected at random from the rearing cages after exposure to *Z. bicolorata* for five days. Each of the leaves had all newly laid frass and eggs removed, grouped per leaf, and placed in sterile eppendorf tubes. All Eppendorf tubes contained 1 ml of sterile distilled water.

To investigate changes in microbial abundance in relation to beetle herbivory, 10 *P. hysterophorus* plants were randomly selected from 50 plants propagated in the outdoor nursery. The third and fourth fully expanded leaves of five plants were sterilised, using 95% ethanol wipes, and the leaves of the remaining five plants' left unsterilised. All 10 plants had the third and fourth leaves enclosed in sterile mesh bags, with the fourth leaf having two adult *Z. bicolorata* beetles, randomly selected from the rearing culture, enclosed within the mesh bag. After a period of five days, 1 cm^2 leaf samples were collected at random from each of the bagged leaves, from all 10 plants (totalling 20 leaves), and placed individually into sterile Eppendorf tubes each containing 1 ml of sterilised distilled water.

All *Z. bicolorata* and *P. hysterophorus* leaf samples (Eppendorf tubes) were vortexed at 3000 rpm for a period of 60 s. Subsequent to vortexing, two 100 μl aliquots were extracted and pipetted onto nutrient agar (NA) and potato dextrose agar (PDA) plates. NA and PDA were used as growth media given their broad selection for differing fungal and bacterial microbes (Venter et al., 2013). Aqueous extracts were immediately spread across the media using a sterile glass spreader. All vortexing and plating procedures were carried out under sterile conditions. All NA and PDA plates were placed into incubators set at 25 °C and 30 °C respectively, for five days (~120 h).

Plates were then removed and refrigerated (~3 °C), to prevent additional growth of colonies, whilst the numbers of microbial colonies were identified and counted per plate. The identification of microbial colonies to a family or genus of bacteria/fungi was done using morphological characteristics, namely colony shape, size, colouration (pigmentation), elevation and margin. In the case that colonies could not be identified to a family or genus, morphologically similar species were grouped and recorded as a morphospecies. A colony was defined as any visible bacterial or fungal spot/cluster growing on the surface of the NA or PDA mediums. Colony counts, per plate, were multiplied by 10 to standardise colony counts to 1 ml of sample.

2.4. Statistical analysis

Defoliation (leaf area removal) amongst the three herbivory treatments (beetle, simulated and control) was compared by means of a one-way repeated measures ANOVA with a Tukey HSD *post hoc*. Gas exchange parameters of photosynthesis (A), stomatal conductance (g_{st}), intercellular CO_2 concentration (C_i) and water-use efficiency (A/g_{st}) as well as SPAD measures were examined using two-way ANOVAs with a Tukey HSD *post hoc.* Microbial presence was assessed using one-way ANOVAs on the cumulative total of microbe colonies (NA and PDA) for *Z. bicolorata* adults, their mouthparts, larvae, frass and eggs as well as sterilised and unsterilised *P. hysterophorus* leaves exposed and unexposed to *Z. bicolorata* herbivory.

3. Results

3.1. Herbivory and leaf defoliation

Both beetle and simulated herbivory treatments significantly reduced *P. hysterophorus* leaf area by 87.3 and 86.8% respectively ($F_{2,18} = 5.47$ $P < 0.01$; Fig. 1). Mean leaf area remaining after herbivory was 2.01 ± 0.27 cm^2 for the beetle treatment, 2.06 ± 0.31 cm^2 for the simulated treatment and 13.42 ± 0.13 cm^2 for the controls. Feeding by individual adult *Z. bicolorata* beetles removed an average of 1.23 ± 0.1 cm^2 of leaf, per day ($y = -2.4507x + 14.421$; $r^2 = 0.96$). Simulated herbivory leaves mimicked the beetle herbivory effectively, with no significant differences in leaf defoliation throughout the course of the experiment (Fig. 1). No change was noted in the mean leaf area for control plant leaves.

3.2. Gas exchange and leaf nitrogen

Parthenium hysterophorus leaves exposed to *Z. bicolorata* herbivory displayed a significant decline in photosynthesis ($F_{2,18} = 38.71$; $P < 0.001$). Conversely, leaves in the simulated herbivory treatment displayed an up-regulation in photosynthesis (Fig. 2A). Leaves adjacent to *Z. bicolorata* folivory showed a significant up-regulation in photosynthetic output (Fig. 2B). No changes in photosynthesis were observed in the control leaves or the adjacent leaves in the simulated treatment.

Leaves attacked by *Z. bicolorata* displayed a significant decline

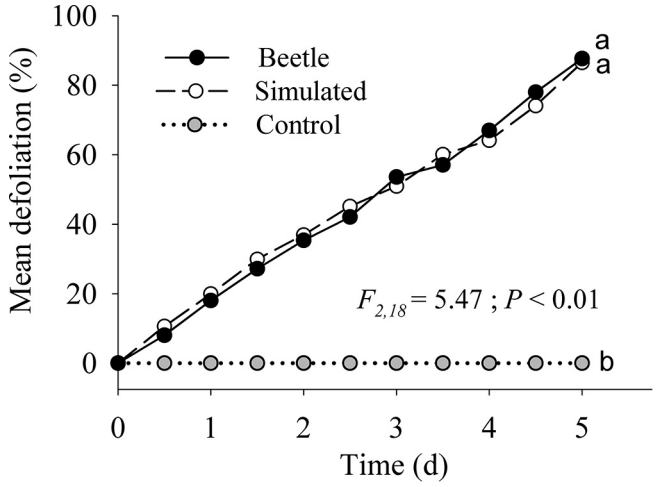

Fig. 1. Mean leaf defoliation (%) over time for *Parthenium hysterophorus* leaves exposed to beetle, simulated and control herbivory treatments ($n = 7$ leaves per data point). F_{stat} and *P*-value indicate overall difference in *P. hysterophorus* defoliation between treatments using a repeated measures ANOVA. Different lowercase letters indicate significant differences between overall treatment defoliation ($P < 0.05$).

($F_{2,18} = 3.91$; $P < 0.05$) in stomatal conductance (g_{st}), whereas simulated herbivory and control leaves showed no change (Fig. 3A). Stomatal conductance increased significantly ($F_{2,18} = 4.17$; $P < 0.05$) for adjacent leaves in both the beetle and simulated herbivory treatments (Fig. 3B). Intercellular CO_2 concentrations (C_i) increased significantly in the beetle and simulated herbivory treatments, for both the 'attacked' and 'adjacent' leaves, whilst controls remained unchanged (Fig. 3C, D). A significant decline in water-use efficiency (A/g_{st}) was found in leaves attacked by *Z. bicolorata*, but not in the simulated herbivory nor control leaves (Fig. 3E). Additionally, significant declines in water-use efficiency of 15 and 21% occurred in the 'adjacent' (undamaged) beetle and simulated leaves respectively (Fig. 3F). Both 'attacked' and 'adjacent' leaves in the beetle and simulated herbivory treatments displayed significant increases in SPAD values, whilst the controls remained unchanged (Fig. 3 G, H).

3.3. Microbial assessments

Microbial assessments on *Z. bicolorata* found that both adult beetles and their larvae carried the highest abundance of microbes, with 5984 ± 543 and 5421 ± 475 colonies ml^{-1} respectively (Fig. 4A). Mean microbe abundance for adult beetle mouthparts (3776 ± 364) and frass (3411 ± 286) was found to be significantly lower than both adult beetles and larvae, but significantly higher than the eggs ($F_{4,20} = 50.17$; $P < 0.001$). Eggs showed the lowest microbial abundance with a mean of 236 ± 83 colonies ml^{-1}.

Mean microbial abundance for sterilised and unsterilised *P. hysterophorus* leaves treated with and without *Z. bicolorata* showed that unsterilised leaves exposed to *Z. bicolorata* had the highest microbial abundance with a mean of 3608 ± 338 colonies ml^{-1}(Fig. 4B). Both sterilised and unsterilised leaves showed significant increases in microbial abundance when exposed to adult *Z. bicolorata* beetles for a period of five days ($F_{3,16} = 89.31$; $P < 0.001$). Lastly, sterilised and unsterilised leaves, which were not exposed to *Z. bicolorata,* had the lowest microbial abundance, with sterilised leaves accumulating the fewest colonies (309 ± 67 colonies ml^{-1}).

A total of 26 916 microbe colonies were recorded growing on NA and PDA plates. Bacterial microbes, from 9 species groups, comprised 72.9% of the total microbes. The majority of these bacteria were recorded on plates with samples from *Z. bicolorata* beetles, their mouthparts, larvae and frass (Table 1). Fungal microbes were classified into 10 species groups and comprised the remaining 27.1% of the total microbes. The highest abundances of these fungi were found on both sterilised and unsterilised *P. hysterophorus* leaves exposed to *Z. bicolorata* beetles (Table 1).

4. Discussion

In addition to the significant removal of *P. hysterophorus* leaf tissue, *Z. bicolorata* folivory reduced the photosynthetic output of attacked leaves by more than 35%. Defoliation frequently results in reduced stomatal conductance (stomatal closure), which in turn diminishes the photosynthetic ability of leaves (Nabity et al., 2009). As expected, adjacent leaves, above the defoliation (beetle), displayed a significant up-regulation in photosynthesis of ~11%. Numerous plants maintain the ability to enhance photosynthesis of neighbouring undamaged leaves in an attempt to counteract the losses incurred by herbivory (Kant et al., 2015). In the case of *P. hysterophorus,* photosynthetic enhancement in adjacent (undamaged) leaves was only a partial compensation and one which is unlikely to overcome continued *Z. bicolorata* herbivory. Simulated herbivory experienced an increase in photosynthesis, suggesting that simulated herbivory (cutting) underrepresents the physiological impact of actual herbivore damage, and more importantly that the indirect effects of folivory are equally, if not more damaging, than removal of leaf tissue itself (Zangerl et al., 2002; Ripley et al., 2008; Venter et al., 2013).

Zygogramma bicolorata folivory metabolically impaired *Parthenium*

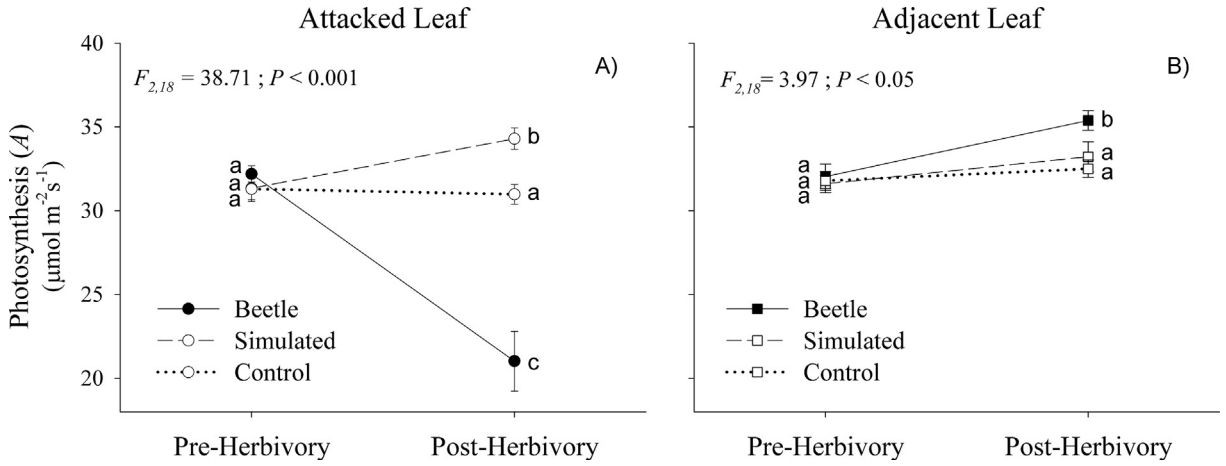

Fig. 2. Mean photosynthesis (± SE) for (A) attacked and (B) adjacent (undamaged) *Parthenium hysterophorus* leaves exposed to beetle, simulated and control herbivory treatments. F_{stat} and *P*-values indicate overall difference in *P. hysterophorus* photosynthesis between treatments using a repeated measures ANOVA. Different lowercase letters indicate significant differences between treatments ($P < 0.05$). ($n = 7$; leaves per data point).

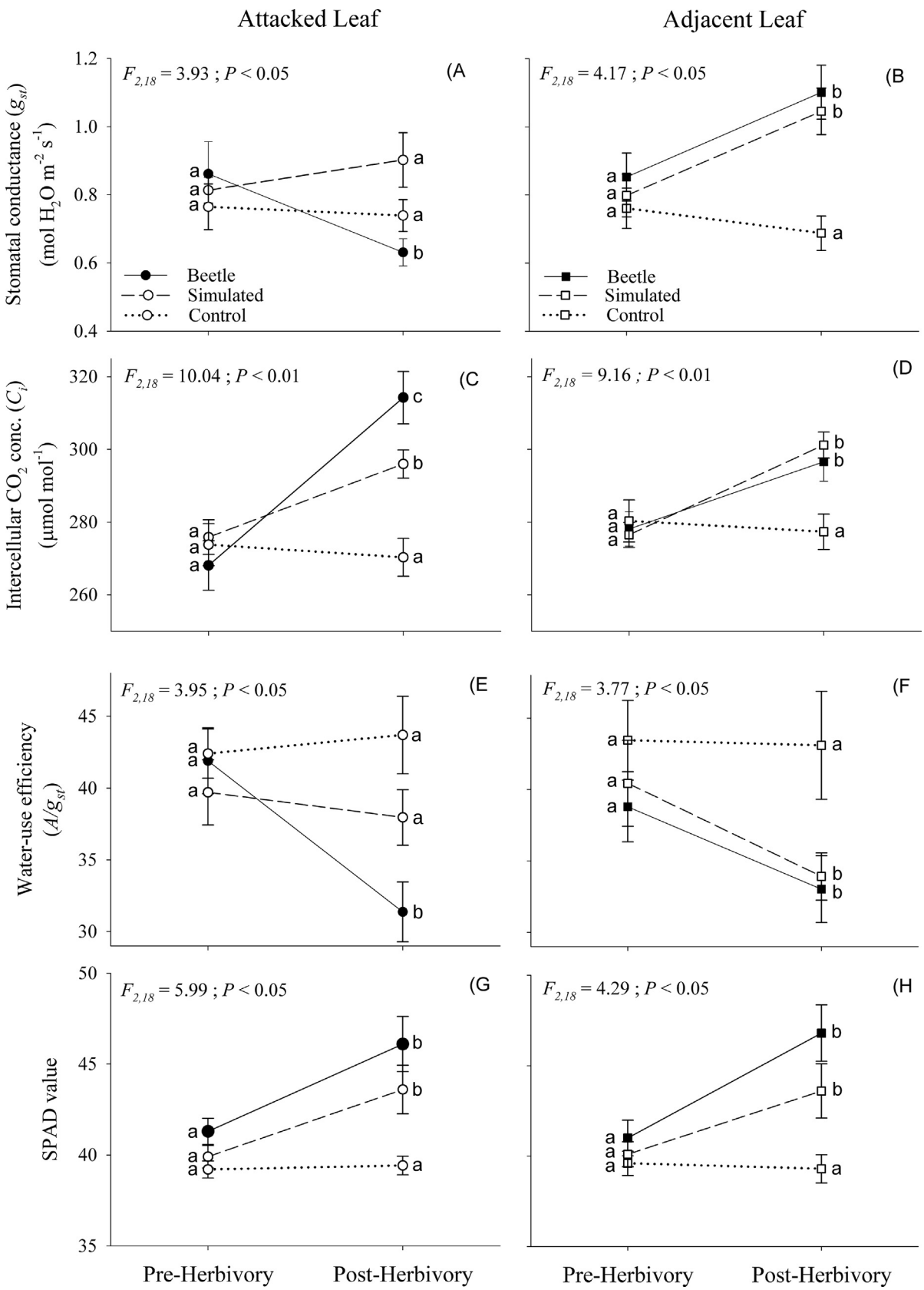

Fig. 3. Mean (± SE) (A,B) stomatal conductance (g_{st}), (C,D) intercellular CO_2 concentration (C_i), (E,F) water-use efficiency (A/g_{st}) and (G,H) SPAD measurements for both attacked and adjacent (undamaged) *Parthenium hysterophorus* leaves exposed to beetle, simulated and control herbivory treatments. F_{stat} and *P*-values indicate overall difference in *P. hysterophorus* gas exchange/SPAD parameters between treatments using a repeated measures ANOVA. Different lowercase letters indicate significant differences between and within (pre- vs. post-herbivory) treatments ($P < 0.05$). ($n = 7$ leaves per data point).

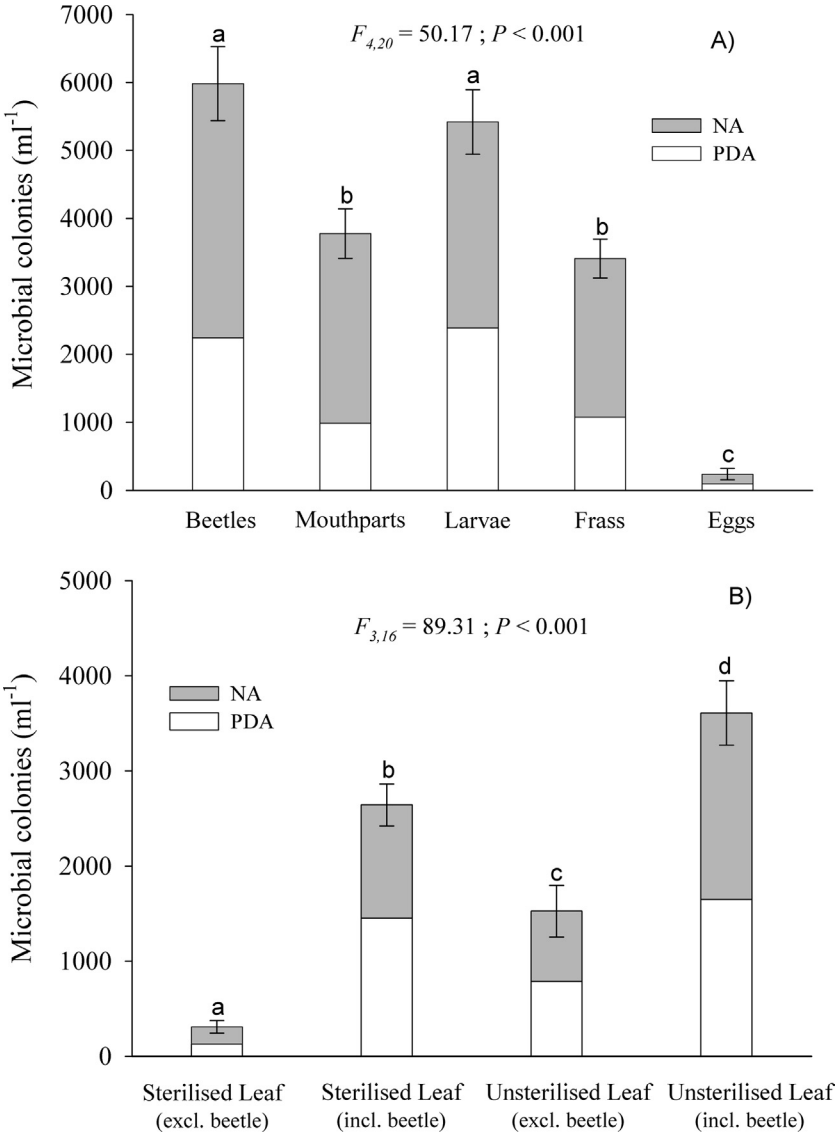

Fig. 4. Mean (± SE) number of microbial (NA + PDA) colonies from A) adult *Zygogramma bicolorata* beetles, their mouthparts, larvae, frass and eggs; and B) from sterilised and unsterilised *Parthenium hysterophorus* leaves exposed and unexposed to *Z. bicolorata* adult herbivory for 5 days. Samples were cultured on nutrient agar (NA) and potato dextrose agar (PDA). F_{stat} and *P*-values indicate overall difference in microbial abundance between treatments using an ANOVA ($n = 5$ per data point).

hysterophorus leaves, as highlighted by increased intercellular CO_2 (C_i) and decreased photosynthetic output (A). This impairment led to alterations in nutrient and water relations of both damaged and adjacent undamaged leaves (Aldea et al., 2005; Delaney et al., 2008). Removal of leaf tissue, even partially, increases evapotranspiration, exacerbating leaf and plant water loss (Aldea et al., 2005). Similar to findings by Dhileepan et al. (2000), severe leaf defoliation by adult *Z. bicolorata* beetles reduced the water-use efficiency (A/g_{st}) of both attacked and adjacent undamaged leaves. Increases in *P. hysterophorus* 'leaf nitrogen/chlorophyll content' (SPAD) occurred in response to *Z. bicolorata* folivory. Plants may frequently re-allocate resources, increasing leaf nutrients in both damaged and adjacent undamaged leaves to minimise the physiological damages due to herbivory (Turnbull et al., 2007; Delaney, 2008; Delaney et al., 2008). The movement and concentration of resources, such as chlorophyll and nitrogen, into leaf tissues is widely known to enhance photosynthesis, as documented in other annuals within the Heliantheae (Panković et al., 2000). This may allow plants to compensate for the losses incurred by herbivory, however, it is ultimately insufficient unless it can completely mitigate the loss (Trumble et al., 1993; Thomson et al., 2003; Wise and Abrahamson, 2005). Furthermore, the reallocation of nutrients into damaged leaves expends additional resources which would otherwise be dedicated towards growth and reproduction (Pratt et al., 2005). In the case of *P. hysterophorus,* increased 'nitrogen' investment not only detracts from plant reserves, increasing the energetic cost of maintaining the leaf, but likely entices further herbivory (Chown and Nicolson, 2004) by insects including *Z. bicolorata*.

Photosynthetic reduction displayed by *Z. bicolorata* damaged leaves, may also be attributed to microbial interactions resulting from herbivory. Leaf-feeding insects, particularly Chrysomelids, enhance the susceptibility of leaves to microbial infection by wounding leaf tissues or suppressing plant responses, via effectors or elicitors present in their saliva (Humphrey et al., 2014; Wielkopolan and Obrępalska-Stęplowska, 2016). Insect folivores harbour various microbial communities, many of which are introduced or spread during and subsequent to their feeding, excretion and locomotion (Mitchell and Hanks, 2009; Sugio et al., 2014). Alternatively, these herbivorised leaves release nutrients which may permit the ingress and proliferation of bacterial or fungal microbes already present on the leaf or from air-, water- or soil-borne origins (Wielkopolan and Obrępalska-Stęplowska, 2016). Thus the high microbial abundance found on *P. hysterophorus* leaves exposed to *Z. bicolorata*, suggests that *Z. bicolorata* may directly, or indirectly, facilitate the increase in leaf microbe abundance. Regardless of whether these microbes are deleterious or non-pathogenic, increased microbial abundance and activity can diminish the photosynthetic capacity of leaves, either via localised necrosis of the leaf and/or by

Table 1
Mean abundance (per ml) of bacterial and fungal microbes cultured on both Nutrient Agar (NA) and Potato Dextrose Agar (PDA). Microbe samples taken from adult beetles, their mouthparts, frass and eggs as well as from sterilised and unsterilised leaves exposed and unexposed to *Z. bicolorata* adult beetles for a period of five days.

Microbe Species	Beetles	Mouthparts	Larvae	Frass	Eggs	Sterilised Leaves		Unsterilised Leaves	
						(excl. beetles)	(incl. beetles)	(excl. beetles)	(incl. beetles)
Bacteria									
Morphospecies A	1788	2144	1482	938	36	68	678	116	892
Morphospecies B	1498	496	1286	378	132	–	284	58	254
Morphospecies C	106	48	14	24	14	6	8	86	46
Morphospecies D	334	210	180	–	4	–	68	–	176
Morphospecies E	256	88	446	154	–	–	46	48	38
Morphospecies F	482	178	1098	596	20	8	88	56	486
Proteus spp.	198	34	178	288	6	–	104	–	192
Pseudomonas spp.	174	2	306	54	4	–	38	4	104
Bacteria Total	4836	3200	4990	2432	216	82	1314	368	2188
Fungi									
Aspergillus spp.	186	94	–	56	–	44	122	68	42
Saccharomycetaceae spp.	132	354	308	344	–	78	516	288	756
Morphospecies A	122	54	–	18	–	–	86	12	82
Morphospecies B	28	–	–	–	8	40	40	122	12
Morphospecies C	346	38	–	378	6	–	86	4	42
Morphospecies D	176	–	–	26	2	–	154	66	144
Mucor spp.	38	–	52	68	–	42	136	32	42
Mycelium spp.	58	–	–	32	–	16	58	130	58
Penicillium spp.	46	28	72	58	4	8	112	434	226
Tricoderma spp.	16	8	–	–	–	–	18	2	16
Fungi Total	1148	576	432	980	20	228	1328	1158	1420
Combined Total	5984	3776	5422	3412	236	310	2642	1526	3608

eliciting plant-immune responses (Berger et al., 2007; Humphrey et al., 2014). The expression of these plant immune responses are beneficial to weed biological control as they suppress photosynthesis and plant growth (Howe and Jander, 2008; Humphrey et al., 2014). The importance of such agent-microbe contributions is being acknowledged in biological control literature, with recent studies showing that microbial interactions may be more damaging than agent herbivory (Ripley et al., 2008; Venter et al., 2013; Sutton et al., 2016).

Leaf-level ecophysiology provides valuable insights towards predicting potential agent-weed interactions at a field level, however, caution should be exercised when up-scaling findings. This research estimates that *Z. bicolorata* incidence in the field would need to defoliate at least a third of *P. hysterophorus* total leaf area continuously, to cause substantial physiological impairment and ensure no photosynthetic compensation occurs. The partial compensation of *P. hysterophorus* to folivory may also be mitigated by adapting agent release strategies for biological control efforts. Extensive releases of *Z. bicolorata*, should be carried out immediately after the onset of spring rains, as this: i) affords *Z. bicolorata* populations time to build-up, increasing agent incidence and subsequent herbivore pressure, and ii) minimises the time available for resource accumulation by developing *P. hysterophorus* plants (Dhileepan et al., 2000). Immature plants, and mature plants which are resource and/or water limited, frequently display poorer compensatory responses and as a result possess a lower tolerance to herbivory (Dhileepan et al., 2000; Wise and Abrahamson, 2005; Suwa and Maherali, 2008). This relationship between herbivory tolerance (compensation) and nutrient availability is an important one and should be explored further, with particular focus on resultant agent performance. Additionally, the ability of *Z. bicolorata* to harbour and potentially introduce microbes may be of further benefit to biological control (Venter et al., 2013; Sutton et al., 2016). Inoculation of these beetles with specific leaf-pathogens, such as the rust fungus *Puccinia xanthii* Schwein. var. *parthenii-hysterophorae* (Pucciniales: Pucciniaceae), prior to release warrants investigation. Although this study focused on the physiological effects of extensive folivory by the most persistent life stage (adult beetles), the effects of lesser folivory damage (%) as well as more thorough identification and culturing of microbial communities associated with *Z. bicolorata / P. hysterophorus*, should be assessed in future research.

In conclusion, *Z. bicolorata* maintains the potential to be a damaging agent despite the ability of *P. hysterophorus* to photosynthetically up-regulate in response to folivory. The physiological implications associated with continued *Z. bicolorata* leaf-feeding, even at low incidences, are unlikely to be overcome by *P. hysterophorus*. These implications, namely photosynthetic reduction, metabolic impairment, decreased water-use and nutrient-use efficiency and increased microbial abundance, are all beneficial towards ongoing biological control efforts, as not only do they increase physiological stress but create and exacerbate resource-loss sinks. These sinks ultimately expend resources that would otherwise be allocated towards growth and reproduction of *P. hysterophorus*. Given the potential effectiveness of *Z. bicolorata* as a biological control agent, efforts should be made to maximise beetle incidence in the field, through mass-rearing and associated implementation activities. More generally, these results may lend partial insight towards the differing levels of *Z. bicolorata* performance and incidence in the field, given the potential compensatory ability of *P. hysterophorus.*

Author contributions

BWC, ETFW, MJB, LWS, JMG and NV conceived and designed the experiments. BWC and NV performed the experiments. BWC, ETFW, MJB, LWS, JMG and NV analysed the data. MJB, ETFW, LWS, JMG and NV provided critical input into data and statistical analyses. BWC wrote the manuscript.

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CHAPTER 6:

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Does host plant quality constrain the performance of the Parthenium beetle *Zygogramma bicolorata*?

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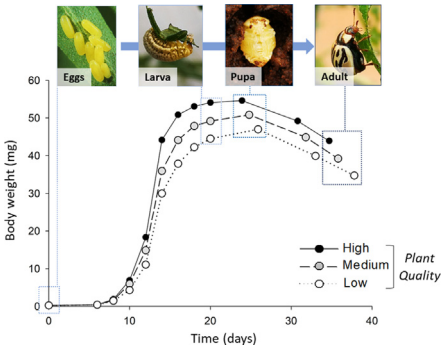
GRAPHICAL ABSTRACT



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ABSTRACT

In South Africa, the leaf-feeding beetle, *Zygogramma bicolorata* Pallister (Coleoptera: Chyrsomelidae), was released in 2013 against the invasive annual herb *Parthenium hysterophorus* L. (Asteraceae: Heliantheae). Poor field establishment and variable incidence of the beetle, during the first few years after release, have led to concerns surrounding potential constraints, including host plant quality. This study assessed the influence of high, medium and low host plant quality, as influenced by fertiliser application, on the survival, development and fecundity of *Z. bicolorata*. Although egg to adult survival was high (~80%) and did not differ between plant quality treatments, larvae developed fastest on plants of high and medium quality. Larval feeding was highly damaging, reducing the growth, reproduction and biomass of *P. hysterophorus* in all three plant quality treatments. Fecundity was associated with plant quality, with larger, more fecund females developing on higher quality plants. Overall, this research may aid current mass-rearing efforts, further field research to verify these findings and direct the selection of more suitable release sites for *Z. bicolorata* in South Africa.

1. Introduction

The annual herb, *Parthenium hysterophorus* L. (Asteraceae: Heliantheae), is a noxious invasive plant and recognised as one of the world's most threatening terrestrial weeds (Adkins and Shabbir, 2014). Originating from Central and South America, specifically regions

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surrounding the Gulf of Mexico, *P. hysterophorus* is now prevalent in at least 92 countries in the tropics and sub-tropics, of which at least 46 are beyond its natural distribution (Dhileepan and Strathie, 2009; McConnachie et al., 2010; Shabbir et al., 2019). In South Africa, *P. hysterophorus* has invaded large swathes of land in the KwaZulu-Natal, Mpumalanga, North West and Limpopo Provinces for several decades and continues to expand its range (Terblanche et al., 2016). *Parthenium hysterophorus* readily invades a wide variety of habitats, ranging from high fertility agricultural lands to degraded and disturbed low nutrient systems (Henderson, 2001). Plants mature rapidly (in approximately six weeks), producing as many as 30 000 seeds per plant, from which extensive, long-lasting seedbanks are established (Navie et al., 2004). The weed is generally unpalatable to wild and domestic herbivores and displays high levels of allelopathy, allowing it to form dense stands which outcompete and displace native species (Adkins and Shabbir, 2014). Major concerns surround the negative socio-economic impacts of *P. hysterophorus* invasions in agricultural, subsistence, communal rangeland, conservation and human habitation settings (Strathie et al., 2011).

Recognising these threats, Africa's first biological control programme against *P. hysterophorus* was initiated in South Africa during 2003, with a total of four biocontrol agents having been released since then. Among these was the leaf-feeding beetle *Zygogramma bicolorata* Pallister (Coleoptera: Chrysomelidae), first released in 2013 (McConnachie, 2015). Releases of *Z. bicolorata* were highly anticipated in South Africa, given substantial damage displayed by the agent in regions of India and Australia (Dhileepan and Strathie, 2009; Dhileepan and Wilmot Senaratne, 2009; Strathie et al., 2011). As adults, the beetles may survive for up to two years, producing as many as four generations in a single summer season (Dhileepan et al. 2000a). Females are highly fecund, laying as many as 900 eggs, singly or in small batches, on the leaves, stems and flowers of *P. hysterophorus* (McClay, 1985; Strathie et al., 2011). Eggs hatch within about five days, with larvae feeding voraciously on leaves as they develop through four larval instars prior to pupation. Pupation occurs in the soil, lasting approximately two weeks, after which adult beetles emerge and begin feeding and copulation (Dhileepan and McFayden, 2012; Dhileepan et al., 2000a). During autumn, adult beetles enter a soil diapause in response to decreasing rainfall, temperature and food availability, which persists through winter (Dhileepan and McFayden, 2012). These beetles then re-emerge during mid-spring, typically three to four weeks after *P. hysterophorus* has begun germinating, and resume feeding (Abels, 2018).

Multiple releases of *Z. bicolorata* in South Africa have been undertaken annually since 2013, at numerous sites throughout the most densely invaded areas within KwaZulu-Natal and Mpumalanga provinces (Strathie et al., 2016a). Despite extensive infestations of *P. hysterophorus* and promising initial persistence of *Z. bicolorata* at release sites, < 13% of selected release sites surveyed were found to have established populations of the beetles in 2016 (Strathie et al., 2016b). Furthermore, sites at which *Z. bicolorata* had established, showed high variability in beetle incidence, although some damaging outbreaks were recorded (Chidawanyika et al., 2017). Poor establishment and variable incidence at release sites has prompted concerns surrounding abiotic and biotic constraints on the beetle's efficacy in the field (Strathie et al., 2016b). Initial studies by King (2008) indicated that *Z. bicolorata* would be well suited to South Africa's climate, and that establishment and development of the beetle was unlikely to be climatically constrained. Although egg and larval predation by generalist predators is suspected to hinder *Z. bicolorata*, these are unlikely to entirely prevent the establishment of the beetle (Abels, 2018). Research by Chidawanyika et al. (2017) demonstrated that *Z. bicolorata* may be more susceptible to thermal stress in the field, particularly in beetles emerging from larvae developing on poorer-quality *P. hysterophorus* plants. This suggests that bottom-up drivers, such as host plant quality, may influence the establishment, abundance and effectiveness of *Z. bicolorata* in South Africa.

The availability of high-quality plant resources is a factor often overlooked in weed biocontrol programmes, yet it may have considerable effects on agent performance (Price, 2000; Manrique et al., 2009; Bownes et al., 2013). Host plant quality typically refers to the relative nutritional value of a plant, particularly its nitrogen status (Chown and Nicolson, 2004; Chown and Gaston, 2010). Linked to this is the 'Plant Vigor Hypothesis', which proposes that more vigorous (nutritious) plants are likely to benefit the development of their insect herbivores (Price, 2000; Stiling and Moon, 2005). Plant quality is a key determinant in the development and survival of both neonate and mature phytophagous larvae (Awmack and Leather, 2002; Heisswolf et al., 2005), with increased survival and shortened development times documented on plants of higher quality (Moreau et al., 2006). Larval food quality also influences the size that adult individuals may attain which has consequences for their subsequent reproductive success (Chown and Gaston, 2010; Van Hezewijk et al., 2008). Fecundity is strongly related to larval host plant quality, with females reared on high quality plants being larger in size, and as a result potentially more fecund than females reared on poorer quality plants (Awmack and Leather, 2002). At a population level, plant quality influences insect-plant interactions and may regulate insect abundance, particularly in specialised or host-specific species (Cotesero et al., 2000; Van Hezewijk et al., 2008).

Given the threats posed by *P. hysterophorus* to biodiversity, food security as well as human and animal health, it is imperative that potential constraints to ongoing biocontrol efforts be investigated and agent efficacy is optimised. Variations in plant quality of *P. hysterophorus*, both spatially and temporally, may impact the establishment and performance of *Z. bicolorata* in the field. Therefore, this study aimed to assess the influence of *P. hysterophorus* plant quality on the survival, development and fecundity of the leaf-feeding beetle *Z. bicolorata*. Additionally, larval feeding damage to the growth, reproduction and biomass of *P. hysterophorus* was examined under high, medium and low plant qualities as influenced by applications of nutrients.

2. Materials and methods

All experiments took place within a temperature-controlled glasshouse section of the insect rearing facility (insectary) at the University of the Witwatersrand, Johannesburg. The glasshouse receives full sunlight (~1800 μmol^{-1}: P.A.R) and follows ambient day-night light patterns. Temperatures were set and maintained at 25 ± 2.2 °C during the day (12 h) and 20 ± 1.3 °C at night (12 h), with relative humidity (RH) maintained between 60 and 70%.

Zygogramma bicolorata individuals used in the study were obtained from ~300 eggs supplied from a laboratory-reared culture maintained by the Agricultural Research Council in their Cedara laboratories at Hilton, KwaZulu-Natal. The culture was originally imported from Queensland, Australia in 2005 (McConnachie, 2015). Freshly laid eggs were divided into batches and placed in Petri dishes (~50 eggs per dish) containing sterilised moistened filter paper. The Petri dishes were stored in the insectary glasshouse but kept out of direct sunlight.

2.1. *Parthenium hysterophorus* growth and *Zygogramma bicolorata* inoculation

Sixty *P. hysterophorus* plants were grown from seed collected in the field during July 2017. Plants were grown in 20 cm pots, filled with a nutrient poor loam soil mixture (15% clay: 25% silt: 60% sand) in full sunlight. Upon germination, plants were randomly split into three fertilisation treatments, namely 'high', 'medium' and 'low' (totalling 20 plants per treatment). Plants were watered daily and fertilised using Seagrow® organic fertiliser (N – 53 g/kg, P – 7 g/kg, K – 17 g/kg). Seagrow® fertiliser was mixed with water at concentrations of 5 ml/ℓ, 2.5 ml/ℓ and 0.25 ml/ℓ for the 'high', 'medium' and 'low' treatments,

respectively. Within each of the nutrient treatments, plants received 100 ml of high, medium or low fertiliser mix applied, using a beaker, directly to the soil of each pot every two weeks. These applications were carried out to encompass a spectrum of *P. hysterophorus* nutrient qualities, ranging from low and moderate fertility systems to relatively high nutrient (fertilised) agricultural areas.

Six weeks after germination, *P. hysterophorus* plants within each of the three nutrient treatments were equally sub-divided into 10 control and 10 beetle treatments. All plants were caged in large mesh cages (0.5 m × 0.5 m × 1.0 m in height) with five plants per cage and four cages per nutrient treatment (i.e. two control cages and two beetle cages). Each of the beetle cages had all plants inoculated with newly-hatched (1st instar) *Z. bicolorata* larvae and set apart within the cages, using clear perspex dividers, to prevent plants from touching and any larval cross-exchange. Control plants were caged and set apart but were not exposed to herbivory by *Z. bicolorata*. To standardise herbivory levels and prevent resource limitation, each of the beetle-treated plants, in the high, medium and low nutrient treatments, received a single larva for every three cm^2 of leaf area calculated at the start of week six (see Equation (1); $n = 100$; $R^2 = 0.95$; Cowie unpublished data), which coincided with egg hatch and larval inoculation. Based on the total *P. hysterophorus* leaf area calculated at week six, the high, medium and low beetle treatments received a total of 42 (4.2 larvae/plant), 33 (3.3 larvae/plant) and 23 (2.3 larvae/plant) neonate larvae, respectively.

$$Leaf\ Area\ (cm^2) = \sum (0.4104[L_1 \times W_1]) + (0.4104[L_2 \times W_2]) + \cdots (0.4104[L_n \times W_n]) \quad (1)$$

L = leaf length (cm); W = leaf width (cm); n = total leaves/plant.

2.2. Development and survival of Zygogramma bicolorata

After inoculation, larval development was assessed daily, with instars classified by head capsule width, with 1st instars = 0.41 ± 0.001 mm, 2nd instars = 0.65 ± 0.016 mm, 3rd instars = 0.98 ± 0.017 mm and 4th (final) instars = 1.44 ± 0.012 mm (Cowie unpublished data). Head capsule widths were photographed under a light microscope (100x magnification) and measured using ImageJ (Ver. 1.5, Wayne Rasband, National Institute of Health, USA). All larvae were weighed (g), using a five-place balance (KERN ABT 100), on alternating days. Developing larvae were collected, using a fine paintbrush, from each plant, weighed and returned to the same plant. Larval body weight measures were concluded when larvae reached their final (4th) instar in each of the treatments. Pre-pupal weights were taken in each of the treatments when 4th instars were observed to attempt burrowing.

Larval burrows were marked using small coloured pins, so pupae could be located easily for later exhumation. Pupae were carefully exhumed from their soil chambers and weighed seven days after the mean first burrowing date of larvae in each of the high, medium and low plant quality treatments. Pupae were then returned to their respective soil chambers and allowed to complete pupation. Newly emerged adult beetles were collected, weighed and measured (total length, width and height – using digital callipers (mm)). All adults collected, from the high, medium and low nutrient treatments, were placed into a single mesh cage (0.5 m × 0.5 m × 1.0 m) containing either three high, medium or low-quality *P. hysterophorus* plants (~7 weeks old), respectively. Pupae/adults which failed to emerge more than a week after the first adults emerged were exhumed and assessed for mortality/survival. Beetle survival to adulthood (%) was calculated as the total number of adult beetles that emerged / total number of larvae inoculated for each of the high, medium and low-quality plants. Once all adults had emerged from their respective treatments, they were used for fecundity assessments (see Section 2.4).

2.3. The effects of larval feeding on Parthenium hysterophorus

To assess the effects of *Z. bicolorata* larval feeding on *P. hysterophorus*, plants in all treatments/cages were measured weekly for a period of six weeks (starting at week 6). Plant height (cm), leaf number, leaf area (cm^2) and flower and seed production were assessed. In addition to morphological and reproductive plant parameters, leaf nitrogen status and chlorophyll content were measured. Leaf nitrogen status was measured, on all leaves, using a single photon avalanche diode (SPAD 502 Plus) (Minolta, Osaka 542, Japan) on two spots per leaf, for all leaves present on the plant. SPAD is frequently used in studies, including the Asteraceae and Heliantheae, as a non-destructive measure of leaf nutrient / nitrogen status (Costa et al., 2001). Similarly, chlorophyll content for all leaves, per plant, was measured using a CCM-300 Chlorophyll Content Meter (Opti-Sciences) on two random spots per leaf.

At the end of the six week period, all plants were harvested for biomass. Each plant was carefully uprooted and washed to remove soil and debris, particularly from the roots. Plants were then sectioned into roots, stems (including floral material) and leaves, and oven dried at 70 °C for five days. Dry biomass for each plant component was then weighed, to three decimal places (g), and recorded. Leaf, stem and root biomass (dry weight) were summed to attain total biomass per plant.

2.4. Zygogramma bicolorata body metrics and fecundity assessments

Beetle surface area was used as a proxy for overall adult body size and was calculated using the formula for an ellipsoid (Eq. (2)). All adult beetles from each of the treatments were then sexed according to the shape of their last abdominal segment (ventrite), with males presenting a narrowed and serrated tip, and females a smooth and entire margin (McClay 1980). Sex ratios were calculated as the proportion of total emerged males / total emerged beetles (see Hasan and Ansari, 2015) in each of the high, medium and low treatments.

$$Beetle\ Area\ (mm^2) = 4\pi\left(\frac{(L \times W)^{1.6} + (L \times H)^{1.6} + (W \times H)^{1.6}}{3}\right)^{0.625} \quad (2)$$

L = half beetle length (mm); W = half beetle width (mm); H = half beetle height (mm).

After sexing, each of the high, medium and low-quality cages had eight female and eight male beetles selected for fecundity analyses. Each of the beetle batches were placed into a single new mesh cage (0.5 m × 0.5 m × 1.0 m) containing either eight high, medium or low-quality *P. hysterophorus* plants (leafy ~7 weeks of age). Beetles were monitored daily for copulation in each of the cages and copulating pairs were transferred onto a single *P. hysterophorus* leaf contained in a large, fine-mesh bag, to allow mating and track initiation of egg laying. Females were monitored daily until initial oviposition which was then recorded as the time from mean adult emergence to the date of first observed egg lay. Once all females within each of the treatment cages were observed to have initiated laying eggs, the mesh bags were removed. All eggs laid per plant, were then collected, using a fine paintbrush, from the high, medium and low-quality cages and counted each week for a period of four weeks. Neonate larvae present on the plants during weekly egg collections were removed to limit defoliation but were included in the egg counts. Eggs found on cage walls were included in egg counts of the nearest plant.

From the eggs collected each week in the high, medium and low cages, 20 were sub-sampled and placed into two Petri dishes (10 eggs per dish) to assess egg hatch (%). Additionally, seven newly laid (yellow) eggs per week were selected from each of the cages and weighed (g), to five decimal places, to investigate the influence of plant quality on egg weight.

2.5. Statistical analysis

Zygogramma bicolorata body weight, developmental time and survival were each analysed using Linear mixed effects regression (LMER) within the 'lme4' and 'nlme' packages in R (R Core Team, 2018). To generate F_{stats} and *p-values*, LMERs were assessed using an ANOVA along with a holm adjusted Tukey multiple comparisons test, within the 'car' and 'multcomp' R packages, respectively. Beetle weight was set as the response variable with nutrient treatment as the fixed effect, time set as a continuous predictor and plant number nested within cage set as the random effect. LMER's for both *Z. bicolorata* developmental time and survival followed the same format but did not incorporate time as a continuous predictor.

The influence of *Z. bicolorata* larval feeding damage on *P. hysterophorus* height, leaf number, leaf area, seed production, nitrogen (SPAD) and chlorophyll content were each examined using LMER along with an ANOVA and a Tukey multiple comparisons test. LMER's had height, leaf number, leaf area, seed production, SPAD or chlorophyll set as the response variable with nutrient-herbivory treatment set as the fixed effect, time as a continuous predictor and plant number nested within cage set as a random effect. Total plant biomass was similarly assessed using LMER with biomass set as the response variable, nutrient-herbivory treatment set as the fixed effect and plant number nested within cage set as the random effect.

The effects of plant quality on adult *Z. bicolorata* body size as well as male and female weight were assessed using LMER with body size / weight set as the response variable, nutrient treatment set as the fixed effect and plant number nested within cage set as the random effect. A Pearson's Chi-square test (χ^2) was used to assess the sex ratios of emerging *Z. bicolorata* adults in relation to plant quality treatment. Female fecundity parameters, namely oviposition, eggs laid per plant, and egg weight were all assessed using LMERs with oviposition, eggs laid, and egg weight set as response variables, nutrient treatment set as the fixed effect, time as a continuous predictor (excluding oviposition) and plant number set as the random effect. Lastly, to assess the effects of nutrients on the egg hatch (%), a repeated measures ANOVA with a Tukey HSD *post-hoc* was used.

3. Results

3.1. Development and survival of Zygogramma bicolorata

Overall, *Z. bicolorata* survival, from egg to adult, was relatively high (~80%), and did not differ significantly ($F_{2,18} = 0.3$; $P > 0.05$) between the high (83.3 ± 4.7%), medium (81.7 ± 5.6%) and low (78.4 ± 7.1%) plant quality treatments (Table 1). Larvae successfully progressed through all four larval instars and the pupal stage on the high, medium and low-quality *P. hysterophorus* plants. However, individuals reared on low quality plants spent significantly longer periods as 4th instars and pupae (Table 1). Mean *Z. bicolorata* development time from egg to adult differed significantly ($F_{2,70} = 60.5$; $P < 0.001$), with larvae reared on high (34.6 ± 1.2 days) quality plants developing fastest, followed by larvae reared on medium (35.5 ± 1.3 days) quality and lastly by those reared on low (37.9 ± 1.5 days) quality plants (Table 1; Fig. 1). Larvae reared on high quality plants produced the heaviest adult beetles (43.9 ± 2 mg) followed by those reared on medium (39.2 ± 1.5 mg) and lastly by those reared on low quality (34.7 ± 1.4 mg) plants ($F_{2,1047} = 221.9$; $P < 0.001$; Fig. 1).

3.2. The effects of larval feeding on Parthenium hysterophorus

Zygogramma bicolorata larval feeding significantly reduced the height ($F_{5,19} = 44.1$; $P < 0.001$) of *P. hysterophorus* in only the high and medium quality treatments by 27% and 24%, respectively (Fig. 2A). Similarly, *Z. bicolorata* reduced the leaf production ($F_{5,19} = 86.6$; $P < 0.001$) of high and medium quality *P. hysterophorus* by 26% and 34% (Fig. 2B). *Parthenium hysterophorus* suffered significant reductions in leaf area ($F_{5,19} = 49.7$; $P < 0.001$), arising from larval feeding, across all three plant quality treatments, with the greatest reduction occurring in the low nutrient treatment (66%) (Fig. 2C). Larval feeding was found to significantly reduce the reproductive output (seeds) ($F_{5,18} = 13.9$; $P < 0.001$) of *P. hysterophorus* plants in the high, medium and low nutrient treatments by 61, 64 and 68% respectively (Fig. 2D).

Leaf nitrogen (SPAD) and chlorophyll content differed between the plant quality treatments, but not between beetle and control plants, with the high-quality plants displaying the greatest leaf nitrogen (SPAD) (39.5 ± 0.9 units) and chlorophyll contents (469 ± 20.2 mg.m^2) (Fig. 3A-B). *Zygogramma bicolorata* larvae significantly reduced the total biomass of *P. hysterophorus* in all three plant quality treatments ($F_{5,13} = 29.1$; $P < 0.001$, Fig. 4). Biomass reductions were inversely proportional to plant quality, with lowest quality plants experiencing the greatest reduction in biomass (38.2%), followed by the medium (28.1%) and lastly the high-quality plants (24%) (Fig. 4).

Table 1
Mean ± SE (*n*) larval and pupal development time (days) and survival for *Zygogramma bicolorata* reared on *Parthenium hysterophorus* plants of high, medium and low quality. Different lower-case letters indicate significant differences between *Z. bicolorata* development stages, overall development time and survival on plants of differing qualities ($P < 0.05$).

	Plant Quality		
Development stage (days)	High (*n*)	Medium (*n*)	Low (*n*)
Egg	5.9 ± 0.3^a (42)	5.9 ± 0.3^a (33)	5.9 ± 0.3^a (23)
1st Instar	5.7 ± 0.4^a (42)	5.7 ± 0.4^a (33)	6.0 ± 0.6^a (22)
2nd Instar	4.1 ± 0.5^a (41)	4.2 ± 0.6^a (31)	4.6 ± 0.6^a (21)
3rd Instar	2.8 ± 0.4^a (39)	3.0 ± 0.3^a (31)	3.3 ± 0.5^a (20)
4th Instar	4.0 ± 0.5^a (38)	4.2 ± 0.5^a (30)	4.7 ± 0.7^b (20)
Pupa	12.1 ± 0.4^a (37)	12.5 ± 0.4^a (29)	13.4 ± 0.5^b (18)
Overall (n)			
Development time (days)	34.6 ± 1.2^a (35)	35.5 ± 1.3^b (27)	37.9 ± 1.5^c (18)
Survival (%)	83.3 ± 4.7^a (35)	81.7 ± 5.6^a (27)	78.4 ± 7.1^a (18)

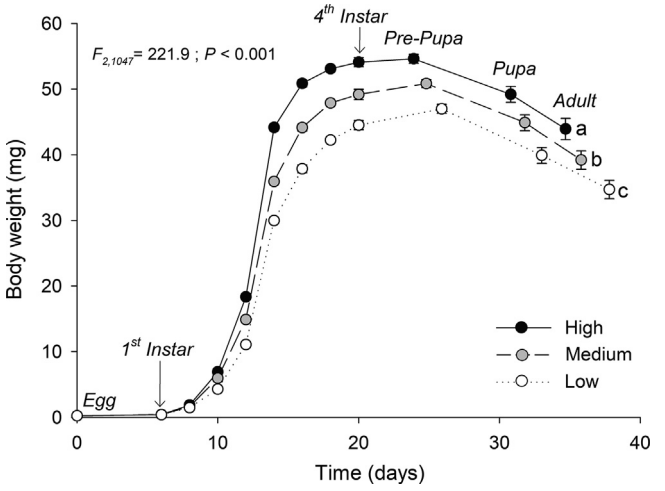

Fig. 1. Mean (± SE) body weight of developing *Zygogramma bicolorata* eggs through to emerging adults reared on high, medium and low-quality treatments. F_{stat} and *P-values* indicate overall difference in *Z. bicolorata* body weights between plant quality treatments using a linear mixed model. Different lowercase letters indicate significant differences between treatment body weights ($P < 0.05$).

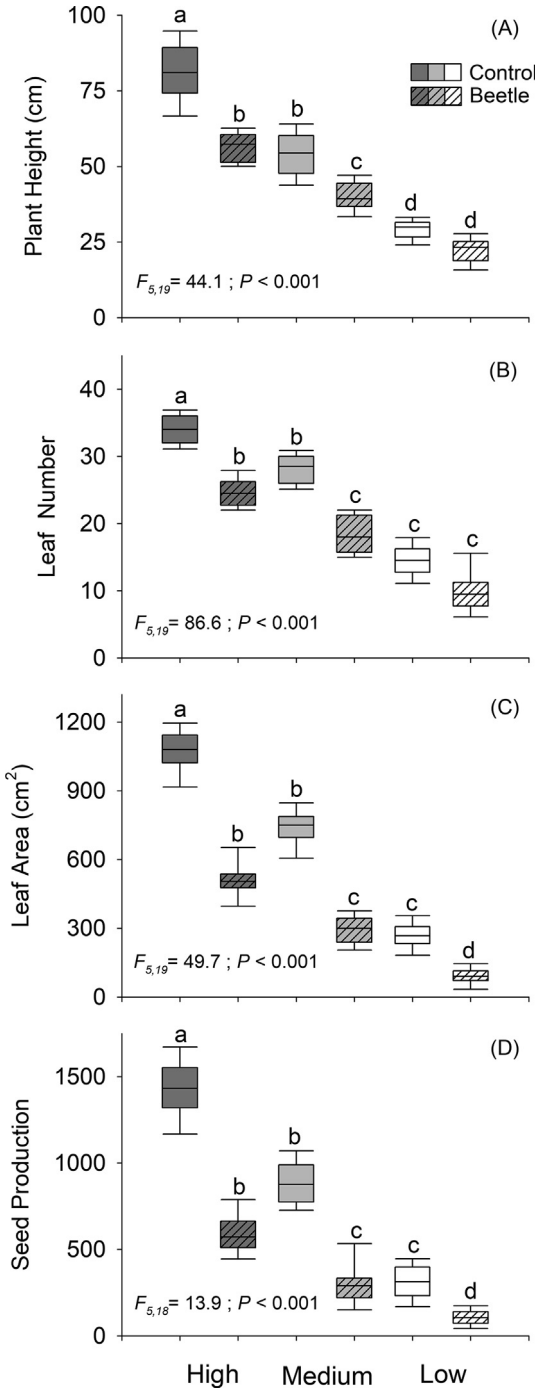

Fig. 2. *Parthenium hysterophorus* (A) height, (B) number of leaves, (C) leaf area and (D) seed production for high, medium and low quality plants after six weeks of herbivory (beetle) or no-herbivory (control) by *Zygogramma bicolorata* larvae. F_{stat} and *P-values* indicate overall difference in *P. hysterophorus* parameters between treatments using linear mixed models. Boxplots show median, 10th, 25th, 75th and 90th percentiles, and differing lowercase letters indicate significant differences between treatments ($P < 0.05$).

3.3. Zygogramma bicolorata body metrics and fecundity assessments

Zygogramma bicolorata reared on high quality plants emerged as significantly larger adult beetles (70.4 ± 2.5 mm^2) when compared to beetles reared on low quality plants (63.2 ± 2.3 mm^2) ($F_{2,69} = 21.6$; $P < 0.001$). Emerging males were also found to be significantly heavier as plant quality increased ($F_{2,28} = 25.4$; $P < 0.001$), whereas the heaviest females developed on high and medium quality plants

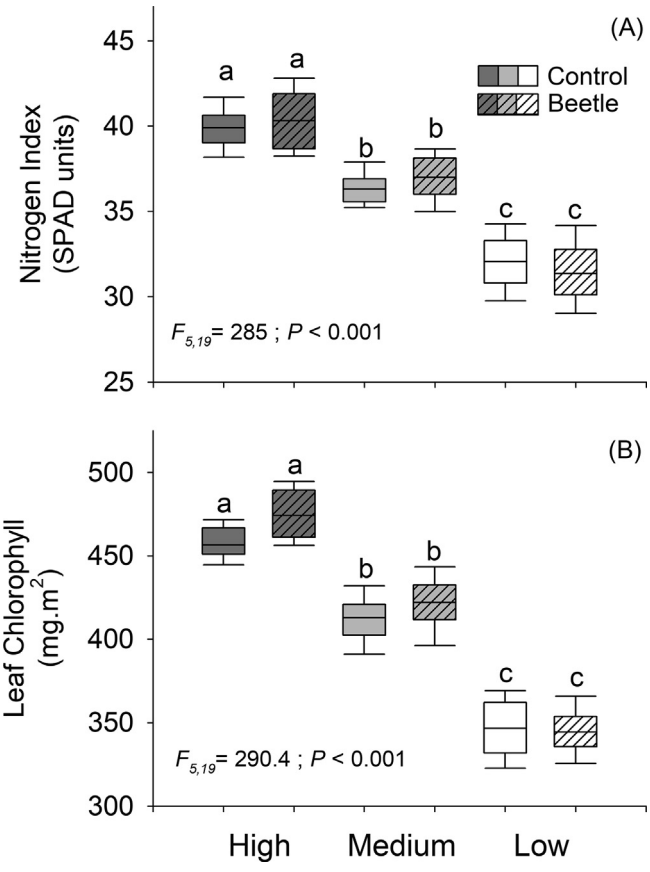

Fig. 3. *Parthenium hysterophorus* (A) leaf nitrogen (SPAD) and (B) chlorophyll context for high, medium and low quality plants after six weeks of herbivory (beetle) or no herbivory (control) by *Zygogramma bicolorata* larvae. F_{stat} and *P-values* indicate overall difference in *P. hysterophorus* leaf nitrogen / chlorophyll between treatments using linear mixed model. Boxplots show median, 10th, 25th, 75th and 90th percentiles, and differing lowercase letters indicate significant differences between treatments ($P < 0.05$).

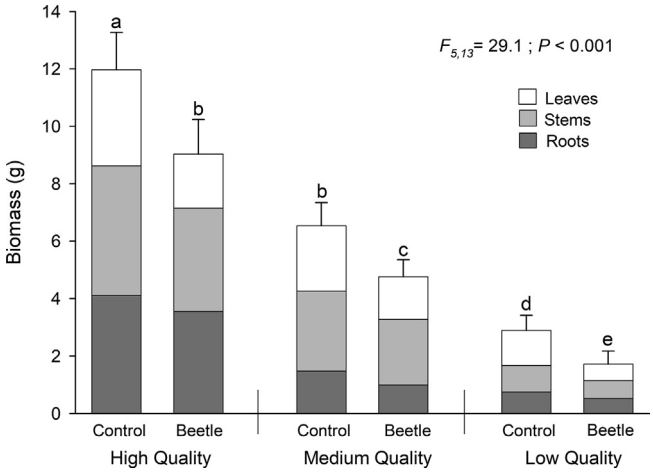

Fig. 4. Mean (± SE) biomass (leaves, stems and roots) for high, medium and low quality *Parthenium hysterophorus* plants exposed (beetle) and unexposed (control) to *Zygogramma bicolorata* larvae. F_{stat} and *P-values* indicate overall difference in total *P. hysterophorus* biomass between treatments using linear mixed model. Different lowercase letters indicate significant differences between treatments ($P < 0.05$).

($F_{2,35} = 19.5$; $P < 0.001$). Sex ratios did not differ between any of the treatments ($\chi^2_2 = 0.02$; $P > 0.05$), but females were more prevalent than males, with an average ratio of 1:1.2 male to females (Table 2).

Table 2
Life history parameters of *Zygogramma bicolorata* (mean ± SE (*n*)) reared on high, medium and low quality *Parthenium hysterophorus* plants. Different lower-case letters per row indicate significant differences between *Z. bicolorata* parameters ($P < 0.05$). Initial oviposition reflects time from mean adult emergence to the date of first egg lay.

	Plant Quality			
Beetle metrics	High (*n*)	Medium (*n*)	Low (*n*)	*LMER/ANOVA/χ^2*
Body size (area mm^2)	70.4 ± 2.5^a (35)	66.4 ± 1.9^b (27)	63.2 ± 2.3^c (18)	$F_{2,69} = 21.6; P < 0.001$
Male weight (mg)	39.7 ± 1.2^a (16)	37.1 ± 0.8^b (12)	32.2 ± 1.0^c (8)	$F_{2,28} = 25.4; P < 0.001$
Female weight (mg)	46.9 ± 1.8^a (19)	43.5 ± 1.6^b (15)	38.3 ± 1.2^c (10)	$F_{2,35} = 19.5; P < 0.001$
Sex ratio (♂:♀)	0.46 (16:19)^a	0.44 (12:15)^a	0.44 (8:10)^a	$\chi^2_2 = 0.02; P > 0.05$
Fecundity parameters				
Initial oviposition (days)	13.5 ± 0.5^b (8)	14.3 ± 0.5^{ab} (8)	15.4 ± 0.6^a (8)	$F_{2,14} = 8.3; P < 0.01$
Eggs laid (per plant)	102 ± 7^a (32)	89 ± 8^{ab} (32)	75 ± 8^b (32)	$F_{2,83} = 22.4; P < 0.001$
Egg weight (mg)	0.25 ± 0.01^a (28)	0.24 ± 0.01^{ab} (28)	0.23 ± 0.01^b (28)	$F_{2,72} = 8.2; P < 0.01$
Egg hatch (%)	68.8 ± 4.0^a (8)	70.0 ± 5.1^a (8)	66.3 ± 5.3^a (8)	$F_{2,21} = 0.1; P > 0.05$

Females from high quality *P. hysterophorus* plants began oviposition significantly earlier than females reared on low quality plants ($F_{2,14} = 8.3; P < 0.01$; Table 2). Additionally, female beetles reared on high quality plants laid significantly more ($F_{2,83} = 22.4; P < 0.001$) and significantly heavier ($F_{2,72} = 8.2; P < 0.01$) eggs than females from the low-quality treatment over a four-week period (Table 2). No difference in egg hatch (%) was found between plant quality treatments ($F_{2,21} = 0.1; P > 0.05$), with an average hatch of ~70% (Table 2).

4. Discussion

This research demonstrated that the development of *Z. bicolorata* is strongly influenced by *P. hysterophorus* plant quality. The prolonged larval development time of larvae reared on low quality plants is a common response to sub-optimal quality plants displayed by many phytophagous insects, particularly amongst the chrysomelids (Obermaier and Zwölfer, 1999). Larvae may compensate for lower quality diets through increased consumption and digestion of poorer quality resources (Wheeler, 2001; Cornelissen and Stiling, 2006). This was evidenced by the feeding of *Z. bicolorata* larvae in the low nutrient treatment as well as their slowed accumulation of body mass and extended time periods spent as instars. Promisingly, no difference in larval mortality was found between the high, medium and low plant quality treatments, suggesting that *Z. bicolorata* is tolerant of a wide range of host plant qualities. This tolerance maintains important implications for the feeding behaviour of phytophagous insects (Obermaier and Zwölfer, 1999; Wetzel et al., 2016), and in a biological control context may be reflected by increases or decreases in damage exerted on target weeds (Heard and Winterton, 2000).

The reduced growth and reproduction of *P. hysterophorus* caused by developing larvae in all three plant-quality treatments reaffirmed the potential effectiveness of the agent. Continued feeding by *Z. bicolorata* larvae on leaves and apical meristems is known to reduce the plant height, leaf production, leaf area and biomass of *P. hysterophorus* (Dhileepan et al., 2000a,b). Whereas reductions in flowering and subsequent seed production are likely to have resulted from the indirect effects of herbivory, namely plant resource depletion and re-allocation (Dhileepan et al., 2000b). The greatest reduction in biomass on *P. hysterophorus* plants of lower quality may be explained by the 'Plant Vigor Hypothesis', in which plants of higher quality readily re-allocate additional resources towards mitigating some of the losses incurred by herbivory (Price, 1991; Manrique et al., 2009; Wetzel et al. 2016). *Parthenium hysterophorus* maintains the ability to partially compensate for *Z. bicolorata* herbivory as shown by Cowie et al. (2018). Although not significant, increases in leaf nitrogen (SPAD) and chlorophyll content, in the high and medium quality treatments, suggest photosynthetic up-regulation as seen in similar experiments (Cowie et al., 2018), which may partially account for the reduced levels of damage experienced by *P. hysterophorus* in higher quality treatments.

Parthenium hysterophorus plant quality also affected the size of emerging *Z. bicolorata* adults. Increased larval food quality, particularly nitrogen (SPAD), is strongly associated with the emergence of larger adult insects (Chown and Gaston, 2010). More importantly, larval plant quality and adult body size frequently reflect the reproductive performance and fecundity of numerous insect species (Awmack and Leather, 2002; Moreau et al., 2006), a trend that has also been recorded for Z. *bicolorata*, with larger females being more fecund (Afaq, 2013). Increases in *Z. bicolorata* fecundity at higher plant qualities may be explained by the size and weight of beetles, with greater fat reserves amassed by larger females. Larger energy stores afford females the ability to provision more resources towards reproductive development, allowing for shortened pre-oviposition periods as well as the production of larger eggs and, or, egg batches (Chown and Nicolson, 2004; Ishihara and Ohgushi, 2006). However, male body weight and size should not be discounted when considering fecundity in *Z. bicolorata*, as Afaq (2013) showed that mating between larger males with larger females produced the most viable and vigorous offspring. Ultimately, improved reproductive outputs are sought in biocontrol programmes as they offer the potential for multiple and larger overlapping generations, which enhance damage to target weed populations (Cotesero et al., 2000; Price, 2000; Van Hezewijk et al., 2008).

Although laboratory-based (individual-level) studies offer focused insights towards the effects of host plant quality, caution should be exercised when extrapolating these findings to field- or population-levels (Bownes et al., 2013). The survival, development and fecundity of *Z. bicolorata* suggests that varying host plant quality is unlikely to directly affect the initial establishment of the agent. However, reduced host plant quality may present underlying hinderances for the biocontrol of *P. hysterophorus*, that should be acknowledged. Prolonged larval development increases the susceptibility of vulnerable immature stages to both biotic and abiotic stresses (Awmack and Leather, 2002; Moreau et al., 2006), which is detrimental to ongoing biocontrol efforts (Stiling and Moon, 2005). In the case of *Z. bicolorata*, egg and larval predation and thermal tolerance as influenced by adult age and feeding status are suspected constraints to *Z. bicolorata* populations in South Africa (Strathie et al., 2016a,b; Chidawanyika et al., 2017; Abels, 2018). Poor quality plants may diminish the physiological (thermal) tolerance of adult beetles, exacerbating heat stresses experienced by *Z. bicolorata* in the field (Chidawanyika et al., 2017). A potential mitigation to the adverse effects of low host plant quality, which prevent initial establishment, may be attained through the fertilisation of relatively small patches of *P. hysterophorus* during and after agent release (Price, 2000). Although seemingly counterintuitive, fertilising target weeds may promote, support and even enhance agent establishment, abundance and performance, as seen and suggested in other weed biocontrol programmes (e.g. Room and Thomas, 1985; Heard and Winterton,

2000; Van Hezewijk et al., 2008). More successful establishment of *Z. bicolorata* in fertilised or recently fallow croplands and their margins, and sites with high-nutrient soils may be expected, barring the influence of other factors constraining establishment.

Overall, these findings may partially explain the poor establishment and varied incidence of *Z. bicolorata* at some field release sites in South Africa, but this requires further field investigation. Although poor host plant quality is unlikely to directly impact the survival of *Z. bicolorata*, the underlying implications may hinder ongoing biological control efforts against *P. hysterophorus*. Prolonged development further exposes larvae to predation, desiccation and thermal stresses as adults. Additionally, reduced fecundity of females in low nutrient settings may also limit the persistence and effectiveness of *Z. bicolorata* populations in the field. These findings offer an improved understanding towards the factors constraining the establishment and performance of *Z. bicolorata*. More generally, this research may aid in mass-rearing efforts as well as provide insight towards the selection or creation of more suitable release sites for *Z. bicolorata* in South Africa.

CRediT authorship contribution statement

Blair W. Cowie: Conceptualization, Methodology, Investigation, Writing - original draft, Data curation, Formal analysis. **Lorraine W. Strathie:** Conceptualization, Methodology, Supervision, Resources, Writing - review & editing. **Jeremy M. Goodall:** Conceptualization, Methodology, Supervision, Resources, Writing - review & editing. **Nic Venter:** Conceptualization, Methodology, Supervision, Resources, Writing - review & editing. **Ed T.F. Witkowski:** Conceptualization, Methodology, Supervision, Resources, Writing - review & editing. **Marcus J. Byrne:** Conceptualization, Methodology, Supervision, Resources, Writing - review & editing.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biocontrol.2019.104078.

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CHAPTER 7:

Feasible or foolish: attempting restoration of a *Parthenium hysterophorus* invaded savanna using perennial grass seed

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Abstract

The annual herb *Parthenium hysterophorus* L. (Asteraceae), remains one of South Africa's most significant invasive weeds, commonly invading savannas and their rangelands, causing severe losses to agriculture, livestock and native biodiversity. Previous studies have suggested that perennial grasses may act as useful competitive species, capable of suppressing the growth and invasion of *P. hysterophorus*. To explore this, small-scale plots were established within an invaded savanna, using a randomised block design, and included treatments with and without the clearing of *P. hysterophorus*, as well as with and without the sowing of native perennial grass seed (*Anthephora pubescens*, *Chloris gayana*, *Cynodon dactylon*, *Digitaria eriantha*, *Eragrostis curvula*, *Panicum maximum* and *Themeda triandra*). Plots were assessed yearly in terms of *P. hysterophorus* density and growth as well as grass composition, basal cover and biomass over a three year period. Clearing alone was found to exacerbate the invasion, allowing *P. hysterophorus* plants to increase their size and density. Whereas the sowing of grass seed, in both the cleared and uncleared plots, increased the abundance of perennial grass species, subsequently reducing the size, reproduction and density of *P. hysterophorus*. In addition, these sowing efforts contributed towards restoration of the plots, enhancing both grass basal cover and biomass production. Overall, this research suggests that sowing of native grass species, with or without clearing, may be a useful supplementary control or restoration tool towards the long-term management of *P. hysterophorus* invasions in certain savannas and rangelands in Southern Africa.

Keywords: Growth suppression; Indigenous grasses; Parthenium weed; Savanna restoration; Veld management; Weed management;

1. Introduction

In Southern Africa, savannas exist as key resource areas which have long supported commercial and subsistence farming, animal husbandry as well as the stocking of livestock and conservation of wild game (O'Connor, 2015; Van Oudtshoorn, 2015). As a result of this intensive and often historic overuse, many of these savannas systems have become degraded, suffering invasion by non-native plants termed 'weeds' (DiTomaso et al., 2010; Roodt, 2011). Numerous factors facilitate the invasion of savannas and their rangelands by weeds, however, the most severe invasions occur predominantly in systems that are poorly managed, particularly when overgrazed (DiTomaso et al., 2010; O'Connor and van Wilgen, 2020). Overgrazing and weed invasions are synonymous given that intensive grazing, without respite, increases disturbance, lowers vegetation cover and limits the growth and recovery of pastures or rangelands, creating ideal conditions for the establishment and spread of invasive plants (Shabbir et al., 2013; Davies and Johnson, 2011). Moreover, the loss of palatable perennial grasses due to overgrazing, increases the susceptibility of an area to further invasion, often exacerbating the extent and impact of weeds (Sheley et al., 2006; Tessema et al., 2016). Invasion by noxious annual weeds remains a continual problem amongst South African savanna systems and rangelands, with one of the most significant weed species being *Parthenium hysterophorus* L. (Asteraceae) (Strathie and McConnachie et al., 2019).

Native to the American tropics, the broad-leaved herb, *P. hysterophorus*, has invaded large swathes of land within the country's eastern high rainfall regions, predominantly the lowvelds of the KwaZulu-Natal and Mpumalanga provinces (McConnachie et al., 2011; Terblanche et al., 2016). Within these regions, *P. hysterophorus* typically germinates during October, with the onset of the first spring rains, forming a leafy basal rosette (Strathie et al., 2011; Tanveer et al., 2015). The weed then bolts, maturing rapidly and producing an abundance of flowers and subsequently seed, often in excess of 30 000 per plant, until it senesces during mid-autumn (Strathie et al., 2011; Cowie et al., 2020a). These *P. hysterophorus* invasions are particularly troublesome in grazed or disturbed savannas / rangelands, where the weed forms recurring and dense monospecific stands, with extensive and persistent seedbanks (Belgeri et al., 2014; Nguyen et al., 2017). These dense stands commonly outcompete and chemically exclude native flora and are toxic and unpalatable to any domestic or wild herbivores, reducing available pastures and furthering agricultural and conservation concerns (Strathie et al., 2011; Adkins and Shabbir, 2014). The weed's annual nature, rapid growth rate, prolific seed production, allelopathic effects and absence of natural enemies all contribute towards making *P. hysterophorus* a severe invader in savanna and rangeland settings (Goodall et al., 2010; McConnachie et al., 2011).

Addressing the degradation of overgrazed and invaded savanna systems, particularly those invaded by *P. hysterophorus*, can be a complex and challenging endeavour (Nigatu et al., 2010). Several approaches have been implemented to manage the invasion of *P. hysterophorus* across South Africa, most notably chemical, mechanical and biological controls (Strathie et al., 2011; Cowie et al., 2018, 2020b). Moreover, the recent development of an integrated national strategy in 2016, with biological control at the core, is promising but yet to offer any notable success in controlling *P. hysterophorus*, with the weed increasing its

range by 480% over the past decade (Terblanche et al., 2016; Van Wilgen and Wilson, 2018). The limited control of *P. hysterophorus* in South Africa, suggests that additional or supplementary, site-specific management approaches will be required to attain control in certain areas (Khan et al., 2013, 2014; Cowie et al., 2020 in prep.). In the case of invaded savannas / rangelands, reducing grazing herbivore numbers and subsequently grazing pressure, has been shown to allow recovery of pastures along with reductions in *P. hysterophorus* density and impact (Dhillepan and Strathie, 2009; Belgeri et al., 2014). However, reducing grazing pressure alone to attain adequate recovery of pastures may take time (O'Conner, 2015), depending on the severity of invasion, advocating instead for the sowing of suppressive plant species, particularly perennial grasses, into invaded areas (Van Der Laan et al., 2008; Khan et al., 2013, 2019).

The sowing of perennial grasses is a common restoration practice used to aid the recovery of savanna and rangeland pastures as well as for the suppression of unwanted invasive plants (Aires et al., 2014; Van Oudtshoorn, 2015). In terms of managing *P. hysterophorus*, numerous perennial grasses have been identified as potential suppressive species, including *Chloris gayana*, *Digitaria eriantha*, *Eragrostis curvula*, *Panicum maximum* and *Themeda triandra* (Van Der Laan et al., 2008; Khan et al., 2013, 2019). These species are erect tufted grasses, suggested to gain their suppressive abilities from their growth properties, mainly rapid establishment, quick growth rate and high levels of biomass production (Shabbir et al., 2013; Khan et al., 2019). However, the sowing and establishment of palatable grasses, such as these, can easily be hindered if stocking rates and grazing pressure are too high, limiting pasture regrowth (Van Oudtshoorn, 2015; Khan et al., 2019). This suggests that sowing native grass seed into *P. hysterophorus* invaded areas with low levels of grazing pressure, may help to mitigate degradation caused by overgrazing and invasion (Strathie and McConnachie et al., 2019).

The ongoing invasion of *P. hysterophorus* in South Africa, particularly within its savannas and their rangelands, poses numerous ecological and socio-economic threats. Furthermore, limited studies exist on *P. hysterophorus* within South African savannas, making this research valuable in understanding and addressing the weed's invasions in these areas. Therefore, this study aimed to assess the potential of sowing native grass seed, with and without clearing, to suppress the growth and invasion of *P. hysterophorus* and aid in the restoration of savanna pastures under field conditions. To investigate this, an invaded savanna reserve with low grazing numbers (i.e. low grazing pressure) was selected and changes in the density and growth of the weed as well as the recovery of the grass were assessed within small-scale treatment plots over a three year period.

2. Methods and materials

2.1 Study site description

The field site selected for the study was a 1 400 ha fenced savanna reserve (25° 33'16.84" S, 31° 37'26.58" E; 282 m.a.s.l.) located in the Malalane (Malelane) region of Mpumalanga, approximately 10 km from the Kruger National Park, South Africa. Described as a granite lowveld savanna with mostly sandy soils, the site maintains a diversity of native savanna trees and grasses (Mucina and Rutherford, 2006). Common tree species at the site included *Terminalia sericea*, *Combretum* spp., *Dichrostachys cinerea* as well as *Vachellia* and *Senegalia* spp. A variety native grasses were also present, most commonly *Eragrostis* spp., *Digitaria longiflora*, *Setaria* spp., *Panicum maximum* and *Chloris gayana*. In terms of invasive species, *P. hysterophorus* was the most widespread and abundant throughout the site, however, small scattered patches of *Amaranthus spinosus*, *Datura stramonium*, *Tagetes minuta*, *Bidens pilosa*, *Ageratum* spp., *Eleusine coracana* and *Dactyloctenium* spp. were also present. Average minimum and maximum temperatures experienced over the study period were 29 °C and 17 °C, respectively (Figure 1A), with the region typically experiencing rainfall of 547 mm per annum, the majority of which falls during spring and summer (Oct-Mar) (Figure 1B).

The reserve was previously used as a cattle farm, likely allowing for the widespread invasion of *P. hysterophorus* recorded on the property. The transition from cattle farm to savanna reserve occurred in 2012, with the majority of cattle (*Bos taurus*) being removed. Only a few grazers currently remain on the reserve, in low numbers (>25 LSU/ha), including Blesbok (*Damaliscus pygargus phillipsi*), Blue Wildebeest (*Connochaetes taurinus*), Impala (*Aepyceros melampus*), Zebra (*Equus quagga*), Common Warthog (*Phacochoerus africanus*) and a few cattle (<15). The low levels of grazing, considerably lower than when it was a cattle farm, have allowed slight recovery of pastures, with *P. hysterophorus* densities averaging 9-12 plants per m^2 each year, since 2015 (Cowie et al., 2020, in prep.). Biological control efforts were undertaken at the site, with releases of the leaf-feeding beetle *Zygogramma bicolorata* Pallister (Chrysomelidae: Coleoptera) during 2015 and 2016, however no establishment, nor impact, of the beetle has been noted at the site since. Therefore, without intervention the recovery of invaded savannas, like this, can take decades (O'Conner, 2015), making the site suitable for trialling small-scale restoration efforts aimed at the recovery of pastures (perennial grasses) along with the suppression of *P. hysterophorus*.

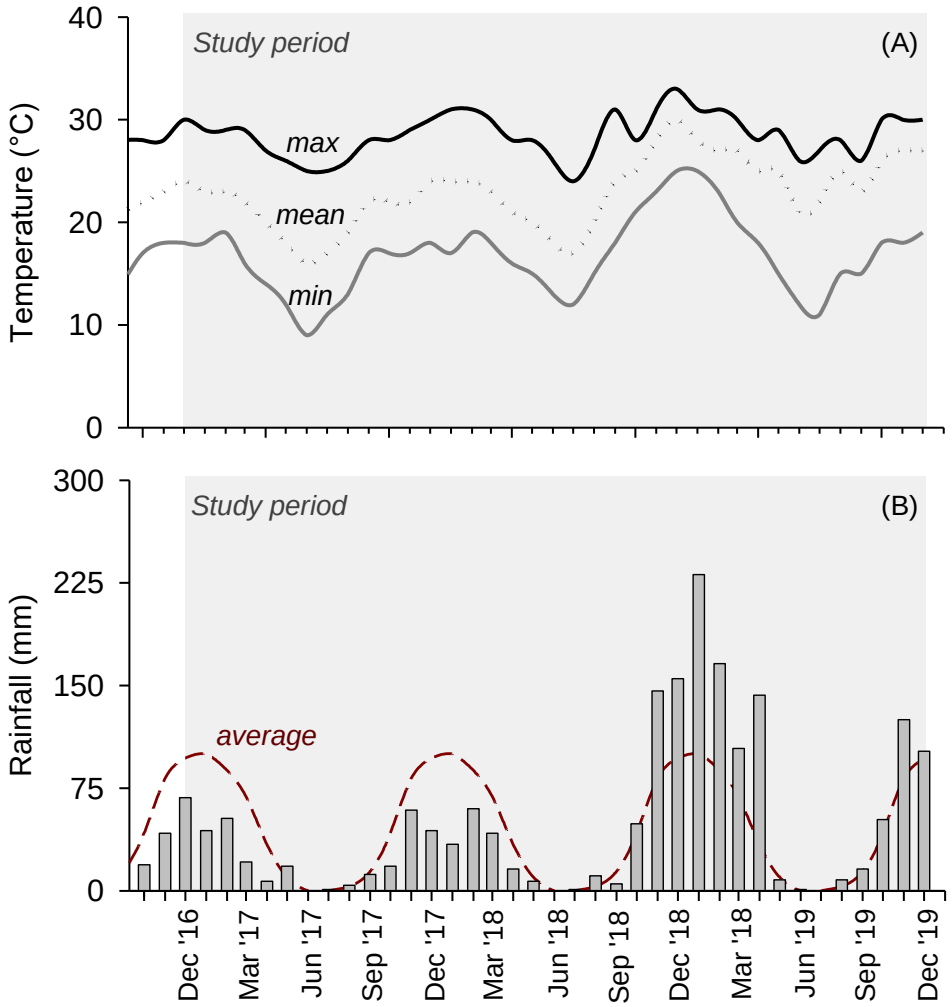

Figure 1. Monthly A) minimum, maximum and mean temperatures as well as B) rainfall (mm) and its long-term average, for the Malelane region of Mpumalanga over a three year period from October 2016 to December 2019. (Data from WWO, 2020).

2.2 Native grass species (seed) selection

Grass seed used in the 'restoration mix' included a combination of seven native perennial grass species, namely *Anthephora pubescens*, *Chloris gayana*, *Cynodon dactylon*, *Digitaria eriantha*, *Eragrostis curvula*, *Panicum maximum* and *Themeda triandra* (Table 1). This particular grass seed mix was selected on the basis of three main criteria: that species within the mix were i) perennial species known to be prevalent and suited to environmental conditions within the area (Roodt, 2011; Van Oudtshoorn et al., 2012, 2015), (ii) have a relatively fast growth rate with moderate to high grazing (nutritional) value and (iii) have been shown or suggested to suppress the growth and reproduction of *P. hysterophorus* or similar annual weeds to some extent (see Van Der Laan et al., 2008; Khan et al., 2019). All species in the native seed mix were already present at the reserve, either above-ground or within the seedbank (Cowie et al., 2020 in prep.), excluding *A. pubescens*, which is a sought after species within arid savannas, although its presence in the Malelane area is scarce (Roodt, 2011, 2015).

Table 1. Indigenous (South African) perennial grass species present within the commercial pasture / restoration mix along with their common name, growth form, grazing (nutritional) value, ecological succession stage (Roodt, 2011; Van Oudtshoorn, 2012) and recommended sowing rates for land rehabilitation / restoration.

Grass species	Common name	Growth form	Grazing value	Succession	Sowing rate
Anthephora pubescens	Wool grass	Erect tufted	High	Climax	6 kg/ha
Chloris gayana	Rhodes' grass	Erect tufted	High	Subclimax	10 kg/ha
Cynodon dactylon	Couch grass	Creeping	High	Pioneer	20 kg/ha
Digitaria eriantha	Smuts finger grass	Erect tufted	High	Climax	8 kg/ha
Eragrostis curvula	Weeping love grass	Erect tufted	Moderate	Sub-/Climax	8 kg/ha
Panicum maximum	White buffalo grass	Erect tufted	High	Sub-/Climax	10 kg/ha
Themeda triandra	Red oat grass	Erect tufted	High	Climax	8.5 kg/ha

2.3 Plot treatments and procedure

The experiment was set-up following a randomised block design, with 12 replicates, during early December (2016) after the arrival of the summer rains, which were late (Figure 1). Steel rods with aluminium tags were used to demarcate the 12 blocks, each measuring 2500 m^2 (50 m x 50 m) in size, within a relatively flat and homogenous grassy-savanna area (~1.5 km^2) of the reserve invaded by *P. hysterophorus*. Within these blocks, four different treatment plots were imposed randomly, which were (i) uncleared and unsown (control-reference plot), (ii) cleared of invasives and unsown, (iii) uncleared and sown with grass seed and (iv) cleared of invasives and sown with native grass seed. Plots each measured 16 m^2 (4 x 4 m) in size and presented similar levels of *P. hysterophorus* infestation (c.a. 9-12 plants/m^2) and grass basal cover (~8-9%, see *Section 2.5*) prior to treatment application. In order to maintain statistical independence within each of the blocks, plots were established at random and kept a minimum of 10 m apart.

Control plots were established as a reference to assess *P. hysterophorus* invasion in the absence of any clearing/restoration (sowing) efforts. The removal of invasive plants (including roots and surface litter (which would contain seeds)), within cleared plots, occurred once (in 2016) and was done following the savanna weed-clearing protocols of Euston-Brown et al. (2007), namely hand-pulling for smaller plants and weeding forks for larger plants. For cleared plots sown with native grass seed, subsequent to initial clearing as above, the soil surface was lightly raked in order to create 1-2 cm furrows for the seed. Grass seed mix was hand sown once, in 2016, evenly across the plots at 1 g/m^2, equivalent to 10 kg/ha (the recommended dosage for restoration). The soil was then re-raked to cover seeds with a fine layer of soil and compacted manually, using a rubber ground rammer (tamper). Similarly, the uncleared and sown plots, had no vegetation cleared, however, all bare soil patches within the plots were raked and sown with grass seed and compacted as above.

2.4 Parthenium hysterophorus density and growth assessments

Parthenium hysterophorus measures were done yearly, during mid-summer (mid-December) and included plant density, height, leaf area and seed production. All plant measurements were made using a 1 m^2 (1 m x 1 m) quadrat in all 16 m^2 and averaged per plot for all 48 plots (i.e. all treatments). Density was assessed as the number of *P. hysterophorus* plants present per m^2 within each of the plots. Plant height was measured from the soil surface to the tallest apical shoot / growing tip, to nearest cm, for all plants growing within the treatment plot. Leaf area was recorded for all *P. hysterophorus* plants within a plot and calculated as per Cowie et al. (2019) using the length and width of each fully expanded leaf present on the plant (Equation 1). Seed production estimates were calculated by multiplying all mature flowers per plant, by five (Mao et al., 2019), which was then averaged per plot.

$$(Eq.1)\ \ Leaf\ Area\ (cm^2) = \sum(0.4104[L_1 \times W_1]) + (0.4104[L_2 \times W_2]) + \cdots (0.4104[L_n \times W_n])$$

L = leaf length (cm); W = leaf width (cm); n = total leaves/plant

To best assess the suppressive ability of the plot treatments over time, *P. hysterophorus* densities were converted to a percentage reduction or increase, by dividing the density from each plot treatment (cleared, sown, and sown and cleared) by control density for each year. In addition, plant height, leaf area and seed production were converted into percentages as above and averaged as to attain an overall growth suppression percentage per year. These percentages were then plotted over time.

2.5 Grass species, basal cover and biomass assessments

Along with *P. hysterophorus* measures in all 12 blocks (48 of the treatment plots), grass species were assessed yearly, from 2017 to 2019, in terms of (a) annual and (b) perennial species density, basal cover and biomass production. During the initial setup (in 2016), a species list of the most common grasses found within all plots, along with those sown, was created and used in the subsequent assessments. Each summer, species were identified within each of the treatment plots using distinguishing morphological features (flowers / leaves) in combination with the relevant literature and classified as annual or perennial species (Van Oudtshoorn, 2012; Fish et al., 2015; Roodt, 2015). Grass basal cover (%), per plot, was calculated using 64 paired grass measurements (i.e. 1 pair per 0.25 m^2 quadrat) in which distance from the centre of each quadrat to the nearest living tuft was measured (cm) as well as the basal diameter of the respective tuft (cm). For grass tufts with basal diameters <1 cm a measure of 1 cm was used. These 64 paired measures were then averaged per plot and final basal cover (%) calculated as per the equation of Hardy and Tainton (1993; Equation 2).

$$(Eq.2)\quad Basal\ cover\ (\%) = 19.8 + 0.39(D) - 11.87(log_e\ D) + 0.64(d) + 2.93\ (log_e\ d)$$

D- mean distance from centre to nearest living tuft (cm) d – mean basal diameter of nearest tuft (cm)

Lastly, grass standing crop (biomass production) was assessed per m^2 using a disc pasture meter (DPM) for all 16 m^2 within each plot. Measurements using the DPM had all non-grass vegetation gently moved aside, to reduce inaccuracies prior to dropping the disc. DPM measures were taken from the ground surface to the resting height of the weighted disc (nearest 0.5 cm) after being dropped once. DPM readings were then averaged per plot and converted into biomass (g/m^2) using the calibrated equation from Zambatis et al. (2006; Equation 3).

$$(Eq.3)\quad Biomass\ (kg.ha) = \left[31.7176 \left(0.3218 \times \frac{1}{DPM} \right) \times DPM^{0.2834} \right]^2$$

DPM - mean DPM (cm) per plot

2.6 Statistical analysis

Parthenium hysterophorus density, plant height, leaf area and seed production were each statistically assessed using a linear mixed effects regression (LMER) within the 'lme' and 'nlme' packages in R (R Core Team, 2018). To generate F_{stat} and p-values, LMERs were assessed using an analysis of variance (ANOVA) along with a Holm adjusted Tukey multiple comparisons *post-hoc* test, within the 'car' and 'multcomp' packages. Plant density, height, leaf area and seed production were set as the response variables with plot treatment set as the fixed effect and plot number nested within block and year set as the random effect. To further explore the suppression of *P. hysterophorus's* density and growth, a standardised major axis test (β_{SMA}) was performed using the 'smatr' package in R. Time (years) was set as the continuous predictor and was used to detect overall differences in destiny and growth suppression (slopes) between each of the four plot treatments over the three year period. To assess the changes in annual and perennial grass species densities between the treatments, a one-way repeated measures ANOVA with a Tukey HSD *post-hoc was used*. Lastly, grass basal cover and biomass were assessed using a LMER with basal cover / biomass set as the response variables, plot treatment as the fixed effect and plot number nested within block and year used as the random effect.

3. Results

3.1 Parthenium hysterophorus density and growth assessments

Restorative efforts against *P. hysterophorus* were strongly affected by the treatments imposed within the plots. *Parthenium hysterophorus* invasion remained relatively consistent within the control plots, over the three year period, with densities ranging between 10-12 plants/m^2 (Figure 2). Clearing alone, intensified the invasion within plots, increasing the *P. hysterophorus* density, on average, by 5 plants/m^2 (40%) ($F_{3,127} = 69.1$; $P < 0.001$; Figure 2). Sowing did not reduce the invasion of *P. hysterophorus*, with plots showing minimal change in average density (10.3 plants/m^2), however, when plots were cleared and sown with seed, *P.*

hysterophorus densities declined significantly, with an average reduction of 2.2 plant/m^2 (17%). When assessing the suppression trends with regards to *P. hysterophorus* density over the three year period, the cleared and sown treatment showed the greatest reduction in *P. hysterophorus*, followed by the sown only treatment and lastly by the cleared treatment (β_{sma} = 16.6; df=3, $P < 0.05$; Figure 3).

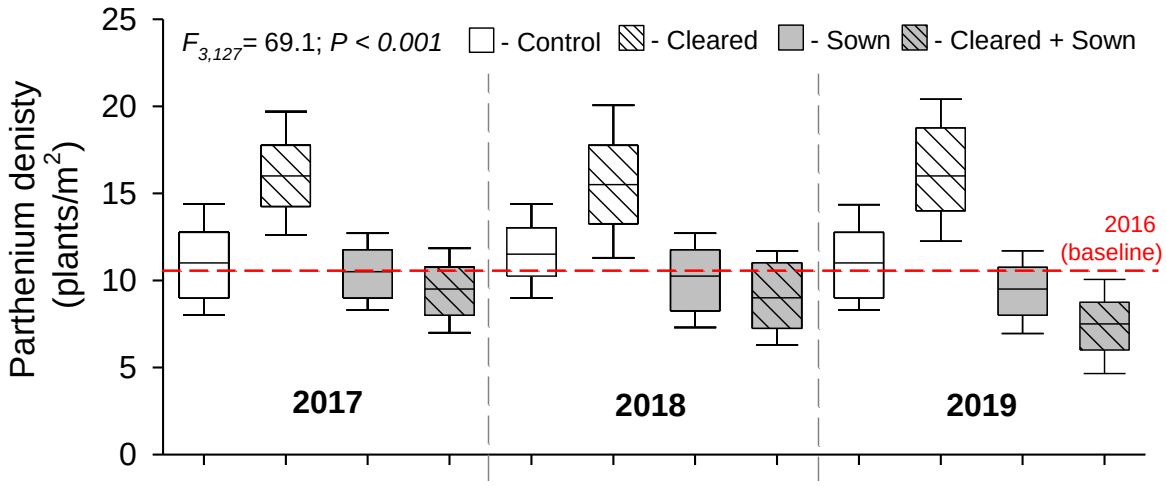

Figure 2. Mid-summer *Parthenium hysterophorus* density within 16 m^2 treatment plots, either (i) uncleared and unsown (control), (ii) cleared only, iii) sown with native grass only or (iv) cleared and sown with grass seed. F_{stat} and *P-values* indicate overall difference in *P. hysterophorus* density between treatment plots using linear mixed effects model (LMER). Boxplot shows median, 10th, 25th, 75th and 90th percentiles, and baseline data originates from all plots (N=48) prior to treatment application in 2016.

Plot treatments also influenced the growth of *P. hysterophorus* in the field, namely the weed's height, leaf area and flower/seed production. In terms of plant height, the tallest plants were found growing within plots which were initially cleared only, attaining heights of ~88 cm (Figure 4A). Whereas plants growing in plots which were sown, as well as those which were cleared and sown, were significantly smaller than the control plots, attaining average heights of 73 and 68 cm, respectively ($F_{3,127}$ = 119.7; $P < 0.001$; Figure 4A). Similarly, *P. hysterophorus* plants within the cleared only plots, accumulated a greater leaf area when compared to the control plots (Figure 4B). Both the sown and the cleared and sown plots were found to have significantly lower the leaf area of *P. hysterophorus*, with leaf areas reduced by 38 cm^2 (6%) and 59 cm^2 (11%), respectively ($F_{3,127}$ = 105.2; $P < 0.001$; Figure 4B). The reproductive output (flowers/seeds) of *P. hysterophorus* was not affected by clearing efforts, with the cleared only plots showing no difference to the controls, averaging similar productions of ~480 seeds/plant over the three years (Figure 4C). Sowing alone reduced the production of *P. hysterophorus* seed by 9%, which was reduced by a further 6% in the plots which were both cleared and sown ($F_{3,127}$ = 138.5; $P < 0.001$; Figure 4C).

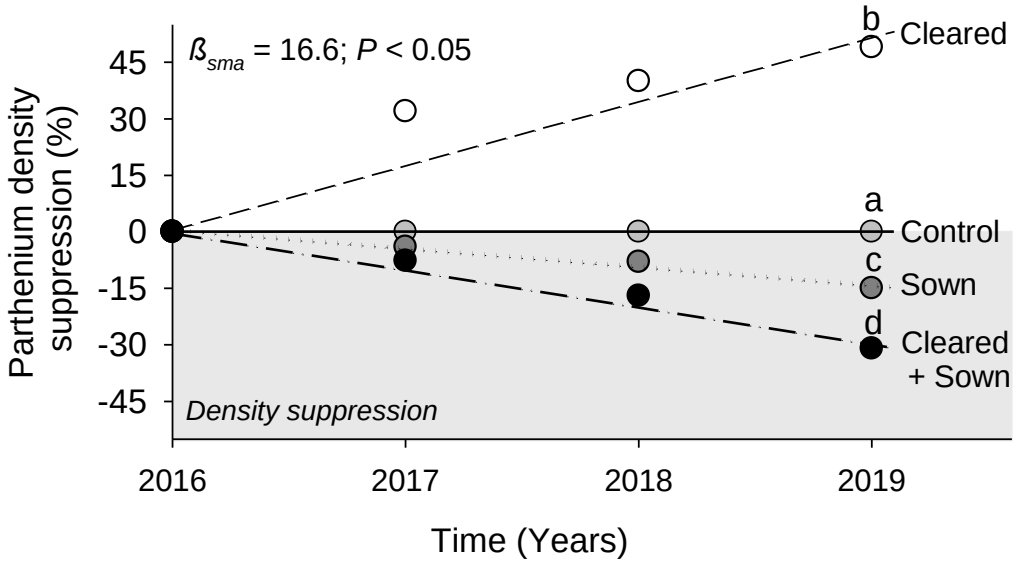

Figure 3. Suppression of *Parthenium hysterophorus* density (%) within 16 m^2 plots, either (i) uncleared and unsown (control), (ii) cleared only, iii) sown with native grass only or (iv) cleared and sown with native grass seed. Different lowercase letters indicate significant differences ($P < 0.05$) between slopes of different plot treatments using a standardised major axis test (β_{SMA}).

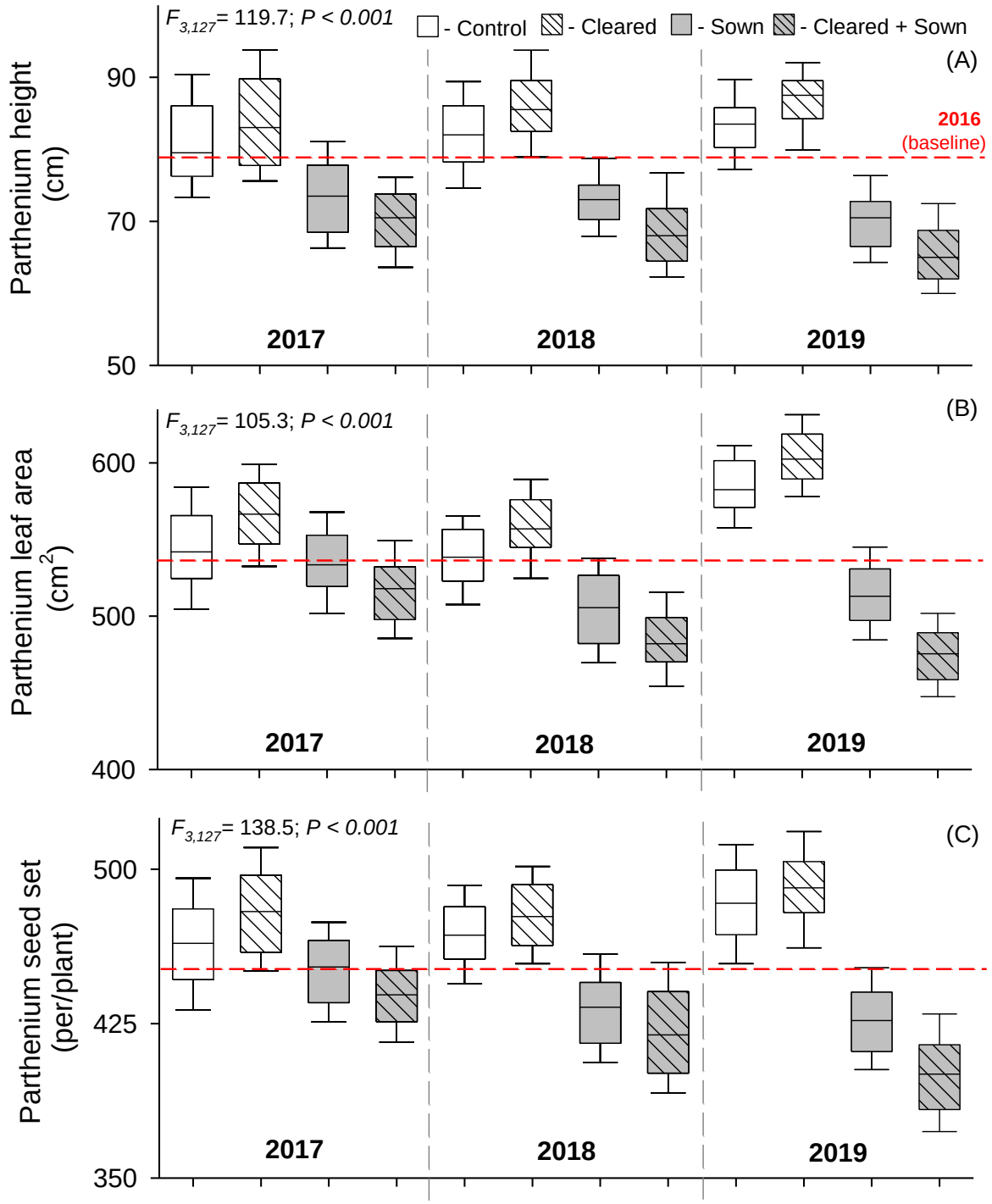

Figure 4. Mid-summer *Parthenium hysterophorus* (A) height, (B) leaf area and (C) estimated seed production within 16 m^2 treatment plots, either (i) uncleared and unsown (control), (ii) cleared only, iii) sown with native grass only or (iv) cleared and sown with native grass seed over a three-year period. F_{stat} and *P-values* indicate overall differences in *P. hysterophorus* density between treatment plots using a linear mixed effects model (LMER). Boxplots show median, 10th, 25th, 75th and 90th percentiles, and baseline data originates from all plots (N=48) prior to treatment application in 2016.

Clearing alone yielded no reductions in the overall growth and reproduction (height, leaf area and seed set) of *P. hysterophorus* across the three years, rather serving to enhance the growth of the weed slightly (Figure 5). The greatest reductions in *P. hysterophorus* growth were found within the cleared and sown treatment, suppressing the weed's overall growth by 19% after three years (β_{sma} = 9.2; df=3, $P < 0.05$). This was followed closely by the sown only treatment, which suppressed the growth of *P. hysterophorus* by 12% (Figure 5).

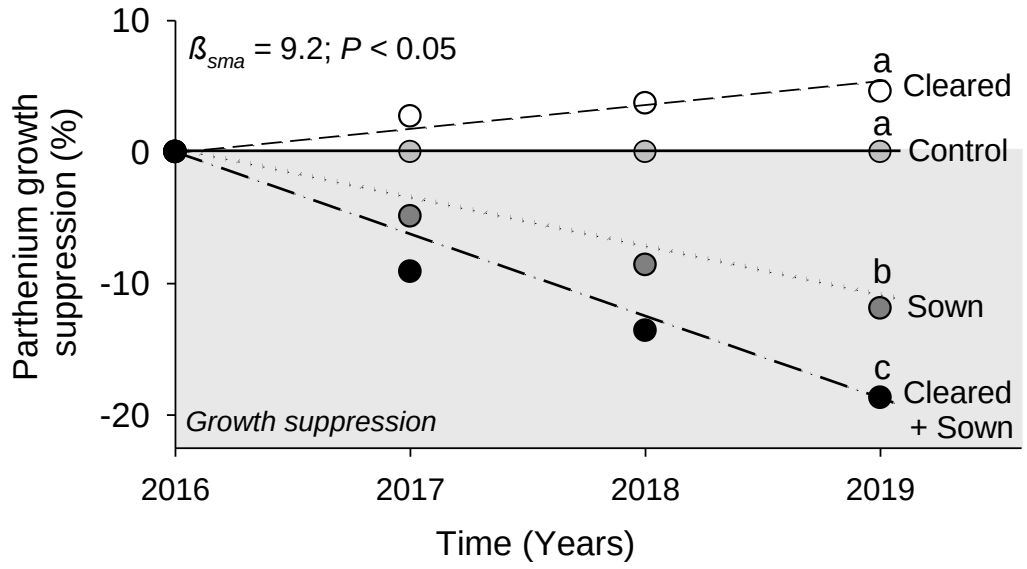

Figure 5. Overall suppression (%) of *Parthenium hysterophorus* growth (height, leaf area and seed production) within 16 m^2 plots, either (i) uncleared and unsown (control), (ii) cleared only, iii) sown with native grass only or (iv) cleared and sown with native grass seed. Different lowercase letters indicate significant differences ($P < 0.05$) between slopes of different plot treatments using a standardised major axis test (β_{SMA}).

3.2 Grass species, basal cover and biomass assessments

Excluding *C. dactylon*, sowing efforts significantly increased the density of perennial grass species in both the sown (26%) and the cleared and sown (42%,) treatment plots, most notably *P. maximum, D. eriantha* and *E. curvula* ($F_{3,9}$ = 11.6; $P < 0.01$; Table 2). Cleared plots had the fewest perennial species present as well as the lowest density of perennial grasses, averaging 6.1 grasses/m^2, after three years. Additionally, the cleared plots presented significantly higher densities of annual grass species, over the three years, with close to double that of any other plot treatment ($F_{3,9}$ = 30.4; $P < 0.001$). Overall, plots cleared and uncleared but sown with native grass seed shifted towards a perennial dominated grass community, compared to cleared only plots which were dominated by annual grasses (Table 2).

Table 2. Mean density of species (m^2) ± SE of annual and perennial grasses present within treatment plots established in a savanna reserve, namely (i) uncleared and unsown (control), (ii) cleared only, iii) sown with native grass only or (iv) cleared and sown with native grass seed assessed over a three year period. Different lowercase letters indicate significant differences between annual and perennial grass densities using a repeated measures ANOVA (*P <0.05*).

	Plot treatment			
Grasses (*species* / m^2) ± SE	Control	Cleared	Sown	Cleared + Sown
Perennials				
*Anthephora pubescens**	-	-	0.1 ± 0.1	0.1 ± 0.1
*Chloris gayana**	0.2 ± 0.1	-	-	0.2 ± 0.1
*Cynodon dactylon**	0.4 ± 0.2	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0.1
*Digitaria eriantha**	1.6 ± 0.7	1.4 ± 0.7	2.7 ± 0.6	3.1 ± 0.8
*Eragrostis curvula**	1.8 ± 0.6	1.3 ± 0.4	2.4 ± 0.5	2.6 ± 0.7
Eragrostis superba	2.3 ± 0.3	1.3 ± 0.3	1.9 ± 0.4	2.0 ± 0.4
Heteropogon contortis	0.9 ± 0.5	0.5 ± 0.1	0.8 ± 0.2	0.6 ± 0.2
*Panicum maximum**	1.9 ± 0.4	1.7 ± 0.5	3.3 ± 0.8	3.9 ± 1.0
*Themeda triandra**	-	-	0.4 ± 0.1	0.7 ± 0.2
Total perennials	9.1 ± 0.3^b	6.5 ± 0.3^a	11.9 ± 0.5^c	13.4 ± 0.5^d
Annuals				
Dactyloctenium giganteum	0.8 ± 0.2	1.1 ± 0.3	0.5 ± 0.1	0.8 ± 0.2
Dactyloctenium australe	0.9 ± 0.1	1.4 ± 0.5	0.7 ± 0.2	0.6 ± 0.2
Digitaria longiflora	1.7 ± 0.3	1.3 ± 0.5	1.8 ± 0.5	1.5 ± 0.6
Eleusine africana	0.8 ± 0.3	1.6 ± 0.3	-	0.5 ± 0.1
Eragrostis rigidior	1.5 ± 0.6	1.9 ± 0.5	1.4 ± 0.5	1.1 ± 0.2
Perotis patens	0.8 ± 0.2	1.4 ± 0.3	0.7 ± 0.2	0.8 ± 0.1
Setaria verticillata	1.2 ± 0.3	1.7 ± 0.4	0.8 ± 0.2	1.1 ± 0.2
Total annuals	7.7 ± 0.2^b	10.4 ± 0.3^a	5.9 ± 0.4^b	6.4 ± 0.3^b
Total	**16.8**	**16.9**	**17.8**	**19.8**

* - grasses present within the native grass mix

Grass basal cover (%) within the control plots showed minimal change over the three years, ranging between 7.7 and 8.8% (Figure 6A). Interestingly, grass basal cover decreased slightly, but not significantly, within the cleared only plots, with an average of 7.9% over the three years. Whereas grass cover, was found to have increased significantly within both the sown and the cleared and sown plots, reaching 8.6% and 9.3%, respectively ($F_{3,127} = 13.7$; $P < 0.001$; Figure 6A). Grass biomass followed a similar trend, excluding the cleared only plots which produced, on average, significantly lower biomass (378 g/m^2) when compared to the control plots, which accumulated 403 g/m^2 over the three years (Figure 6B). Increases in basal cover within the cleared and the cleared and sown plots were also reflected in grass biomass, with plots producing 5% (422 g/m^2) and 9% (435 g/m^2) more biomass, respectively ($F_{3,127} = 34.8$; $P < 0.001$; Figure 6B).

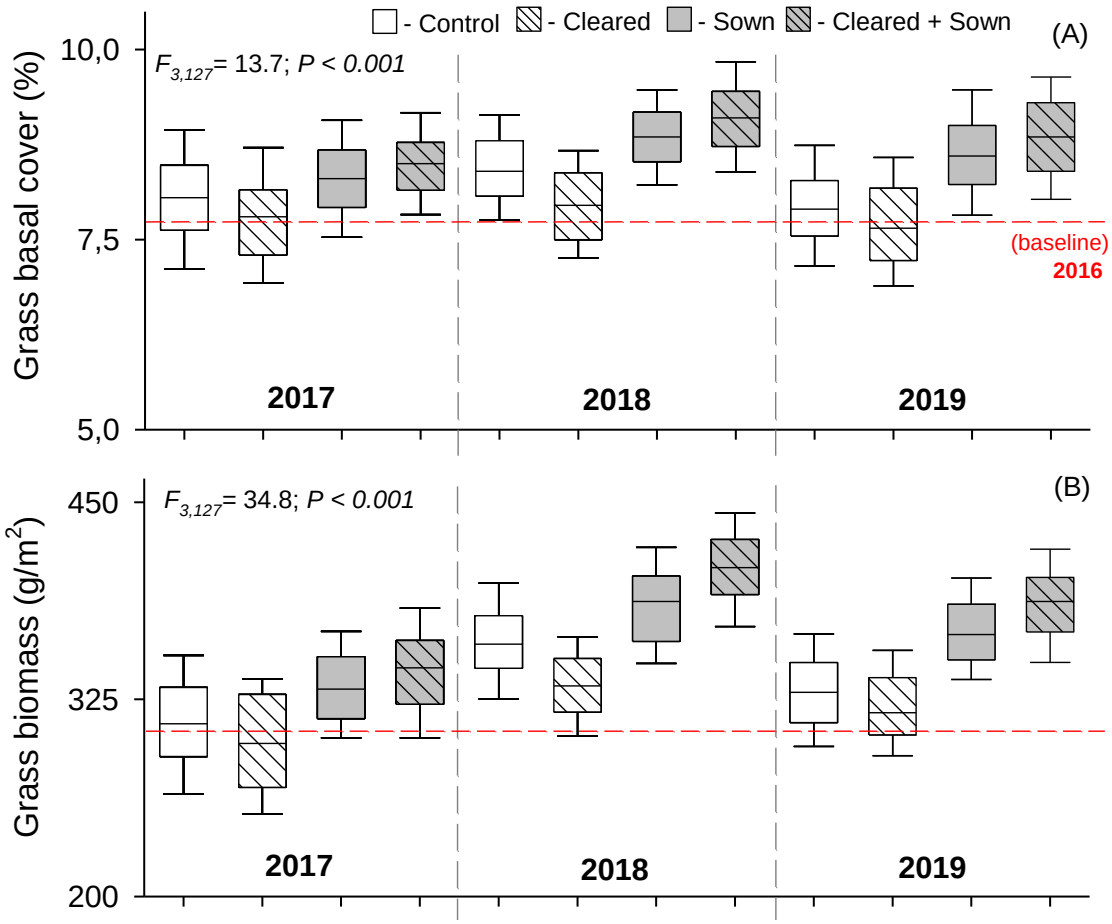

Figure 6. Mid-summer grass (A) basal cover percentage and (B) biomass production within 16 m^2 treatment plots, either (i) uncleared and unsown (control), (ii) cleared only, iii) sown with native grass only or (iv) cleared and sown with native grass seed over a three-year period. F_{stat} and *P-values* indicate overall differences in grass cover / biomass between treatment plots using a linear mixed effects model (LMER). Boxplots show median, 10th, 25th, 75th and 90th percentiles, and baseline data originates from all plots (N=48) prior to treatment application in 2016.

4. Discussion

Parthenium hysterophorus remains a serious annual invader of South African savannas and rangelands, with no single control measure able to manage the weed at present (Goodall et al., 2010; Terblanche et al., 2016). However, the sowing of native grass species successfully reduced the invasion (density) of *P. hysterophorus*, in both the cleared and uncleared plots, over time, highlighting the potential of this technique to be employed as a long-term supplementary control. Contrastingly, the large (~40%) increases in *P. hysterophorus* density recorded within plots which were cleared without the addition of native grass seed, are accounted for by the resulting effects of this technique. Disturbances caused by intensive

alien clearing activities frequently open patches in the landscape, facilitating the invasion and/or densification of noxious annual species, such as *P. hysterophorus* (DiTomaso, 2000; Higgins et al., 2000). Moreover, soil perturbance, resulting from clearing efforts, is known to enhance the germination and recruitment of *P. hysterophorus* (Navie et al., 2004) and is highly likely given the weed's dominance within the site's seedbank (Strathie and McConnachie, 2019; Cowie et al., 2020 in prep.). This reaffirms that mechanical / manual controls alone should be avoided when managing noxious annual weed infestations, given their health hazards and likelihood to exacerbate the invasion (DiTomaso, 2000; Sheley et al., 2006). Although these sowing and/or clearing efforts directly affected the density of *P. hysterophorus*, they also influence the weed's growth and reproduction (Shabbir et al., 2013).

The suppression of *P. hysterophorus's* growth, namely plant height and leaf area, occurred only in the plots which were sown or cleared and sown. This growth suppression is likely to have resulted from the lowered resources available to *P. hysterophorus* given increases in competition imposed by surrounding vegetation, particularly grasses (Strathie and McConnachie, 2019; O'Connor and van Wilgen, 2020). Under low to moderate grazing pressure, such as this, perennial grasses are highly competitive, growing rapidly and establishing large tufts which acquire various above- and below-ground resources (Van Der Laan et al., 2008; Khan et al., 2013, 2019). This may account for the heightened growth of *P. hysterophorus* within the cleared only plots, given the loss of perennial grasses and their associated levels of competition. Decreases in the reproductive capacity of *P. hysterophorus* within the sown as well as the sown and cleared plots is likely to have arisen from the limited available resources (Cowie et al., 2019; Khan et al., 2019). However, *P. hysterophorus* still produced an abundance of seed, despite suffering reductions in vegetative growth and resource availability. This may be attributed to the plant's annual and ephemeral nature, in which abiotic or biotic stressors, resulting from competition, may cause *P. hysterophorus* to forgo vegetative growth, favouring investment in flowering / seed production and offering reproductive assurance (Cowie et al., 2020a).

Along with the changes in the density and growth of *P. hysterophorus*, grass species composition was also influenced by treatments within plots. The clearing of plots alone facilitated an increase in the abundance and proportion of annual grass species, which is a typical occurrence when overgrazed areas are further disturbed (Van Oudtshoorn et al., 2012; 2015). Whereas increases in the density and proportion of perennial species within the sown and the cleared and sown plots was expected, given the likelihood for grass establishment and persistence following initial sowing efforts as well as the low grazing pressure at the site (Belgeri et al., 2014; Khan et al., 2019). Furthermore, the increases in *P. maximum, D. eriantha* and *E. curvula* density, in these plots, aligned with findings by Van Der Laan et al. (2008), in which the species were found to successfully persist within a *P. hysterophorus* invaded savanna. The lower density of perennial grasses within the sown compared to the cleared and sown plots may have been due to the presence of *P. hysterophorus* plants, with established plants known to limit the growth and germination of surrounding species though competition and allelopathy (Van Der Laan et al., 2008; Shi and Adkins, 2018).

The sowing of native perennial grasses, particularly erect tufted species, was not only valuable in competing with and suppressing *P. hysterophorus*, but also contributed towards the restoration of the pastures, as desired. Increases in the abundance and basal cover of grass, were as a result of the sowing efforts and reduced grazing pressure and are well accepted to aid in an area's recovery by improving water infiltration, nutrient retention, reducing run-off and minimising soil erosion in savannas (Van Oudtshoorn, 2012; Roodt, 2015). Similarly, increased biomass production by palatable, perennial grasses, in the sown and the cleared and sown plots, is sought in savanna and rangeland settings, given the improvements in grazing quality, availability and capacity (Shabbir et al., 2013; Van Outshoorn, 2015). Increases in grass cover and biomass serve to make areas' more ecologically resilient by limiting the intensity and severity of disturbance events and lowering their susceptibility to overgrazing and invasion (Sheley et al., 2006; DiTomaso, 2010). In the case of *P. hysterophorus*, the recovery and maintenance of pasture grasses is believed to be a key factor in negating and reversing the negative impacts of the weed in natural areas (Van Der Laan et al., 2008; Dhileepan and Strathie, 2009).

Overall, restoration efforts are often long-term endeavours prone to seasonal changes within regions and over time, which must be considered along with the goals of the area (Sheley et al., 2006; Davies and Johnson, 2011). Thus, assessments over a longer time period may showcase improved levels of restoration, as seen in other studies (e.g. Khan et al., 2019). Nevertheless, these findings offered various insights towards the management of *P. hysterophorus* when using perennial grass seed. The underlying influences that climate, namely rainfall, maintains on grass growth and subsequently restoration efforts must be considered, with below-average rainfall years likely to offer limited restoration potential as seen here (Shabbir et al., 2013). Likewise, the sowing of grass seed into different localities may require changes in the composition of species selected within seed mixes to better suit the local conditions. However, caution must be exercised when using competitive grasses outside of their natural ranges, given the potential risk of extralimital invasions (Khan et al., 2013, 2014). More importantly, the grazing species and resulting grazing pressure at a site will strongly affect both the suppression of weeds as well as the recovery of grass communities (Khan et al., 2019). Mismanagement, particularly overstocking and overgrazing, will negate any weed suppression or grass recovery efforts, suggesting that sowing grass seed is not suited to poorly or unmanaged reserves as well as any unfenced communal rangelands (Terblanche et al., 2016; Strathie and McConnachie, 2019). Overall, the sowing of native perennial grasses is a promising control which has the potential to be integrated with other management approaches in South Africa, such as chemical or biological control (Shabbir et al., 2013; Tanveer et al., 2015).

Taken together, the findings from this study conclude that the sowing of native grass species, with or without clearing efforts, may serve as a promising supplementary control towards managing *P. hysterophorus* invasions in low to moderately grazed savannas and rangelands. Ideally, this approach is best suited for use in actively managed / monitored areas of conservation concern at risk of further *P. hysterophorus* invasion, such as the Kruger National Park and Hluhluwe–Imfolozi Park (Strathie et al., 2011). This research also

discourages the use of mechanical / manual clearing against *P. hysterophorus*, without the appropriate follow-up controls, given the likelihood to intensify invasions. More broadly, the clearing of *P. hysterophorus* along with the sowing of native perennial grasses may aid in the suppression of *P. hysterophorus* and recovery of pastures within similarly invaded savannas in southern Africa and abroad.

Acknowledgements

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CHAPTER 8:

SYNTHESIS & CONCLUSION

General overview

Parthenium hysterophorus remains one of the most damaging and problematic invasive annual species globally, prevalent in numerous sub-tropical and tropical countries, particularly in South Africa (McConnachie et al., 2010; Shabbir et al., 2019a). The ecological and socio-economic impacts of *P. hysterophorus* in South Africa are well documented (Reynolds et al., 2020), and several control measures have been initiated. Mechanical and chemical controls were deemed unsustainable for large-scale management and if executed poorly can easily become counter-productive, exacerbating the problem (Strathie et al., 2011; Bajwa et al., 2016). Land management alone has emerged as a potential control tool but is likely only to slow the invasion and spread of *P. hysterophorus* (Terblanche et al., 2016; O'Connor and van Wilgen, 2020).

As such research efforts have remained focused on biological control, one of the few long-term and cost-effective options available for controlling *P. hysterophorus* at a national scale (Dhileepan and Strathie, 2009; Strathie et al., 2011). However, biocontrol efforts have had limited success to date, likely owing to the relatively recent release of agents (Strathie et al., 2016). With the recent drafting of a national management strategy, control efforts are now being pushed towards integrated management (Strathie and McConnachie, 2019). Although these integrated strategies are yet to be formally initiated, they hold promise for limiting the spread and impact of *P. hysterophorus* in South Africa (Terblanche et al., 2016).

Implications of *Parthenium hysterophorus* invasion in South Africa

Insights into the spread and impacts of the invasion

Parthenium hysterophorus has increased its range by ~500% over the past 10 years, particularly within savannas, grasslands and their rangelands (van Wilgen and Wilson, 2018).

This ongoing invasion remains a major threat to South Africa's rangelands, which comprise more than 70% of the country's land surface and support R30 billion worth of domestic livestock and wild game per annum (Mucina and Rutherford, 2006; O'Connor and van Wilgen, 2020). Therefore, understanding the extent of *P. hysterophorus's* potential invasion and identifying the regions likely to be impacted within South Africa is crucial. It is well accepted that much of South Africa remains climatically well-suited to invasion by *P. hysterophorus*, particularly areas with > 500 mm of rainfall / annum (McConnachie et al., 2010; Bajwa et al., 2017). However, ecophysiological research in Chapter 2 highlighted the tolerance of *P. hysterophorus* to water stress, suggesting that the weed is likely to persist and establish viable populations / seedbanks in regions with lower summer rainfalls, potentially as low as 400 mm per annum. This may allow for the intensification of invasion within areas already infested with *P. hysterophorus*, and more concerningly, broaden the weed's potential invasion range (Figure 8.1). This highlights the importance of addressing current *P. hysterophorus* invasions as well as limiting the spread of the weed across South Africa.

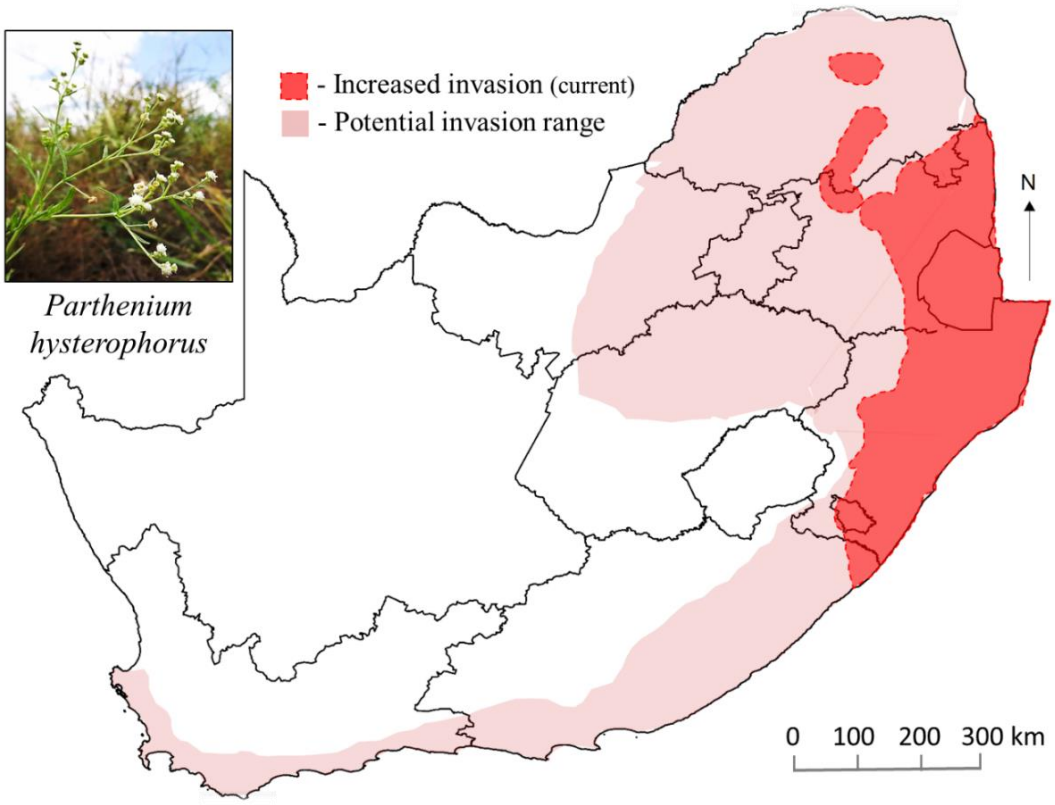

Figure 8.1. Current and potential *Parthenium hysterophorus* distribution in South Africa. (map generated using Climex with data taken from McConnachie et al., 2011; Henderson, 2017; Cowie et al., 2020a).

Reducing the invasion through land management

Several studies demonstrate that losses in grass cover, primarily due to overstocking and the associated high levels of grazing and disturbance, allow *P. hysterophorus* to drastically increase in abundance and impact (Dhileepan and Strathie, 2009; Nigatu et al., 2010; Belgeri et al., 2014). The poor management of numerous savannas, grasslands and their rangelands remain an ongoing concern, and one which will need to be addressed if problematic rangeland invaders, such as *P. hysterophorus*, are to be effectively managed (DiTomaso et al., 2010; O'Connor and van Wilgen, 2020). One of the management strategies proposed to limit the invasion and impact of *P. hysterophorus*, is the stocking of domestic livestock and wild game at sustainable rates within reserves and communal rangelands (Van Oudtshoorn, 2015; Strathie and McConnachie, 2019; Figure 8.2). To estimate such stocking rates, landowners typically divide the size of their property by the number of livestock or game present, commonly referred to as hectares per large stock unit (ha/LSU). This indicates the number of hectares required to sustain an animal (of 450-500 kg) for a year-long period, without depleting or degrading the area's natural resources (DAFF, 2017; Figure 8.2).

Although ha/LSU is a useful metric, it should not be used as an absolute. Depending on the quality of the land (i.e. levels of invasion), the type(s) of herbivores (grazers vs browsers) present and the overall the needs of the landowner, this may be altered further (Van Oudtshoorn, 2015; DAFF, 2017). Reducing the number of grazers allows for the recovery of native vegetation, particularly grasses and their associated basal and aerial cover, which gradually displaces and limits *P. hysterophorus* as seen in Chapter 3 and other studies (e.g. Nigatu et al., 2010; Belgeri et al., 2014). Furthermore, lowered grazing pressure is not only able to reduce *P. hysterophorus* within invaded properties but is likely to constrain the weed, slowing its spread to nearby uninvaded regions. However, this may be challenging to implement at a provincial or national scale for a variety of reasons including: (i) large swathes of rural land, used to sustain livestock, are often communal and unlikely to be fenced, (ii) high numbers of livestock are perceived as a "symbol of wealth" for many South African communities and enforcing reductions in livestock numbers may be a culturally sensitive topic and (iii) resources needed to enforce these reductions are unlikely to be made available, owing the country prioritising access to clean water, food and housing in many of these areas (Strathie et al., 2011; Terblanche et al., 2016; Strathie and McConnachie, 2019).

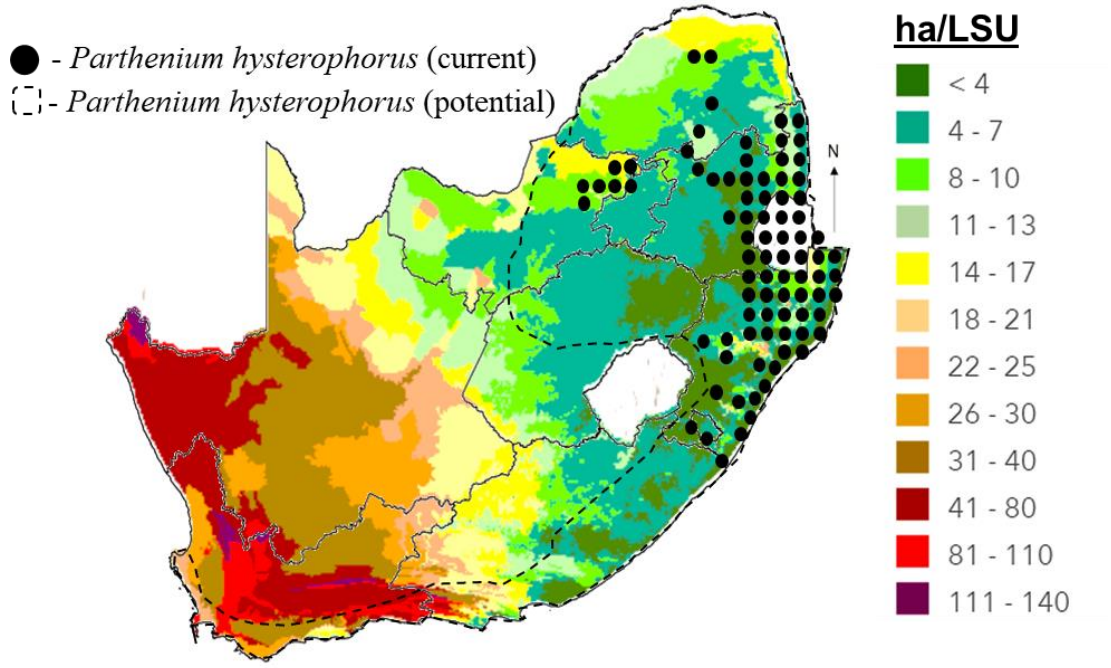

Figure 8.2. Recommended grazing capacity (ha/LSU), for the sustainable upkeep of livestock / game across South Africa as well as the potential prevention or limitation of *Parthenium hysterophorus*. (Data adapted from DAFF, 2017; Henderson, 2017).

Insights into the control of *Parthenium hysterophorus* in South Africa

Implications for chemical control

The use of registered pre- and post-emergent herbicides remains a useful small-scale control for the management and eradication of *P. hysterophorus* in areas, such as commercial agriculture, urban areas as well as road and rail-sides (Goodall et al., 2010; Shabbir et al., 2014; Bajwa et al., 2016). Research in Chapter 4 highlighted potential reductions in herbicide (glyphosate) efficacy in the future, under elevated CO_2 conditions. In order to maintain efficacy as well as limit the potential for herbicide resistance, it is imperative that dosages along with the correct spraying procedures be strictly adhered to (Bracamonte et al., 2016; Heap and Duke, 2018). Likewise, the rotation of herbicides, with different formulations as well as modes of action, such as Picloram, should further reduce the probability of developing populations less susceptible to herbicide in the future (Campbell et al., 2019; Bajwa et al., 2019).

The ongoing invasion of *P. hysterophorus* will undoubtably see an increase in the demands for chemical control in South Africa, however, it is unlikely that the country will be able to meet these financially or logistically (Strathie and McConnachie, 2019). This shortfall will require the integration of chemical control programmes to be prioritised, such as those

outlined by Terblanche et al. (2016). This also suggests that in the interim, South Africa will need to optimise the remaining *P. hysterophorus* controls, such as biological control, to limit the invasion and impact (Strathie et al., 2011).

Improving our current biocontrol efforts (viz. Zygogramma bicolorata)

South African biocontrol efforts against *P. hysterophorus* are yet to attain their full potential, owing to the recent release of agents, their poor establishment and limited impact, particularly from the leaf feeding beetle *Zygogramma bicolorata* (Strathie and McConnachie, 2019). Although disappointing, researchers should not be completely discouraged, nor abandon, *Z. bicolorata* as an agent, as similar struggles were experienced and overcome in Australia. Much like South Africa, initial releases of *Z. bicolorata* in Australia were met with poor establishment (~10% success) and low levels of impact (Dhileepan and McFadyen, 2012). Despite these initial establishment concerns, Z. *bicolorata* populations gradually adapted to the drier conditions and over a decade, became more widely established, contributing to the substantial reduction of *P. hysterophorus*, with ~ 12 000 km^2 of the weed being defoliated to date (Dhileepan and McFadyen, 2012).

Furthermore, ecophysiological studies undertaken in Chapter 5 highlight and re-affirm the potential of *Z. bicolorata* to be an extensively damaging agent of *P. hysterophorus* in South Africa, should constraints including plant quality and predation be addressed (McConnachie, 2015a; Chidawanyika et al., 2017). Quick and effective solutions to some of these biocontrol constraints may be attained through alterations to the release strategy of the agent (Cowie et al., 2018a). The fertilising of relatively small patches of *P. hysterophorus* prior to, and during, the release of *Z. bicolorata* is likely to overcome the hinderances associated with low plant quality, aiding in the development, persistence and establishment of larger and more damaging *Z. bicolorata* populations (Chapter 6). Immediate reductions in the predation of *Z. bicolorata*, may be achieved through the release of only adult beetles, the least susceptible stage to predators (Abels, 2017). Likewise, increasing the number of agents released at a site, proportionally limits the losses to predation, increasing the probability for establishment and persistence (Gupta et al., 2004). Furthermore, carrying out releases during mid-spring, just as *P. hysterophorus* germinates and when predator populations remain low, may further improve the effectiveness of the beetle (Schulz et al., 2019).

Pursuing additional biocontrol agents

Ongoing biocontrol research, both in South Africa and abroad, suggests that a suite of agents will be required to achieve effective control over *P. hysterophorus* at a national level (Dhileepan and Strathie, 2009; Strathie et al., 2011). Failing to consider or shelving potentially safe and effective agents remains a concern expressed in nearly all biological control programmes (Downey and Paterson, 2016). Therefore, native range explorations for new candidates as well as the revision of potential candidate agents is likely to be of benefit to ongoing *P. hysterophorus* biocontrol efforts in South Africa (see Table 8.1). Research into new agent(s) should focus on either (i) increasing biocontrol impacts by acquiring new highly damaging agents or (ii) by establishing new agents in regions that are unsuited to or experience little impact from current agents (Cowie et al., 2018a).

The most promising impacts are likely to be made with the addition of agents known to be damaging in other similarly affected countries, particularly Australia (Strathie et al., 2011). Two such agents, yet to be released in South Africa, include the root-crown feeding moth *Carmenta* nr. *ithacae* Beutenmüller (Lepidoptera: Sesiidae) and the stem-galling moth *Epiblema strenuata* Walker (Lepidoptera: Tortricidae) (Dhileepan et al., 2019; Table 8.1). The root-crown feeding moth *C.* nr. *ithacae* is believed to be highly host-specific and damaging, with high numbers resulting in plant mortality, however, concern surrounds the moth's localised establishment and initially low abundances which take time to build up (Dhileepan and McFadyen, 2012). In contrast, *E. strenuana* maintains the potential to be widespread, abundant and damaging but the moth is not monophagous, found to utilise two exotic plants (*Ambrosia artemisiifolia* L. and *Xanthium strumarium* L.) as well as an oil-seed crop (*Guizotia abyssinica* Cass.) during host-specificity trials in South Africa (Strathie et al., 2011; McConnachie, 2015b). The complete development of *E. strenuana* on various cultivars of *G. abyssinica*, despite the fact the crop has no commercial value in southern Africa, remains a concern and one which is likely to preclude its release in South Africa until host-specificity issues can be resolved (McConnachie, 2015b; Dhileepan et al., 2019).

The remaining agents which were not considered for importation into South Africa (Table 8.1), given their poor establishment and/or low levels of perceived damage after release in Australia, and included a leaf-mining moth *Bucculatrix parthenica* Bradley (Lepidoptera: Bucculatricidae), a stem-galling weevil *Conotrachelus albocinereus* Fiedler (Coleoptera: Curculionidae), a stem-boring moth *Platphalonidia mystica* Razowski & Becker

(Lepidoptera: Tortricidae) and a sap-sucking planthopper *Stobaera concinna* Stål (Homoptera: Delphacidae) (Dhileepan et al., 2019). However, the moth *B. parthenica* and weevil *C. albocinereus* may warrant reconsideration, as they can be moderately damaging and are likely to establish alongside the current suite of biological control agents released in South Africa (Dhileepan and McFadyen, 2012; Table 8.1).

The feasibility of restoration efforts as a control method?

The restoration of weed invaded landscapes is a challenging, and frequently overlooked, aspect associated with the management of invasive plants (Shabbir et al., 2013; Van Oudtshoorn, 2015). In the case of *P. hysterophorus*, few restoration studies have been conducted in Southern Africa, likely owning to the fact the weed remains largely uncontrolled. Although the findings from Chapter 7 suggest that the sowing of native perennial grasses may be a worthwhile endeavour to aid in the suppression of *P. hysterophorus*, the practicality of this control must be considered. The establishment of grasses, from seed, can be challenging and costly, requiring intensive labour as well as low grazing levels (Khan et al., 2013, 2019), which may deter landowners.

This suggests that should large scale restoration efforts be attempted, they should be focused on grassland or savanna reserves, which are already appropriately stocked (i.e. > 4 ha/LSU in grasslands and > 10 ha/LSU in savannas) (Van Der Laan et al., 2008; O'Connor and van Wilgen, 2018). In addition, the selection of grasses will require careful consideration as to balance restoration efforts with the long-term goals of the property / landowner (Shabbir et al., 2013; Khan et al., 2014; Van Oudtshoorn, 2015). More importantly, when utilised alone restorative efforts are unlikely to completely manage *P. hysterophorus* and may take prolonged periods of time to become effective, as seen in Chapter 7. Therefore, the integration of restorative efforts with other controls such herbicides, land management or biocontrol, is strongly advocated to increase the efficacy of native grasses in suppressing *P. hysterophorus* (see Khan et al., 2014, 2019; Shabbir et al., 2013, 2019b, 2020).

Table 8.1 Potential candidate agents which may be re-considered for biological control efforts against *Parthenium hysterophorus* in South Africa (data from Klein, 2011; Strathie et al., 2011; Dhileepan and McFadyen, 2012; Dhileepan et al., 2019).

Species	Order: Family	Damage	Status	Concern(s)
Priority candidates				
Carmenta sp. nr ithacae	Lepidoptera: Sesiidae	Root-feeding	Under investigation	Untested / localised damage
Epiblema strenuana	Lepidoptera: Tortricidae	Stem-galling	Under investigation	Host-specificity
Reconsideration				
Bucculatrix parthenica	Lepidoptera: Bucculatricidae	Leaf-mining	Shelved	Low damage
Conotrachelus albocinereus	Coleoptera: Curculionidae	Stem-galling	Shelved	Low damage
Reconsideration (low priority)				
Platphalonidia mystica	Lepidoptera: Tortricidae	Stem-boring	Shelved	Poor establishment
Stobaera concinna	Homoptera: Delphacidae	Sap-feeding	Shelved	Poor establishment
Released (South Africa)				
Listronotus setosipennis	Coleoptera: Curculionidae	Stem-boring	Released (2013)	Localised damage
Puccinia xanthii	Pucciniales: Pucciniaceae	Leaf rust	Released (2010)	Localised establishment
Puccinia abrupta	Pucciniales: Pucciniaceae	Leaf rust	Locally occurring	Localised establishment
Smicronyx lutulentus	Coleoptera: Curculionidae	Seed-feeding	Released (2014)	Localised establishment
Zygogramma bicolorata	Coleoptera: Chrysomelidae	Leaf-feeding	Released (2013)	Poor establishment / damage

Integrating management to enhance *Parthenium hysterophorus* control

To date, the biological and chemical control of *P. hysterophorus* have attained limited success in South Africa suggesting that *P. hysterophorus* is best suited for an integrated management approach (Terblanche et al., 2016; Strathie and McConnachie et al., 2019). Although South Africa drafted a national management strategy for the control of *P. hysterophorus* in 2016, which calls for integrated approaches, it is yet to be formally adopted (van Wilgen et al., 2020). The inclusion of biocontrol within an integrated management context was aimed at (i) reducing the invasion and spread of *P. hysterophorus* in areas that are large or costly to clear, (ii) minimising the impacts of the weed and (iii) lowering the costs of current and future clearing efforts (Pimentel, 2011; Terblanche et al., 2016; Figure 8.3). The combination of manual, chemical and biological controls is complex, but conceptual frameworks are useful in selecting and integrating the most appropriate controls in the field (see Figure 8.4).

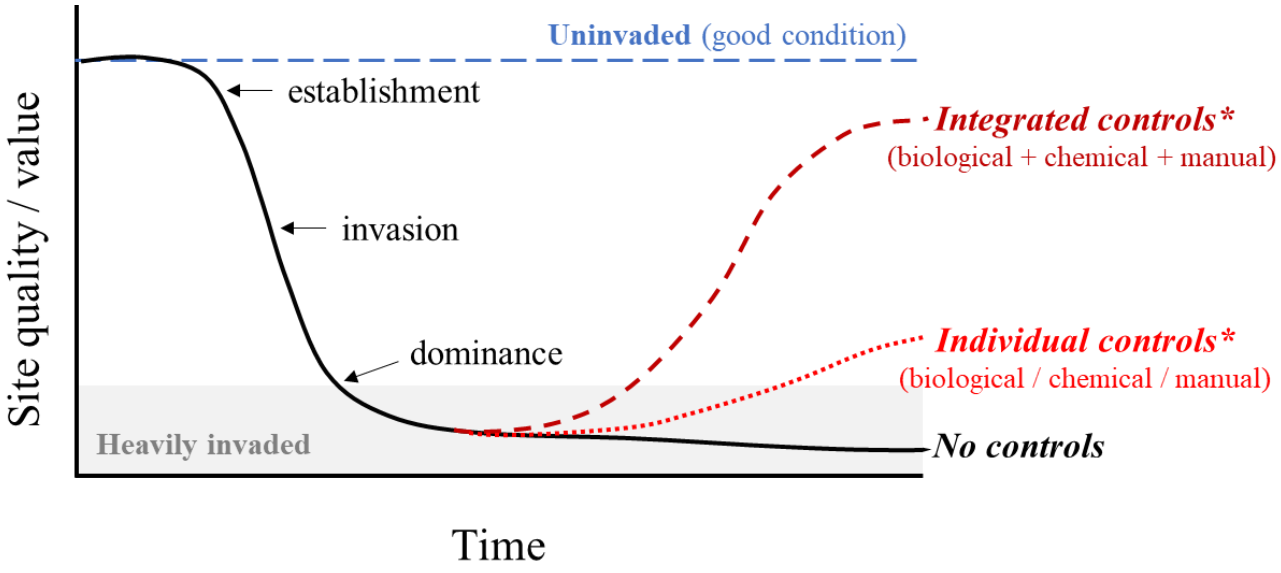

Figure 8.3. Theoretical diagram highlighting the importance of integrating controls to reduce *Parthenium hysterophorus* invasion and restore the quality and value of invaded land (adapted from DiTomaso et al., 2010). *- assuming controls are carried out following best practice guidelines.

The detection of *P. hysterophorus* is done using roadside surveys (Henderson, 2001; 2017), however, advances in remote sensing techniques are likely to aid in monitoring invasions and identifying sites or areas in need of control (Terblanche et al., 2016; Kganyago et al., 2017). Based on the size of these *P. hysterophorus* infestations as well as the economic

or ecological value of the site, controls can be implemented (Figure 8.4). Manual removal of *P. hysterophorus*, although unsustainable at large-scales, remains a key tool for many subsistence farmers in rural areas of South Africa. Chemical clearing is carried out using registered foliar herbicides, which are applied to young, non-flowering, plants during the early spring and summer months (Goodall et al., 2010). Biological control efforts involve the mass-rearing and release of agents, predominantly *Z. bicolorata, L. setosipennis* and *S. lutulentus*, into suitable infested areas (Strathie and McConnachie, 2019). Although biological control is the core element of an integrated control, it is likely to be aided good land-use practices, where possible (Terblanche et al., 2016; Figure 8.4).

Combining the most effective controls is often the primary focus of integrated control, however, education and awareness surrounding *P. hysterophorus* invasions remains an equally important component of these programmes. Increases in awareness and education not only serve to warn the public about the detriments of the weed (Byrne et al., 2020), but aid in the slowing and prevention of further invasions, through the adoption of best management practices, ultimately allowing for more rapid and effective responses (Terblanche et al., 2016; Campbell et al., 2019). Although researchers are often reluctant to engage with the public, this practice can be highly beneficial, as seen with the effective re-distribution of *Z. bicolorata* by the public and landowners in both Australia and India subsequent to awareness campaigns (Dhileepan and Strathie, 2009; Dhileepan and McFadyen, 2012).

The significance of *P. hysterophorus* as a global weed, presents the opportunity for various collaborative efforts towards effective management, as previously seen between South Africa and Australia (Strathie et al., 2011). Maintaining good relations with Australia is paramount, given the country's long-standing history and insights into the management of *P. hysterophorus* (McFadyen et al., 2019). Likewise, advancing South African - American relations are particularly important, as they offer the opportunities including (i) native range explorations for new, potentially more damaging agents and (ii) the ability to conduct open-field tests of new or previously shelved agents within their native range(s). Such collaborations may identify new promising agents or support the release of agents, currently hindered by ambiguous host-specificity data (Cowie et al., 2018a), such as the damaging stem-galling moth, *E. strenuana* (McConnachie 2015b). Although building relations is undoubtably a valuable endeavour, this may prove challenging for many South American countries, including Brazil and Argentina, given their current political climates.

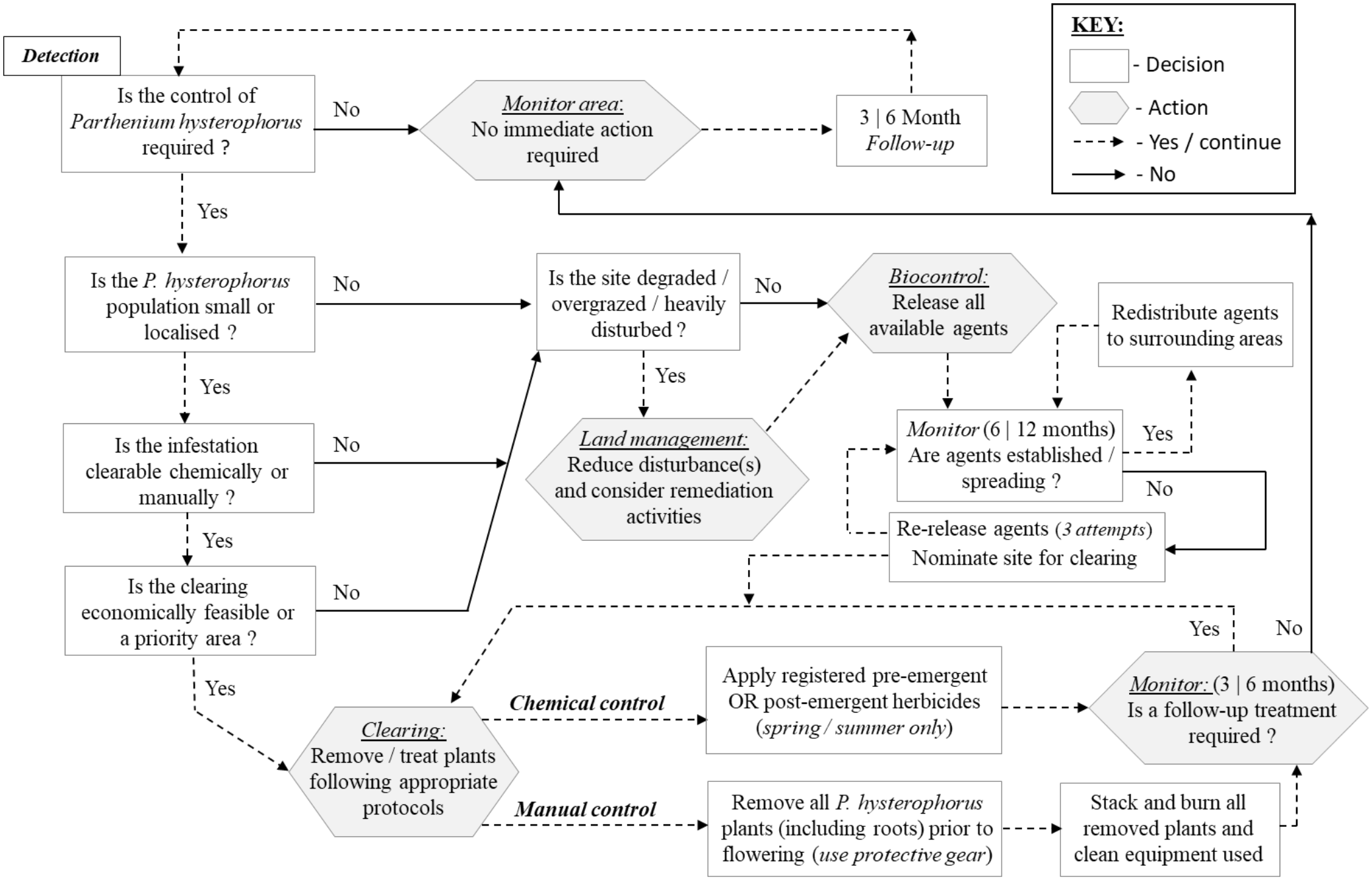

Figure 8.4. Conceptual decision diagram proposing integrated management of *Parthenium hysterophorus* in South Africa, incorporating biological (biocontrol), chemical and manual controls as well as land management and remediation efforts (Adapted from Cowie et al., 2018a).

Conclusion

Overall, it is hoped that this research will serve as a useful resource, contributing towards the understanding and control of *P. hysterophorus*, not only within South Africa, but in other similarly affected countries on the African continent (Table 8.2). This thesis highlights not only the detriment of *P. hysterophorus* but crucially provides insights into improving the current and potential control efforts in South Africa, including land management, herbicide (viz. glyphosate), biological control and restoration. Although biological and chemical controls still hold promise, they will need to be formally integrated to increase their impact and subsequent success at a regional / national level. Likewise adopting good land management practices, throughout South Africa may serve to limit the spread, invasion and impact of *P. hysterophorus*. Ultimately, achieving control over *P. hysterophorus* in South Africa will be a long-term and challenging endeavour, and one which will require continued support and investment from government, researchers, landowners and the public.





Table 8.2 Literature summary pertaining to the invasion and control of *Parthenium hysterophorus* in Africa. *- Key knowledge gaps addressed or contributed to by this thesis.

Subject	Author(s)	General focus
Parthenium hysterophorus		
	(Belz et al., 2007)	Allelopathic potential
	(Belz et al., 2009)	Allelopathic effects
	(Cowie et al., 2020a)*	Effects of water-stress
	(Henderson and Wilson, 2017)	South African distribution (update)
	(Kganyago et al., 2017)	Detection (remote sensing)
	(McConnachie et al., 2010)	Current / Future distribution
	(Nigatu et al., 2010)	Impacts on grazing
	(Reynolds et al., 2020)	Impact on livelihoods
	(Strathie and McConnachie, 2019)	History and management
	(Tamado et al., 2002)	Germination ecology
	(Tefera, 2002)	Allelopathic effects (native grass)
	(Wabuyele et al., 2014)	East African distribution (road survey)
	(Wakjira et al., 2009)	Allelopathic effects (food crop)
Biological control		
	(Abels, 2018)	Predation effects (constraints)
	(Cowie et al., 2018b)*	Ecophysiology assessment (impact)
	(Cowie et al., 2019)*	Plant quality (constraints)
	(McConnachie, 2015a)	Host-specificity (*Z. bicolorata*)
	(McConnachie, 2015b)	Host-specificity (*E. strenuana*)
	(King, 2008)	Climatic suitability (*Z. bicolorata*)
	(Retief et al., 2013)	Host-specificity (*P. xanthii*)
	(Shoba, 2011)	Climatic suitability (*L. setosipennis*)
	(Strathie et al., 2011)	General overview (biocontrol initiation)
	(Strathie et al., 2016)	Update on establishment and impact
	(Dhileepan and Strathie, 2009)	General overview (biocontrol initiation)
	(Wood and Scholler, 2002)	Biocontrol (*P. abrupta*)
Other controls		
	(Goodall et al., 2010)	Efficacy assessment (general controls)
	(Cowie et al., 2020b)*	Herbicide efficacy (current/future)
	(Cowie et al., unpublished)*	Influence of land management
	(Cowie et al., unpublished)*	Sowing native grass seed (restoration)
	(Terblanche et al., 2016)	National integrated control strategy
	(Van Der Laan et al., 2008)	Interference potential of grasses

Popular articles: were also produced during the PhD to aid in disseminating information on the impacts of *P. hysterophorus* as well as our biological control efforts to the general public:

General information: https://theconversation.com/how-south-africa-is-keeping-its-worst-invader-famine-weed-at-bay-89783

Biocontrol efforts: https://sciencetoday.co.za/2017/12/01/beating-famine-with-a-beetle/

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