Invasion of *Campuloclinium macrocephalum* (Less.) DC in highveld grassland: ecology, control and non-target impacts

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Declaration

I declare that this thesis is my own, unaided work, unless otherwise noted within the text. It is being submitted for the Degree of Doctor of Philosophy at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.

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24 day of MAY 2016 in PIETERMARITZBURG

Abstract

This thesis reveals previously unknown facts concerning the invasion, ecology and management of the perennial alien forb *Campuloclinium macrocephalum* (Less.) DC. (Asteraceae, pompom weed) in the grassland biome of South Africa. All these areas of research are of critical importance to identify the causes of invasion and prescribe best management practises aimed at reducing the density and spread of the weed and restoring these ancient grasslands. Grassland biomes throughout the world are receiving international attention because of their vulnerability to transformation, a history of ignorance regarding their evolution and prejudice as evidenced by their exploitation.

There are many theories as to why alien plants become invasive outside of their native range but most are controversial, except those that support the absence of natural enemies as the primary reason for invasiveness. Few studies have attempted to empirically measure environmental and ecological factors that facilitate invasion, not to dispute the Absence of Predators Hypothesis (APH); APH regulates invasiveness but the external factors affecting habitat vulnerability to invasion remain unchanged. Based on the assessments of 80 invaded grasslands in Gauteng Province prior to the release of any host-specific biocontrol agents, it was concluded that *C. macrocephalum* favours disturbed grasslands. Numerous agencies of disturbance were identified; the most important being heavy grazing, abandonment (e.g. old lands) and modification (e.g. draining of wetlands). Herbivory by generalist insects was insignificant. The main drivers of native species composition in the invaded study sites were rainfall, topography and soil texture. The weed was most problematic in grasslands with a basal cover of <19% and in poor condition from a pastoral perspective. Other alien and native invasive species were also found in grasslands with *C. macrocephalum*. High fire frequency also appeared to exacerbate weed density.

The Novel Weapons Hypothesis postulates that some invasive species transform vegetation for establishment, densification and expansion because they are allelopathic. Stems and leaves of *C. macrocephalum* have both rigid multicellular hairs and glandular trichomes that exude an unidentified substance. It has been speculated that allelopathy may be an important trait aiding its rapid expansion in invaded grasslands. The importance of allelopathy and competition was investigated under controlled conditions using *Eragrostis curvula* (perennial grass), *E. tef*, (annual grass) and *Lactuca sativa* (lettuce) as test species. Petri-dish studies proved that root and shoot extracts of adult *C. macrocephalum* plants had zero inhibitory effect on the seed germination in all three test species. Stunting of radicles was evident in treatments comprising leaf extracts at 10 and 25% w/v; with *E. tef* displaying a higher tolerance than *E. curvula*. *Eragrostis curvula,* because it was the most sensitive of the test species, was used in a pot study together with *C. macrocephalum* to evaluate allelopathy and interference. The biomass and growth of *E. curvula* was not affected by *C. macrocephalum* at densities of one or five plants per pot. The weed on the other hand incurred density-dependant trade-offs in size, biomass and mortality. In a separate pot study, the incorporation of weed residues into the potting medium had no impact on the growth of *E. curvula*. The process went one step further by analysing the association between *E. curvula* and *C. macrocephalum* from the 80 grassland assessments. *Eragrostis curvula* had a narrower ecological niche and was only found in disturbed grasslands on well drained soils. *Campuloclinium macrocephalum* invaded a broad gradient of soil types including poorly drained wetland soils not amenable for *E. curvula*. Competitive exclusion between the two species was not apparent. Pot studies and field observations support a degree of tolerance between *C. macrocephalum* and *E. curvula* that lends support to coexistence at a range of weed densities. The conclusions from this study were (a) *C. macrocephalum* is not allelopathic and allelo-chemistry cannot be inferred as a causal mechanism for the weed's invasiveness, and (b) *C. macrocephalum* and *E. curvula* have different limiting resource requirements that enables coexistence in areas where the latter can grow.

In 2006 *C. macrocephalum* infestations in Gauteng Province were severely damaged by *Puccinia eupatorii* Dietel (Pucciniaceae), a biotrophic rust identical to the *P. eupatorii* strain in quarantine that was imported into South Africa from Argentina as a potential biocontrol agent. An investigation of the rust's impact on *C. macrocephalum* was carried out in the 80 rangeland infestations previously assessed and how pathogen pressure affected the weed's realised niche. The rust caused premature senescence of the stems in late summer with compensatory regrowth in autumn. In contrast disease-free plants senesced in late autumn; the rootstocks remained dormant throughout winter and did not produce compensatory regrowth. No significant changes in weed density were detected and *C. macrocephalum* retained its realised niche. The weed remains adapted to the rust despite a 40-year separation from the pathogen.

Registered herbicides should provide 80% control of the targeted plants, providing label directions concerning concentration, mixing, application and environmental considerations are adhered to. Herbicide trials were conducted at two contrasting sites in Gauteng, a wetland and a rocky grassland, to test the efficacy of picloram and metsulfuron-methyl on the control of *C. macrocephalum*. Both sites had dense infestations of *C. macrocephalum* at the beginning of the study in 2005. Herbicides were applied in either February (summer) or April (autumn) annually for three consecutive years. Monitoring continued for an additional three years after spraying was terminated. *Puccinia eupatorii* established at both study sites from the second year of study. Both sites also experienced wildfires and drought. As these uncontrolled factors were not anticipated in the design their individual effects could not be factored out. Mean mortality however was <80% expected of registered herbicides which can only be attributed to uncontrolled factors acting as natural constraints. The efficacy of picloram was not significantly different ($P<0.05$) to that of metsulfuron methyl within a season. Autumn applications are not recommended because rust damage on the leaves is too advanced to ensure adequate herbicide uptake and translocation. Herbicide persistence in the topsoil was not detected by gas chromatography. Three applications of herbicide were inadequate to bring *C. macrocephalum* under control in plots. It is estimated that five to seven years of herbicide application are needed to reduce the weed density to <1 plant per plot (25 m^2). This effectively renders chemical control in medium to dense infestations uneconomic. Chemical control of *C. macrocephalum* will only be effective if there is a commitment to follow-ups and remedial vegetation management practices.

The herbicide trials also looked at the impact of picloram, metsulfuron methyl and hoeing on native species and other alien species that occurred in plots. Three plant functional groups were analysed, namely native grasses, native forbs and alien broadleaf species. The herbicides were applied as broadcast sprays over three years and therefore their effect on all broadleaf species was non-selective. Hoeing twice a year for three years only targeted *C. macrocephalum.* High forb richness coupled with low abundance and patchy distribution meant there was a high turnover between replicate plots across the study sites. Herbicides had a significant impact on the native forb functional group compared to hoeing which had a superficial effect. Hoeing did not suppress *C. macrocephalum*. The parasitic forb *Thesium utile* was killed in all treatments except the untreated control. The target-specific hoe treatment also eliminated *T. utile*, suggesting a new association might exist between parasite and *C. macrocephalum*. Hoeing also facilitated the establishment of alien annual weeds.

In South Africa the grassland biome is under considerable threat from mining, afforestation, agriculture and urban development. Although alien plants pose a minor threat in contrast to these land-transforming activities they remain the greatest threat to grasslands that are not threatened by exploitation. *Campuloclinium macrocephalum* is not a pioneer species, but rather a long-lived perennial herb with evolutionary adaptations to fire, herbivory and disease. These characteristics have enabled it to establish and spread in grasslands that have been maintained in a variety of disturbed states by a range of agencies that reduce grass basal cover and exacerbate soil erosion. Addressing poor land use practises that foster *C. macrocephalum* invasion and spread are as important as the remedial activities necessary to control the species. Grassland restoration/rehabilitation practises have not been investigated in the context of *C. macrocephalum* management and is of critical importance to the integrated control of the weed.

Although comprehensive literature about *C. macrocephalum* have recently been published gaps in our understanding of its biology, ecology and control still exist that prevent the development of best management practices. These areas for new research make ideal projects for post-graduate students. Future research should focus on (a) rust–herbicide interactions, (b) fire and seedbank dynamics, (c) integrated weed management incorporating biological control, fire, selective herbicide application techniques (e.g. spot-spraying) and ecosystem restoration practises. Grazing strategies promoting grass species adapted to frequent non-selective defoliation restrict pompom weed better than degraded underutilised rangelands, as evidenced by low levels of *C. macrocephalum* in communal areas, and warrants further investigation, including fence-line contrasts in invaded and un-invaded road reserves.

Key words: allelopathy, altered growth, efficacy, environmental factors, fire, hoeing, interference, invasion, non-target impacts, pathogen, pompom weed, registered herbicides, regrowth, seedlings, uncontrolled factors, vegetation assessments

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I would like to dedicate this work and the years spent on it to my daughter Casey and my son Liam. I only wish I had spent more time with you while you were still at home. With love, Pops.

Glossary of abbreviations & terms

APH - Absence of Predators Hypothesis

Decreaser species (D) – Palatable species which dominate pristine grasslands under ideal conditions but decline under selective grazing pressure

EICA - Evolution of Improved Competitive Ability Hypothesis

ERCA - Evolutionary Reduced Competitive Ability Hypothesis

ERH - Enemy Release Hypothesis

Highveld – South African inland plateau which has an altitude above 1500 m with characteristic veld types (see veld)

Increaser species (I1-I3) – Species of varying palatability and fodder value which increase under suboptimal management, e.g. under-grazing (I1), overgrazing (I2) and selective grazing (I3)

NWH – Novel Weapons Hypothesis

Veld - rural landscape in Southern Africa covered in grass or low scrub (see highveld)

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1. INTRODUCTION

PROJECT RATIONALE

Campuloclinium macrocephalum (Less.) DC. (Asteraceae, tribe Eupatorieae; common name pompom weed), a plant of South American origin, is a declared weed in South Africa in terms of Regulation 15 of the Conservation of Agricultural Resources Act (Act 43 of 1983) and section 97(1) of Alien and Invasive Species Regulations (2014) of the National Environmental Management: Biodiversity Act (Act No. 10 of 2004). It remained a sleeper weed for about 30 years after its introduction in the 1960s, probably from Argentina based on its genetic diversity in South Africa (Gitonga *et al.* 2015), when it was first planted around Pretoria city in Gauteng (G in Fig. 1) for ornamental purposes (Goodall *et al.* 2011). Sleeper weeds are alien species that display a lag phase of several years or decades before becoming invasive (Grice & Ainsworth 2003). The plant is only a weed problem in South Africa and its presence and invasive status in other parts of the world is currently unknown.

The distribution of *C. macrocephalum* in South Africa (black dots in Fig. 1) includes the provinces of Gauteng (G) where it is most abundant, Mpumalanga (M), KwaZulu-Natal (K) and Limpopo (L), with localised infestations in North West (NW), Free State (F) and Eastern Cape (EC). It is already ubiquitous in Gauteng but its potential distribution in the remainder of South Africa is enormous (grey dots in Fig. 1) and displays a high affinity with the grassland and savanna biomes (Fig. 2), which are the most susceptible to invasion (Trethowan *et al.* 2011). The weed is also predicted in invade the neighbouring counties of Lesotho (Ls) and Swaziland (S) (Fig. 1). Much like other members of the Eupatorieae (e.g. *Chromolaena odorata* (L.) R.M.King & H.Rob.), it could become a seriously problematic weed in similar environments globally.

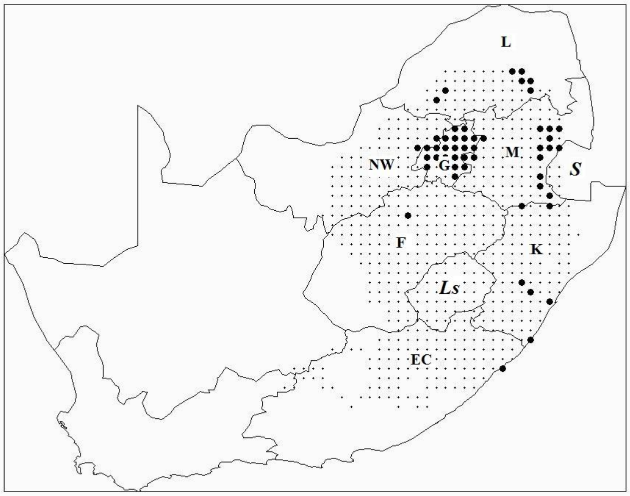

Fig. 1: Distribution of *Campuloclinium macrocephalum* in South Africa (black dots). Grey dots illustrate the potential distribution. Provinces mentioned above are denoted by the letters G, M, K, L, NW, F and EC. The sovereign states of Lesotho (Ls) and Swaziland (S) are included in its potential distribution. Courtesy of Lesley Henderson, administrator of the South African Plant Invader Atlas (SAPIA), Agricultural Research Council, URL: http://www.arc.agric.za/arc-ppri/Pages/Weeds%20Research/Geographical-distribution-of-IAPs-in-southern-Africa-(SAPIA)-.aspx.

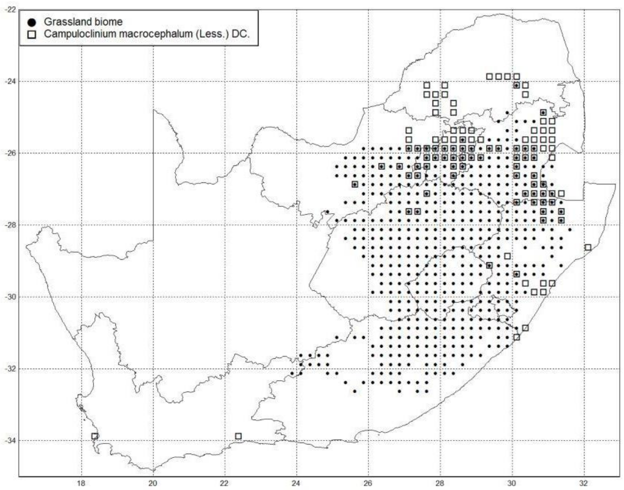

Fig. 2: The overlap in distribution of *Campuloclinium macrocephalum* and the extent of the grassland biome in South Africa. Courtesy of Lesley Henderson, administrator of SAPIA, Agricultural Research Council (2015).

The main hub of invasion is in Gauteng Province (G in Fig. 1). This infestation is disconnected from infestations in surrounding provinces (Fig. 1) and it covers a relatively large area (± 12 000 km^2). The grassland biome is well represented in six of South Africa's eight provinces, the exceptions being the Western and Northern Cape provinces. The study of the ecological impact and control of a highly invasive alien plant under different environment conditions in an isolated mega-infestation is a unique opportunity to quantify the problem and provide solutions that could mitigate spread. This is the first time *C. macrocephalum* has been the focus of ecologically-based field research in South Africa.

Research into the ecology and control of *C. macrocephalum* would help prevent this recent weed problem reaching disastrous proportions in South Africa. This in itself it a daunting task because once an Invasive Alien Species (IAP) has established, it is almost impossible to eradicate and control efforts, including the research and development phase of integrated weed management, are costly (Panetta *et al.* 2011; Panetta 2015). Hence if we can fully understand its ecology and its control in South Africa, although we may be unable to pre-empt its invasion on other continents, we would be in a strong position to provide assistance in the form of best management practices and biological control agents.

LITERATURE REVIEW

Campuloclinium macrocephalum is an understudied species whose invasion behaviour, ecology, environmental impact and control remain unknown. Consequently, more questions are raised than answered. The Gauteng infestations occur mostly along roadside (93%), the remaining infestations are found in wasteland, urban open space and veld[1] (Lesley Henderson pers. comm., SAPIA co-ordinator). Roadside / road reserve appears to be the primary habitat and facilitator of spread. Veld remains largely unaffected by pompom weed, < 7% of infestations occur in rangelands. Does highveld[2] grassland possess innate qualities that have functional resistance traits to invasion? Are these resistance traits arranged along hierarchical lines, i.e. the community as a collective whole (super-organism), functional groups or key / dominant species? Is the potential for invasion into veld just a question of time, i.e. is all veld unconditionally vulnerable? Are specific environmental factors required for invasion to take place? What factors are (positively or negatively) associated with invasion pattern? Are these factors external / environmental, intrinsic traits of the weed or both? Why is there such a dichotomy in the rate of spread between infestations on the highveld and the east coast? Clearly more than one ecological theory about invasiveness and invasion are relevant.

Evolution

One of the most well recognised principles in weed ecology is alien species become invasive because they were introduced without their natural enemies. Central to this theory and the practice of biological control is the Absence of Predators Hypothesis (APH) (Huffaker *et al.* 1976), or the Enemy Release Hypothesis (ERH) (Keane & Crawley 2002). Invasive alien plants profit from a lack of host-specific enemies, e.g. insects and fungal pathogens, and therefore have

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[1] "Veld is a type of rural landscape in Southern Africa covered in grass or low scrub with few trees similar to the Australian outback, North American prairies, South American pampas and the steppe of Central Asia." URL: https://en.wikipedia.org/wiki/Veld

[2] "Highveld is a portion of the South African inland plateau which has an altitude above roughly 1500 m. It is a region containing some of South Africa's most important commercial farming areas, as well as its largest concentration of metropolitan centres, especially the Gauteng conurbation." URL: https://en.wikipedia.org/wiki/Highveld

competitive advantages over indigenous species in every facet of their life cycles. Cronk and Fuller (1995) theorised that alien species would have a greater reproductive potential than native species, which are preyed upon by herbivores, pathogens and diseases, thus producing greater quantities of seed and consequently have large residual seed banks.

The Evolution of Improved Competitive Ability (EICA) hypothesis predicts that the lower abundance of herbivores in introduced countries causes evolutionary changes in the invasive species for reduced anti-herbivore defences (Blossey & Nötzold 1995). Subsequently plants invest more into growth and reproduction than into compounds that deter herbivory. This theory does not win unanimous approval amongst invasion biologists. Firstly, disinvestment in defensive mechanisms, e.g. reduced alkaloid levels in alien populations, would render them more prone to attack by generalist herbivores in the introduced range, creating equal pressure on both the alien and native competitors (Keane & Crawley 2002). Secondly, the change in allocation from defence to growth in introduced populations may be more the exception than the rule (Joshi & Vrieling 2005). Willis *et al.* (2000) tested the EICA hypothesis using growth experiments of four invasive alien weed species sampled from introduced and native habitats and concluded that there was no genetic basis for increased plant size, rather, changes were attributed to a plastic response to a new environment. The response of generalist invertebrate herbivores from native and introduced weed ranges offers more compelling evidence than a hypothetical evolutionary change of alien weed resistance to herbivory (Schaffner *et al.* 2011).

Bossdorf *et al.* (2004) not only refuted the EICA hypothesis but provided an alternative hypothesis called the Evolutionary Reduced Competitive Ability hypothesis or ERCA. In their experiments they found no differences between the progeny of native and invasive populations of *Alliaria petiolata* grown without competition, but the natives outperformed the invasives when they competed against each other. Under the ERCA hypothesis, if the ability to compete involves a trade-off in fitness traits, under conditions where there is less competition in the introduced range, natural selection acts against it and even reduces intraspecific interactions. It is possible that evolutionary adaptation and adaptive plasticity have become muddled. The capacity of some alien species with the same genotype to differ in phenotype or behaviour under novel or manmade environmental conditions occurs too rapidly to allow for an evolutionary response (Sultan 2004). Adaptive plasticity could explain why certain species occupy such broad ecological niches without the lag time required for local adaptation, making them more invasive than in their natural environment.

In experiments comparing the size and fecundity of native and invasive California poppies, Leger and Rice (2003) found the invasive poppies were better at maximizing growth and reproduction and concluded that a genetic shift had occurred, but did so without comparing genetic differences between the two. Maron *et al.* (2003) investigated *Hypericum perforatum* L. in its native range in Spain and Sweden (the species is indigenous to Europe, the Mediterranean and Asia), and introduced populations in California and Washington in the USA. They did not find differences in size or fecundity between alien and native populations but they did detect differences within introduced populations from different latitudes in the USA. Pre-adaptation was cited as not being the only reason for differences in clinal patterns in leaf area and plant performance. The authors also suggest evolutionary adaptation to large-scale environmental conditions in the introduced country. Large genetic variation could simply be genotype differences in traits selected geographically (climate, day-length) for growth following multiple introductions from Mediterranean and temperate parts of Europe. The call for rapid evolution is also inconclusive if what appears to be inadequate sample size is valid. Rapid phenotypic evolution can be caused by a reduction in genome size. Lavergne *et al.* (2010) found alien invasive genotypes in the introduced range had smaller genomes than the native genotypes in their home range. The smaller genome size produced phenotypes with increased invasive potential and faster juvenile growth rates. The phylogeographical relationships between alien invasive and native populations suggest genome reduction is by natural selection during the invasion phase. Reduced genome size could result in rapid changes in phenotypic attributes that improve invasiveness.

Evidence from manipulative experiments on invasive alien plants that attribute rapid evolution to increases in growth, size and fecundity are not unequivocal. In the absence of genetic studies that verify observed rapid evolution as a step towards speciation these experiments did not nullify natural selection, adaptation or the absence of natural enemies as possible reasons for increased vigour. Case studies on the biological control of invasive plants attest to the validity of the APH / ERH in aquatic and terrestrial ecosystems in South Africa. The complete control of floating aquatic species such as *Azolla filiculoides* Lam. (McConnachie *et al.* 2004), *Pistia stratiotes* L. (Cilliers 1991b) and *Salvinia molesta* D.S.Mitch. (Cilliers 1991a) with single insect agents are definitive examples. Yet external factors have also imposed restrictions on the efficacy of the biological control of other aquatic species. In the case of *Eichhornia crassipes* (Mart.) Solms climate incompatibility, high eutrophic levels, episodic flooding and exposure to herbicide were responsible for constraining the impact of several arthropod agents (Hill & Cilliers 1999).

Most of the success stories involve aquatic weeds but there are examples of complete biocontrol of terrestrial species. The family Cactaceae has the highest incidence of success, four out of 15 species, with one to two agent species responsible for causing complete control (Moran *et al.* 2011). Complete control of the following terrestrial weeds has been achieved biologically: *Ageratina riparia* (Regel) R.M.King & H.Rob. (one agent, pathogen), *Hypericum perforatum* (two insect agents) and *Sesbania punicea* (Cav.) Benth. (three insect agents) are effectively controlled by the synergistic interaction of insect agents. Empirical data from post release evaluations of 42 invasive alien species in southern Africa subjected to biological control show that 10 species are under complete control, 19 species still require integration with other control methods and 13 species remain completely reliant on other control methods. In some cases, predation/parasitism of biological control agents by generalists (Goeden & Louda 1976, McFadyen & Spafford 2004) or unfavourable environmental conditions (Byrne *et al.* 2002) are given when agent establishment fails, or insufficient suites of agents result in suppression of some phases of the plants life cycle (Hoffmann & Moran 2004). We can deduce from this that invasiveness in terrestrial species is strongly affected by the establishment of natural enemies; however, it may be more complex than the regulation of populations by organisms only. Environment and climate, especially climate change, may impose limits on the efficacy of agents and/or enhance invasiveness of alien weeds (Hellmann *et al.* 2007; Tylianakis & Binzer 2014).

Adaptation and niche

Many of the alien species that were intentionally introduced into South Africa do not become invasive. Eucalypt and pine species, for example, are not all invasive and serve an important role in agriculture and forestry (Poynton 1979a, 1979b; Henderson 2001). If evolutionary adaptation was the only reason for altering species behaviour from benign–beneficial to costly–invasive, it could be argued that many more species should be displaying invasive traits. Many invasive alien species do not spread immediately after introduction. Invasive species are believed to undergo a lag-phase of variable duration before they display invasive behaviour (sleeper weeds). Some theories, not necessarily exclusive, for the lag-phase include the generational time required for the development of genotypes with increased invasiveness (Hobbs & Humphries 1995, Erfmeier & Bruelheide 2005) and spread being limited by the availability of suitable habitat (Cousens & Mortimer 1995; Sakai *et al.* 2001; Barlow & Kean 2005,). Pyšek and Hulme (2005) contend that the lag-phase is an artefact, because the phenomenon may be scale-dependant, plants may be spreading at a constant rate and as populations increase spread becomes exponential over time. Part of the problem lies in the fact that invasive species are studied at some point in their spread when they are already having an impact, i.e. hypotheses are based from patterns that were not specified *a priori*. A general theory is one of low initial spread, a stage when species become naturalised as scatted populations in a few areas (nascent foci) and are often overlooked (observer error), followed by rapid range expansion or exponential spread and a third phase where the range reaches a plateau (Lonsdale 1993).

Pre-adaptation, i.e. environmental conditions are similar in native and introduced ranges, and ecological niche are also considered to be important factors for invasiveness. *Rhododendron ponticum* invasion in Great Britain has been partly attributed an ecological release from environmental constraints in its native habitats by more favourable conditions in its introduced territory (Cronk & Fuller 1995, Erfmeier & Bruelheide 2005). King and Wilson (2006) found invasive alien plants in xeric grassland were successful because by pre-adaptation, they could cope with the environment in the same way as native species and they also grow faster; not because they are better adapted. Multiple introductions of the Eurasian grass *Aegilops triuncialis* L., pre-adapted to serpentine soils, into California is the primary cause for the rapid invasion of serpentine habitats in the USA (Meimberg *et al.* 2010). Conversely, in island ecosystems alien species are reported to be better adapted to the environmental peculiarities (human impacts, disturbance, small native populations) than less competitive native species due to long evolutionary isolation (Küffer *et al.* 2003; Fleischmann *et al.* 2003). The effect of release from enemies is still easier to demonstrate than intrinsically superior adaptive and niche dominance qualities causing the displacement of native species by alien species.

The competitive exclusion principle of two species not being able to coexist together indefinitely if they compete for the same resources (Gause 1936, Giller 1984), and the Hutchinsonian niche concept (Hutchinson 1957), have been applied to this review on the ecology of plant invasions. The term “vacant niche” (or “empty niche” or “niche space”) is irrelevant under Hutchinson’s (1957) niche concept. The niche is defined as a multidimensional hypervolume corresponding to the full range of resource gradients along which species are distributed, i.e. the fundamental niche. Niches are species’ properties, not *vice versa*, and therefore species have to be present to validate the existence of niche. How does one identify / predict a potential niche when the objects required to “fill” and “be filled” cannot be estimated or comprehended? Hutchinson recognized the misuse of the niche principle in the case of introduced species whose rapid spread was not caused by so many “empty niches”, but rather because invasions were characterised by disturbed conditions. If vacant-niches were valid, it would assume alien invasive species “fit in” rather than to competitively “push out” native species in affected communities. This theory is not supported by empirical evidence, alien species modify ecosystems and reduce species diversity (Higgins *et al.* 1999), not add to it. The pressure of living in communities with other species (interspecific competition) and being subjected to predation, produces a much narrower hypervolume space, the realised niche (actual living space), that a species must be adapted to for existence and replacement. It is important to understand the application of realised niche and niche width in invasion biology, i.e. how resource gradients affect alien species (spread, distribution, impact) in the introduced range (relative to home range) and how ecological integrity (diversity, structure, function, process, environment) relates to resistance or susceptibility.

Smith (1985) believed invasive alien species infest their fundamental niche in introduced areas, having escaped from factors that limited them to the realised niche of their native environment. This would be extremely difficult to prove. (A) The extent to which pre-adaptation occurs in invasive alien species can be inferred from niche position along various environmental gradients in the introduced range using Ellenberg indicator values (Prinzing et al. 2002). They showed that the species’ niche position within the native range matched that of the conditions in the introduced range. Escape from constraints such as natural enemies may simply increase an invader’s niche width / breadth in the introduced range, but is still disproportionate to its fundamental niche due to the persistence of other unknown limiting factors. (B) The invader species’ niche-overlap will narrow at some point along the invasion sequence as it meets new species, to the point where there is no overlap, and the superior competitor in that situation / or at that stage will prevent it filling its fundamental niche. (C) Fundamental niche is a theoretical concept that was not intended to be an entity for quantification, species are the entities required to ascertain the existence of a niche (Hutchinson 1957). (D) Invader species may just have bigger realised niches in introduced ranges on the premise of predator absence, pre-adaptation, evolutionary adaptation, rapid evolution, environmental plasticity, peculiar traits to name just a few.

The term "niche opportunity" (Morton & Law 1997, Shea & Chesson 2002) is arguably the most logical application of the "niche" in invasion biology. A niche opportunity is defined as "the potential provided by a given community for alien organisms to have a positive rate of increase from low density" (Shea & Chesson 2002) and is dependent on factors assumed to improve invader performance, viz. resource opportunity, escape opportunity (from natural enemies), the physical environment, interactions between these factors and concomitant spatiotemporal fluctuations. Disturbance, especially human activities, is associated with freeing-up resources and offering opportunities for invaders to establish. An important aspect of the "niche opportunity" concept is that invasive alien species are not elevated to "super species" status, but they may respond faster than local resident species to events that release resources in any given place and time. In some alien species their "natural niche" (in the country of origin) may have a role in producing a localised effect, e.g. allelopathy. Niche opportunity breadth is believed to result from species diversity, with high diversity communities offering low niche opportunity for invasion. The evidence provided, however, is highly contradictory, and there appears to be very little connection between diversity and invasibility, despite concerted efforts to establish one. Establishing an underlying pattern in invasion resistance of plant communities has been elusive but could be confounded by external covariables (scale and time dependent environmental variables) arising in field studies. New field techniques and / or statistical procedures are needed to partial out the influence of extrinsic effect in the internal analysis of within-community invasion resistance.

Elton's (1958) balance of nature hypothesis suggests species-rich communities with complex interactions are more resilient to invasion. Empirical evidence on the role of diversity as an important factor in ecological resistance is inconclusive at small and large scales. In experimental grassland plots species, Kennedy *et al.* (2002) found that diversity improved invasion resistance by reducing the number and size of invaders establishing through increased crowding and species richness in localised plant neighbourhoods. In a greenhouse experiment, Dukes (2002) tested how species composition, richness and community age affected the susceptibility of grassland communities to invasion by the Asteraceous weed *Centaurea solstitialis*. Results showed invasibility and impact were limited by diversity at the neighbourhood level. In contrast, the invasion of the alien herb *Melilotus officinalis* in grassland is facilitated by its abundance and dominance, not because of reduced grass richness (Smith *et al.* 2004). Reduced grass dominance was brought on by increasing weed competition and crowding out of native grasses and was not associated with reduced richness. Yurkonis *et al.* (2005) showed diversity declining under invasion and increasing alien plant cover.

It would be difficult to quantify the relationship between the diversity and invasion by alien species on a landscape scale because there are so many uncontrollable external factors that come into play. Scale of inference has a strong positive affect on all aspects of ecological heterogeneity (species, communities, vegetation types, biomes) and environmental heterogeneity (climate, topography, geology, human activities etc.). Islands appear to be more invasible than mainlands (Lonsdale 1999; Denslow 2003). Resistance to alien plant invasions on islands does not appear to be related to geographical position, species richness or diversity (Levine & D'Antonio 1999). Current theory indicates that resource availability, dispersal mechanisms and disturbance are important factors aiding naturalisation of exotic plants in island vegetation (Rodgers & Parker 2003; Bellingham *et al.* 2005). Levine *et al.* (2004) did not find evidence that resistance is caused by species interactions that repel invasions, only that abundance of invasive species is constrained once they have successfully established. In a study of non-native plant invasions in vegetation at multiple spatial scales, Stohlgren *et al.* (2004) found that species rich vegetation was often highly vulnerable to invasion, alien species richness was positively correlated to increasing non-native plant cover and the same environmental variables that maintain high native plant species richness also help maintain non-native plant species richness. Alien invader plants are known adversely to affect biological diversity (Drake *et al.* 1989) but very little has been reported on how individual species affect plant community composition, structure, and function. Grassland resistance to invasion may be dependent on landuse (DiTomaso 2000), yet there is evidence suggesting natural resistance occurs and may be reliant on the presence and abundance of specific key species (Thomsen & D'Antonio 2007).

Environmental factors

In plant community ecology release from natural enemies is not regarded as a universal law upholding populations, densities and assemblages of invasive species. Extrinsic factors may exert even stronger mechanistic roles in cause and effect of plant invasions than specific traits of species. Wells *et al.* (1986) cited 1653 invasive taxa that compete with indigenous flora, of which 711 included naturalised exotics. The remaining 43% were indigenous species, presumably under their own suit of natural enemies, yet became invasive under certain conditions. For example, our understanding of bush encroachment has been challenged in recent years and traditional beliefs that heavy grazing and fire as being the principle causes may not be true (Ward 2005). In arid savanna infrequent rainfall may be more important than fire for woody plant recruitment (Higgins *et al.* 2000). Carbon dioxide has been shown to have an important role in tree-grass relations. Under low CO_2 levels typical of the last glacial period (180 ppm) recurrent fires in mesic savannas would have suppressed tree saplings, affecting tree dominance to favour the spread of grasses (Bond *et al.* 2003). The current elevated CO_2 levels (360 ppm) greatly favour increases in woody plant densities at the expense of grasses, and recurrent fires are unable to suppress bush encroachment. Hence the role of climate change, i.e. altered rainfall, temperature and CO_2 levels, may be a far greater driver of vegetation change than the intrinsic properties of invasive species or anthropogenic disturbances. Evidence from Australia shows that increasing atmospheric CO_2 levels would allow *Acacia nilotica* (L.) Willd. ex Delile to invade more xeric sites in the continent’s interior, while elevated temperatures would facilitate the weed’s expansion into cooler regions (Kriticos *et. al.* 2003). The consequence of global warming and increased CO_2 is therefore likely to reduce the influence that limiting factors, e.g. soil moisture and temperature, have on some problematic (native and alien) and facilitate invasion into more marginal areas.

Although our mechanistic understanding of invasions is somewhat limited, much work has been done on some of the physical causes of invasion. Essentially human activities (van Sittert 2002; Mack 2003; McKinney 2003; Rouget & Richardson 2003a) are the cause for most, if not all, biological invasions worldwide. Having stated that, disturbance is possibly the most cited factor causing invasions of alien plants in natural ecosystems (Lozon & MacIsaac 1997). Examples of disturbance facilitating invasions of alien plants include grazing (Mensing & Byrne 1998, Brooks 1999, Kimball & Schiffman 2003), soil disturbance, afforestation (Richardson

1998), agriculture (Meiners et al. 2002), invasion after fire (D'Antonio et al. 1993) and the creation of vast tracts of roadside habitat. The role of roads as both corridors for the spread of alien plants, and roadside as nursery habitat for invading adjacent vegetation, are increasingly becoming more prominent in the literature (*sensu* Henderson & Musil 1984, Saunders & Hobbs 1991; Tyser *et al.* 1998; Pauchard & Alaback 2004). Results from these studies are ambiguous as to the invasibility of grasslands from adjacent infestations of roadside weeds. One study reported adjacent grasslands being vulnerable even under low levels of livestock grazing and other anthropogenic disturbances (Tyser & Worley 1992). Another study showed alien roadside weeds were less likely to invade adjacent grassland but there may be a phylogenetic connection as to which species successfully cross this transition (Kotanen *et al.* 1998) and the levels of disturbance in the grassland. In this study Asteraceous species were the most prominent roadside weeds, followed by members of the Poaceae, but none of these species invaded adjacent grassland habitat. Asteraceous herbs are notorious invaders of disturbed vegetation adjacent to roads, e.g. *Ageratum houstonianum* Mill., *Coreopsis lanceolate* L., *Cirsium vulgare* (Savi) Ten., *Parthenium hysterophorus* L. and *C. macrocephalum* (Henderson 2001). Patchiness may also be a factor contributing to invasibility of grasslands. Fragmented grasslands are prone to invasion by a variety of species and life forms of invasive alien plants, irrespective of their distance from roadside (Morgan 1998).

The process of biological change or altered successional direction in plant communities following invasion is poorly understood. When confronted with data from heavily invaded plant communities it is almost impossible to separate the cause from the effect, despite significant correlations between alien density and response variates from the affected community, e.g. native species richness. The respective roles of different causal mechanisms of native plant population declines are even more difficult (Didham *et al.* 2005). MacDougall and Turkington (2005), using a manipulative field experiment with a factorial design, found alien plants to be ‘void-fillers’ because native species dominance did not occur once the alien species were removed. They concluded that habitat disturbance was the main cause affecting native species, with little evidence of interaction between invasive and native species. This study did not adequately address the temporal aspect to invasion and how altered dominance roles between alien and native species fluctuated over time. Results after two years in a limestone grassland invasion experiment in England indicated that invasion was strongly promoted by disturbance and less so by increased soil fertility (Thompson *et al.* 2001). After 5 years non-invasive cover was positively corrected to increasing fertility and invasive cover had declined, however, all fertility-disturbance treatments were invaded at some point. Early successional traits of invaders were typical r-type, viz. seed mass and germination characteristics. Late successional species were grasses and no single trait could be used to predict invasion success. Increasing the availability of resources through disturbance and nutrient supplementation facilitated the establishment of invader species.

Repeated measures experiments with multifactorial designs in natural ecosystems could provide details about the interactions between composition, diversity, invasion intensity and key environmental variables, e.g. fertility, moisture, soil type, grazing, fire and undisturbed (moribund). No experiments of this nature have been conducted, which creates large gaps in our knowledge about the temporal changes taking place in vegetation throughout an invasion process, and the external factors that facilitate or mitigate invisibility (refer to Chapter 2). Direct-gradient analysis is an alternative method to manipulative experimentation, but the inferences about the causes and effects of invasions are relative, not absolute. Simple experiments yield results that are often inconclusive and unable to challenge ecological theories (O'Connor 1985). For example, increasing species richness associated with declining chromolaena populations following annual burning in coastal grasslands (Goodall *et al.* 2006) showed that chromolaena was sensitive to fire. The study could not determine *a posteriori* if chromolaena dominance was the cause or the effect because the role of fire frequency / intensity was not tested against invasibility. Did the absence of burning merely provide the opportunity for a highly invasive, fire-sensitive, alien species to establish? Were there other mechanisms involved such as secondary forest succession shading out grassland species, followed by chromolaena invasion that ultimately led to competitive replacement (sequential succession) and exclusion? As a consequence, the role of chromolaena in altering succession away from grassland – coastal forest (Acocks 1953), both being high diversity habitats, to grassland – monotypic thicket could be hypothesised but not tested. It is recognised that fire is an important variable maintaining grassland in the False Grassland Biome (Mentis 1984).

General consensus is that alien species are opportunists taking advantage of disturbance but are not the cause of ecological changes. This may be an over-simplistic opinion of cause and effect. Remove the disturbance and theoretically you remove the problem (symptom) is not a practical consideration and does not advance science. Disturbance is a natural regulator of stability and resilience of ecosystems but it involves episodic periods of instability, e.g. fire and grazing in grassland, canopy tree deaths (gap dynamics) in forest. Mechanisms explaining invader effects on community diversity can be divided according to the demographic stage that is impacted by the invasion (Yurkonis *et al.* 2005). The invader can either affect resident species established within the community (species displacement), inhibit the establishment of new individuals (establishment limitation), or both. Disturbance is a component of vegetation pattern, arguably the cause, and so it is an oversight to infer the role of invasive aliens as a minor effect. Once established the influence of invasive alien species are likely to increase over time and their impacts on community structure, composition and function are likely to be substantial, e.g. grassland species are unable to survive under the canopies of invading Australian acacias. If we still cannot prove the connection between alien plant invasions and changes in species diversity, it is unlikely that their role in species extinctions can be quantified either because their role cannot be separated from other factors that impact negatively on biodiversity (Gurevitch & Dianna 2004, Didham *et al.* 2005). Alien plants are reportedly more likely to displace species and alter community composition than cause extinctions (Brown & Gurevitch 2004). The exact cause of extinction becomes even vaguer when species are already categorised on the IUCN Red List as critically endangered or endangered (Gurevitch & Dianna 2004). Endangered species face more than one threat; hence extinction of a remaining population in the wild cannot be attributed to its habitat being modified by an alien species when human population pressure was the principle determinant in the loss of suitable habitat.

Species traits

In theory organisms lie on a continuum between two extremes of resource allocation representing two strategies for survival (MacArthur 1962, Pianka 1970). Species with r-selected traits are considered to have evolved in unstable or newly formed habitats, e.g. disturbed conditions. The K-selected species, conversely, evolved at or near the carrying capacity of the environment. As a result, K-selected species use resources more efficiently, and their competitive ability is high. Sequential succession occurs when life history traits of early and late (successional) species are inversely correlated (Huston & Smith 1987) and strategic allocation of resources results in a tradeoff between below and above ground tissue. Species that establish and reproduce the fastest at a specific level of a limiting resource will be competitively dominant. Thus in early succession when resources are ‘abundant', r-selected species can establish as large populations of low diversity. In late succession resources are limited, realised niches are narrow, species diversity is at a premium, and the lack of empty niche space will prevent species with r-selected traits from establishing.

Extreme K-selected species are characterised by being long-lived, reaching greater sizes, having prolonged vegetative stages, require low resource allocations for reproduction and are usually found occupying the later stages of succession. Extreme r-selected species, in contrast have shorter life-spans (e.g. annuals), smaller and largely herbaceous growth forms, place large resource allocations into reproduction and are early occupants in disturbed sites. Asteraceous weeds in with typical r-selected life-history traits occur in the tribe Eupatorieae, viz. *Ageratina*, *Ageratum* and *Chromolaena*. Other Asteraceous weeds include *Cirsium*, *Parthenium* and *Xanthium*. Physical factors (e.g. perturbations) regulate populations of r-selected species, whereas K-strategists are maintained by biotic factors (e.g. pathogens, diseases, predators). These extreme scenarios are unlikely in practice, but the gradient between the two suffices for most species with inversely correlated traits (Radosevich & Holt 1984, Huston & Smith 1987). Central to McCook's (1994) review on succession is the idea that correlations between traits, especially growth form, growth rate and shade tolerance, can explain autogenic succession. In other words, r-selected species characterising secondary succession cannot occupy the same space in a continuum ending in a stable state. This may be true for native species but may not hold for alien invasive species that appear to establish and persist at any stage in succession because of other underling factors.

Abundance, distribution and their relation with each other in the country of origin have been linked to invasion success; i.e. species traits that determine propagule pressure (Williamson & Fitter 1996). Inconclusive findings from long-term monitoring studies indicate that fluctuation in resource availability could be important in governing site-susceptibility to invasion, but these episodic conditions must coincide with availability of invading propagules as the first step in the invasion process (Rouget & Richardson 2003b). Mechanisms geared for long-distance dispersal, wind and transportation allow for the rapid spread of certain species above others, for example the wind-dispersed spread of chromolaena in South Africa after 1940 (Goodall & Erasmus 1996) compared to the river-dispersed seeds of black wattle introduced into Natal in 1886. The relative importance of reproductive biology, mechanisms of dispersal, availability and mobility of propagules and environmental stress on seed production in the process of invasion remains general conjecture.

Allelopathy has been cited as being an important trait for successful invasion, the so-called "novel weapons hypothesis" (Callaway & Ridenour 2004, Bais *et al.* 2003). While acknowledging the importance of APH / ERH in invasion biology, these authors proposed that certain invader species are able to transform vegetation because they possess allelochemicals. Under this hypothesis the processes of adaptation allow natural neighbours to cohabit an area with an allelopathic species without suffering negative side effects, but in new environments native species may be intolerant to the same chemical compounds responsible for the allelopathic effect. There could also be neighbourhood effects on overall community composition and structure. Also proposed is the advantage of having an allelochemistry includes the possibility of selection trade-offs of competitive traits for greater quantities of toxins. Like other species in the Asteraceae, it may be possible that pompom weed contains allelopathic compounds that kill or suppress neighbouring species. *Parthenium hysterophorus* for example contains sesquiterpene lactones, an important group of compounds involved in allelopathy (Kanchan & Jayachandra 1980). Leaf extracts of this weed are apparently strongly phytotoxic to plants, inhibiting germination and affecting seedling development of many species (Adkins & Sowerby 1996; Reinhardt *et al.* 2004). Other alien invasive species in the tribe Eupatorieae (Henderson 2001, Retief 2002) exhibiting allelopathy are *Ageratum conyzoides* (Hu & Kong 1997), *Ageratina adenophora* (Kaul & Bansal 2002) and *C. odorata* (Sahid & Sugau 1993; Ambika 2002; Navaz *et al.* 2003).

Harper (1977) refutes the role of allelopathy in plant-on-plant interactions, calling experiments that testify to allelopathic effects "laboratory artefacts". "Any species can, by appropriate digestion, extraction, and concentration, be persuaded to yield a product that is toxic to one species or another." Allelopathy effects from laboratory studies should only be accepted if they are supported by field experiments. Under field conditions organic toxins produced by an allelopathic species could be rendered unavailable to neighbouring species or broken down by the combined interactions of soil texture, organic matter, temperature, irradiance and microbial breakdown. Most allelopathy experiments, including all the ones cited above, failed to take this crucial step of in-field validation. Any unknown numbers of studies that claim allelopathy are potentially rejecting a true null hypothesis.

Control

In agricultural croplands the indirect impact of herbicides on bird species through the elimination of cover and as food plants for insects has been well documented (Chiverton & Sotherton 1991; Moreby 1997; Taylor *et al.* 2006). Crops with weeds supported more beneficial arthropods (predators of insect pests and weed seeds) and a larval food-source for young birds than fields of monocultures where herbicides had been used to kill off non-crop species. The control of emergent weeds caused increased mortality of game bird chicks (partridge and pheasant) living in arable ecosystems due to the rapid elimination of insect host plants. As a consequence, populations of game birds also declined on farms where only chemical weed control was practiced as apposed to farms using integrated pest management practices and intercropping with other species.

Several studies have been conducted in the USA on the impact of selective herbicides on invasive alien forb species in grassland and associated not target effects, especially release of graminoids from competitive alien species and herbicide-impact on native forb species. In one study where herbicides suppressed the target weed, grasses responded positively to release from exotic forb competition and non-target forbs in treated plots were not significantly different to the untreated control plots three years after spraying (Rice & Tony 1998). Similarly, selective broadleaf herbicides applied in plots to control leafy spurge (*Euphorbia esula* L.) in grassland did not affect the cover or biomass of graminoids (Butler 1994). Native forb richness and cover, however, were severely affected by herbicides; richness declined by 86% (1/7 species survived) and aerial cover was reduced from 3.7% before application to 0.9% one year later. There was no evidence to suggest graminoids benefited from competitive release from leafy spurge. Detrimental herbicide effects were detected on native forb cover and frequency in three important grassland families in the US, viz. Asteraceae, Apiaceae and Fabaceae (Morghan *et al.* 2003). Their results also indicated that some species were not adversely affected by herbicide in the long term, despite initial setbacks in the first year after treatment. This suggests that selective herbicides affect native forb species in a variety of ways, viz. differing herbicide-tolerance thresholds, growth form may favour some species over others (e.g. therophytes – short term effect) and role of soil seed reserves in buffering the effects of herbicide-induced mortality of parent populations. In a recent study testing selective herbicides and hand-removal to control *Potentilla recta*, grass cover and biomass increased and native forb density decreased with herbicide application, but species richness and diversity were not adversely affected (Sheley & Denny 2006). Mechanical hand-removal caused an increase in total richness but more so with native forbs than graminoids. Studies on the impact of herbicides and mechanical control on native plants in grasslands provide inconclusive evidence concerning the risks, cost-benefits and trade-offs of integrated control.

Studies are urgently required to address the potential non-target impacts of chemical and mechanical control of invasive alien weeds in the grassland biome in light of large-scale alien plant clearing initiatives, e.g. the Working for Water Programme. Data from sequential (annual) herbicide and mechanical control applications in grassland and wetland studies can be used to model risks, trade-offs and cost-benefits of control under different species diversity and weed density scenarios. This would be a valuable tool for grassland science and conservation initiatives in the grassland and savanna biomes.

THE PROBLEM SPECIES

Botanical and ecological characteristics

Campuloclinium macrocephalum (Less.) DC is an alien hemicryptophytic herb that aggressively invades grassland in Gauteng Province. Infestations become conspicuous when the plants are in flower in summer. This is attributed to its fluffy pink pom-pom like flowers, borne upon long stalks, sticking above the grass layer. Its common name, “pompom weed”, aptly describes a new weed species that is rapidly becoming a threat to grasslands in South Africa.

Not much is known about pompom weed. It is a member of the daisy family (Asteraceae) and is closely related to *C. odorata* (chromolaena), an invasive alien shrub, of the tribe Eupatorieae. Chromolaena is a shrub that invades forest, grassland and savanna in frost-free parts of South Africa’s eastern seaboard. Chromolaena seeds are wind-dispersed, and since its introduction in the 1940’s, this species has invaded extensive tracts of land from Port St Johns in the Eastern Cape to Louis Trichardt in Northern Province (Goodall & Erasmus 1996). Pompom weed’s initial rate of spread suggests a similar pattern, however, its ecological niche appears more suited to Highveld conditions where frost is prevalent. *Campuloclinium macrocephalum* originates from the central Americas where it is native to Mexico, Argentina, Bolivia, Columbia, Guatemala, Honduras and Paraguay (Foster 1958, Turner 1997, Williams 1976). The plant was probably introduced as an ornamental species before 1970 (spreading in Fountains Valley, Pretoria, in the early 1970's) but became naturalised shortly thereafter.

Pompom weed is a species adapted to living in fire-driven grassland and savanna ecosystems. Shoots are produced from perennating buds on the root crown at or below the soil surface in spring / early summer (November) and senesce at the end of the growing season (April / May); i.e. shoots are annual. The plant has a tuberous perennial root system without perennating buds, i.e. tubers are for storage and do not regenerate when severed. The plant survives winter by dying back to its underground structures. Over winter buds on the root crown produce short white shoots which grow to just below the soil surface and remain dormant until spring. Shoots appear in spring and produce dense leafy shrublets up to 0.5 m over a period of about two months. Stems and leaves are covered with rough, bristly hairs. Leafless inflorescences rapidly elongate once vegetative growth reaches completion and plants in full flower stand up to 1.3 m high. The pink flowers consist of hundreds of tiny, star-shaped florets surrounded by purple bracts. Mature florets each produce a single-seeded dry fruit (achene) with a tuft of hairs (pappus) that are adapted for dispersal by wind. Peak flowering is usually from January to March followed by rapid seed set. Although wind appears to be the main vector of dispersal; vehicles are probably also responsible for long-distance dispersal of roadside infestations. By mid-April most of the seeds have been disseminated and plant senescence is quite advanced.

The plant is apparently unpalatable to livestock, which means increased grazing pressure is exerted on the remnant vegetation, accelerating veld degradation. The ecological implications of *C. macrocephalum* on diversity and ecosystem function in the grassland biome are unknown, but are likely to be serious.

Distribution

Pompom weed is currently (2015) found predominantly in Gauteng Province (epicentre around Pretoria), but is also locally abundant in KwaZulu-Natal (KZN), Limpopo, Mpumalanga and Northwest Province (Figs. 1&2). The species also occurs in North West Province, Free State and Eastern Cape where its current incidence is rare. In 1987, it took a concerted effort to find specimens of this plant outside Pretoria in Gauteng (pers. comm. Prof. Charles Stirton), but since then, the population has increased exponentially. Infestations are concentrated mostly around the Pretoria - Irene area. The potential distribution of the weed in South Africa encompasses the grassland biome (Fig. 2) and parts of the savanna biome (Trethowan *et al.* 2011) and poses a threat to the neighbouring countries of Lesotho and Swaziland (Fig. 1).

Legislation and control

Research into the control of any invasive alien species is preferable when the invasion is at an early stage. *Campuloclinium macrocephalum* is a declared weed (Category 1) under the Conservation of Agricultural Resources Act (Act 43 of 1983). Landowners are compelled to control pompom weed. Two selective herbicides were recently registered for the control of pompom weed, viz. metsulfuron methyl 600 g ai kg^{-1} (BRUSH-OFF®) and picloram 240 g ai L^{-1} (ACCESS®). Mechanical control recommendations for pompom weed include uprooting/hoeing, ploughing and cutting of flower heads but the effectiveness of these techniques have not been established. The initiation of biological control of pompom weed should be complimented by research on herbicide and mechanical control, as was the case with chromolaena (Erasmus 1985, 1988), with a view to the future integrated control of the weed. Herbicide and mechanical control studies usually focus on treatment efficacy and cost, i.e. percentage control achieved and amount of product used (Erasmus & Clayton 1992). These trials involve marking treated plants and only provide data on the average efficacy. What they don’t provide are before and after data on weed population dynamics following treatment episodes and changes in community composition resulting from these applications, i.e. ecological trade-offs and cost-benefits of control.

Research into the biological control of pompom weed is in progress. The biotrophic rust *Puccinia eupatorii* Dietel (Pucciniaceae) is a candidate biocontrol agent and has been managed under quarantine conditions since 2003 (McConnachie *et al.* 2011). In 2006 *C. macrocephalum* infestations around Pretoria were severely infected by the rust. The rust subsequently spread rapidly after its initial detection and by 2010 infestations throughout the weed’s range in South Africa had become infected with urediniospores. The phylogenetic relationship between *P. eupatorii* isolates in the field and specimens under quarantine have not yet been concluded so it is too early to say that the rust escaped quarantine. The rust may also have been unintentionally introduced into South Africa via contamination of materials, e.g. clothing, during the exploratory phase of potential agent detection and collection in South America.

Liothrips tractabilis Mound and Pereyra was approved as a host-specific biocontrol agent against pompom weed in 2013 following extensive host-range testing in quarantine (McConnachie & McKay 2015). The thrips agent was first released on pompom weed in KwaZulu-Natal and Gauteng in the summer of 2013/14 with subsequent multiple releases. The thrips has the potential to inflict significant reductions in the weed's biomass and flower production by affecting plant height and photosynthetic cover through feeding damage.

Pompom is expanding its distribution in the grassland biome and other forms of control are needed. Biological control and herbicidal control, integrated with sound veld management, are tools that would benefit agriculture and nature conservation in the fight against pompom weed. While biological control is target-specific, herbicides, even selective herbicides, have the potential of not only killing pompom weed but also a broad spectrum of desirable native species. Herbicide impact assessments are important to (1) identify formulations that are (cost-) effective on pompom weed, (2) quantify the non-target impacts, (3) conduct a cost-benefit analysis and determine acceptable tradeoffs / thresholds (under what conditions are non-target species impacts tenable / untenable).

Motivation

Pragmatically, the success of invasive alien plants in displacing native species is attributed to superior fitness / competitiveness due to two key factors, viz. introduction from other continents with similar or matching climate (Bastow Wilson *et al.* 2000, Rouget *et al.* 2004, Thuiller *et al.* 2005) and introduction without natural enemies (Huffaker *et al.* 1976). After introduction as an ornamental species pompom weed became invasive in grassy ecosystems for a host of plausible reasons: (a) absence of natural enemies, (b) pre-adapted for subtropical conditions in the southern hemisphere, and (c) similar habitat, environment and niche opportunities in both home and host countries. Evolutionary shifts in plant traits could explain why the weed is more invasive on the Highveld than at the coast, but less than optimal environmental conditions on the humid eastern seaboard could also be responsible for reduced rates of invasion in KZN.

The indirect impact (direct impact infers a causal mechanism) of pompom weed on species composition, species diversity, vegetation condition and grazier carrying capacity remains unknown. Also unknown are: (a) extrinsic factors that facilitate invasion (e.g. vegetation type, physiognomy, disturbance, soil variables, topography, climatic variables, landuse, herbivory and proximity to roads) (Chapter 2); (b) the existences of plant traits that produce harmful neighbourhood effects, i.e. allelopathy (Chapter 3), (c) the efficacy of biological control (Chapter 4), (d) the efficacy of registered herbicides in pompom weed suppression (Chapter 5) and (e) the effect of herbicides and mechanical control on other plants species (Chapter 6). Landusers urgently require control recommendations against pompom weed. Knowledge gained from these studies will be important for implementing management strategies aimed at reducing pompom weed density, mitigating spread and providing recommendations that balance pompom weed control with grassland conservation (Chapter 7).

STRUCTURE OF THE THESIS

The thesis structure includes an introductory chapter / literature review (Chapter 1), the core research chapters as a compilation of four published papers (Chapters 2-5), an unpublished chapter (Chapter 6) and a synthesis / conclusion (Chapter 7). In this thesis repetition of past research relevant to the context and aims of the five individual research papers is unavoidable.

AIMS & OBJECTIVES

The study of the ecological impact and control of a highly invasive alien plant under different environment conditions in an isolated mega-infestation is a unique opportunity to quantify the problem and deliver solutions that could mitigate spread. This study has five broad aims:

Aim 1 (Chapter 2)

The identification of factors or conditions that are facilitative and facultative in nature (e.g. perturbation, allelopathy, species diversity, soil cover and competition) from those that drive invasion (e.g. absence of natural enemies, climatic pre-adaptation), are important in understanding the ecology of invasive species. Few studies are able to quantify the impact that invasive alien plants have on biodiversity with factors that best explain the causes of invasion. Ecological data could be used to identify susceptible “invasion states” from “resistant states” and to test the theory that natural enemies have an overriding role in invasion success.

The aim of this study was to gain a better understanding of the drivers of *C. macrocephalum* invasion in the grassland biome by quantifying invasion intensity with grassland species composition and factors that facilitate invasion. The study was carried out in 80 grassland sites across Gauteng province, South Africa and had the following objectives:

- Carry out vegetation assessments to obtain reliable estimations of proportional grassland species composition in relation to variation in pompom weed densities and environmental gradients.
- Measure weed variables such as density and height (cm) of rooted plants in quadrats, including shoot, leaf condition and feeding damage assessments which provide important baseline data of natural stress factors prior to the release of biological control agents.
- Quantify relevant environmental factors such as vegetation physiognomy, rainfall, soil erosion, shading, grass cover, soil stoniness, terrain morphology, soil texture and fertility to determine their role in facilitating pompom weed invasion.
- Evaluate land use management including type of enterprise, past and current management practises, grazing animal categories, grazing intensity/stocking density and fire frequency to determine their role in facilitating pompom weed invasion.

This chapter has been published: Goodall J, Witkowski ETF, Morris CD & Henderson L (2011) Are environmental factors important facilitators of pompom weed (*Campuloclinium macrocephalum*) invasion in South African rangelands? *Biological Invasions* **13**:2217–2231.

Aim 2 (Chapter 3)

In line with Chapter 2 the aim of this study was to determine the role that allelopathy and competition play in *C. macrocephalum's* ability to invade grassland. The hypothesis is that chemical compounds in the weed and/or the weed's competitive ability may affect the germination, growth, survival and recruitment of native grasses and forbs in invaded communities. To achieve this aim the following objectives were implemented:

- Expose seeds of monocot and dicot test species to weed extracts *in vitro* to establish allelopathic potential on the seed germination and early seedling development.
- Establish the role of interference and weed residue persistence in separate pot trials by growing *C. macrocephalum* with *Eragrostis curvula*.

- Establish the relation, association and interaction between *C. macrocephalum* density and *E. curvula* in invaded grasslands in Gauteng.

This chapter has been published: Goodall J, Witkowski ETF, Ammann S & Reinhardt C (2010) Does allelopathy explain the invasiveness of *Campuloclinium macrocephalum* (pompom weed) in the South African grassland biome? *Biological Invasions* 12:3497–3512.

Aim 3 (Chapter 4)

Campuloclinium macrocephalum was introduced into South Africa in the 1960s without biological control agents and has benefitted from enemy release for >50 years. In 2006 the rust *Puccinia eupatorii* Dietel was first observed on *C. macrocephalum* in the field. Whether it escaped quarantine or was introduced unintentionally, e.g. contaminated clothing is not the important issue here. The aim of this study was to determine if susceptibility and changes in realised niche to host-specific predation has been influenced by duration of separation (years) and successive generations growing under predator-free conditions. To achieve this aim we revisited the 80 grassland sites discussed in Chapter 2 (Aim 1) and repeated the weed density measurements and included pathogen damage ratings.

This chapter has been published: Goodall J, Witkowski ETF, McConnachie AJ & Keen C (2012) Altered growth, population structure and realised niche of the weed *Campuloclinium macrocephalum* (Asteraceae) after exposure to the naturalised rust *Puccinia eupatorii* (Pucciniaceae). *Biological Invasions* 14:1947–1962.

Aim 4 (Chapter 5)

At present the control of *C. macrocephalum* is completely reliant on the application of registered herbicides. Registered herbicides generally offer about 80% control of sprayed plants in an area because precise application is never achieved. Some plants will not receive adequate spray coverage to cause death and unfavourable environmental conditions can affect herbicide uptake and translocation, e.g. heat and/or moisture stressed plants. In addition to 20% error in the control of visible weeds there is the residual weed seedbank that also needs to be accounted for in a herbicide control programme, implying that the actual reduction in the weed population could be as low as 50-60% in an invaded area. In this context chemical control can only offer rapid and effective control in situations where infestations are light or as a rapid-response tool for controlling species in the early phase of invasion.

The aims of this chapter were to determine if the efficacy of registered herbicides for the control of *C. macrocephalum* translated into similar reductions in field populations, how many follow-up operations are required to bring the weed to a maintenance level (<5%), the optimum time of application and the role of external / environment factors on herbicide efficacy. To achieve these aims the following methods were implemented:

- Laid out herbicide trials at two sites (grassland and wetland) each with a randomised block design that included three treatments (two herbicides and an untreated control), two application times (summers vs. autumn) in 5×5 m plots and monitored from 2005 to 2010.
- Herbicides were applied at registered rates as foliar sprays in plots annually for three consecutive years (2005-2007), soil samples from residue plots were collected from time zero and thereafter at 1, 7, 14, 28 and 56 days after treatment and analysed for herbicide persistence on a gas chromatograph.
- The number of *C. macrocephalum* plants in each plot was counted prior to the application of treatments for the first 3 years (2005-2007), with a final count in year four (2008), 1 year after the third and final application of herbicide.
- Seedlings were monitored in permanent sample units, 10 units per plot, once per season from 2005-2010.

This chapter has been published: Goodall J & Witkowski ETF (2014) Testing the performance of registered herbicides on the control of *Campuloclinium macrocephalum* (Asteraceae) in South African grasslands. *Weed Research* **54**, 274–284.

Aim 5 (Chapter 6)

The aim of this study was to determine the impact registered herbicides and manual weeding have on grassland species composition from the same experiment described in Chapter Five. To achieve these aims the following objectives were implemented:

- Herbicide trials – same as Chapter Five.
- Application – same as Chapter Five.

- Vegetation assessments in summer using line intercept measurements of all species bisecting permanent transects from 2005 to 2008 (four years).

This chapter has been submitted to Weed Research and is currently under review: Goodall J & Witkowski ETF (2016) Impacts of chemical and physical control of *Campuloclinium macrocephalum* (Asteraceae) on grassland species composition. *Weed Research*, under review.

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ORIGINAL PAPER

2. Are environmental factors important facilitators of pompom weed (*Campuloclinium macrocephalum*) invasion in South African rangelands?

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Abstract The absence of natural enemies being keystone to the success of invasive alien plants (IAPs) can only be accepted once all the factors governing the invader and the ecosystems it invades have been established. Few studies have attempted this approach. This study reports on the relations between the invasive alien forb *Campuloclinium macrocephalum* (Less.) DC. (pompom weed), herbivory and the ecology of the rangelands invaded in South Africa. Eighty invaded rangelands in Gauteng Province were studied. Pompom weed herbivory was insignificant with the greatest damage being caused by native grasshoppers. Rainfall, topography and soil texture were the primary drivers of vegetation pattern. Pompom weed exhibited disturbance-mediated invasion strategies, favouring rangelands affected by non-sustainable commercial grazing practices, abandoned agricultural fields and drained wetlands. It invaded vegetation in poor condition with sward basal cover <19%. Weed density was exacerbated by high fire frequency. Vegetation susceptible to pompom weed was also invaded by other alien and native pasture weeds. The absence of natural enemies could give pompom weed a competitive advantage over native pioneer cohorts in disturbed rangelands, but is unlikely to increase fitness enabling its establishment in vegetation in good condition. Grazing strategies that promote the dominance of grass species adapted to frequent non-selective defoliation appear to restrict pompom weed better than degraded rangelands that are not utilised. Biotic resistance of rangelands to pompom weed invasion will be greatest in vegetation in good condition and under good management. The low abundance of pompom weed in rangelands in communal areas and fence-line contrasts in invaded and un-invaded road reserves warrants further investigation.

Keywords Disturbance · Environmental heterogeneity · Fire · Herbivory · Range management · Species composition · Sward basal cover · Vegetation condition

Introduction

Few quantitative plant ecological studies investigate how external factors explaining native species

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composition in rangelands contrast with factors that explain invasive alien plant (IAP) composition in these same communities. Interactions between range condition and invasion susceptibility, including drivers of vegetation pattern, degradation and invasion, are poorly understood, but remain very important for conservation and the sustained utilisation of rangelands. Rangelands can attain alternative plant species assemblages under the dominance of discrete ecological groups of plants in response to grazing (Dyksterhuis 1949; Westoby et al. 1989, Tainton 1999) and climate (O'Connor 1994; Fynn and O'Connor 2000); namely the 'decreaser' and 'increaser' Ecological Response Groups (ERG). When increaser groups assume dominance certain species form almost pure stands that are very difficult to reverse in terms of improving range condition. What is not known is whether there is a connection between IAP susceptibility and the drivers of vegetation pattern, including the state of range condition.

The absence of natural enemies or enemy release is often cited as the primary reason for the invasiveness of naturalised alien plants and many successful biological control programmes against weeds demonstrate that introduced natural enemies can be effective on particular species (Hoffmann and Moran 1998; McConnachie et al. 2004). But current opinion assigns the enemy release hypothesis (Colautti et al. 2004) alongside several other theories of invasiveness (Catford et al. 2008). If the absence of natural enemies were the principle cause of invasiveness in alien plants, why do some native species, with more elaborate guilds of natural enemies also become invasive? *Acacia karroo* Hayne is a good example (Miller 1996), being an encroacher species in its native African range (Stuart-Hill and Tainton 1989; O'Connor 1995; Roques et al. 2001) and a naturalised species in Australia (Scott 1991). The properties of IAPs are, for example, influenced by life history traits (Sutherland 2004), enemy release and rapid adaptation during range expansion (Joshi and Vrieling 2005; Müller-Schärer et al. 2004). On the other hand properties that affect ecosystem susceptibility to invasion include weed propagule pressure (Lockwood et al. 2005), biotic resistance (Elton 1958; Levine et al. 2004), human activities (Vitousek et al. 1997; Sax and Brown 2000) and disturbance (Lake and Leishman 2004).

Studies that quantify IAP impacts on species composition with comparative accounts of factors driving plant communities from those causing non-native invasions could be used to identify susceptible "invasion states" from "resistant states" of ecosystems and to test the theory that natural enemies as keystone species have an overriding role in invasion success. This rationale was applied to the alien forb *Campuloclinium macrocephalum* (Less.) DC. (Asteraceae: pompom weed), a highly invasive rangeland weed in South Africa. The natural distribution of pompom weed extends from Mexico to Argentina in South America. Although infestations occur in several South African provinces (Fig. 1) (Henderson 2001), the infestation in Gauteng Province is sufficiently disjunct for the aim of discriminating between factors that facilitate invasion from those that influence vegetation dynamics, and secondly, to determine the impact that pompom weed density has on plant community composition in an isolated mega-infestation. The Gauteng infestation, being the original point of introduction in the 1960s (Goodall et al. 2010), is considerably larger and more concentrated than in other provinces. By the 1970s it was already widespread around the city of Pretoria with populations ranging from 30,000 to >100,000 plants per site (pers. com. C. Stirton's field records: Pretoria News 23.08.1980). Prolific production of wind dispersed seeds and growing conditions with high levels of disturbance and human activity provided an ideal opportunity for naturalisation (Lonsdale 1999) and subsequent expansion into the surrounding provinces. Vehicles are important vectors of dispersal with roadsides serving as nursery sites for infestations in new areas.

Pompom weed produces annual stems from a perennial woody rootstock below the soil surface (suffrutex). It is a declared weed in South Africa and is an invader of road-reserve, grassland, wetland and savanna ecosystems in the central, northern and eastern parts of South Africa (Fig. 1), which receive the majority of their rainfall in summer. At present South Africa is the only country where pompom weed has become naturalised. It is not an allelopathic species (Goodall et al. 2010) and hence the novel weapons hypothesis (Callaway and Ridenour 2004) does not apply in its invasion strategy. The aims of the study were to: (1) conduct vegetation assessments to provide a reliable estimate of proportional species composition in relation to environmental gradients, taking into account the variation in pompom weed

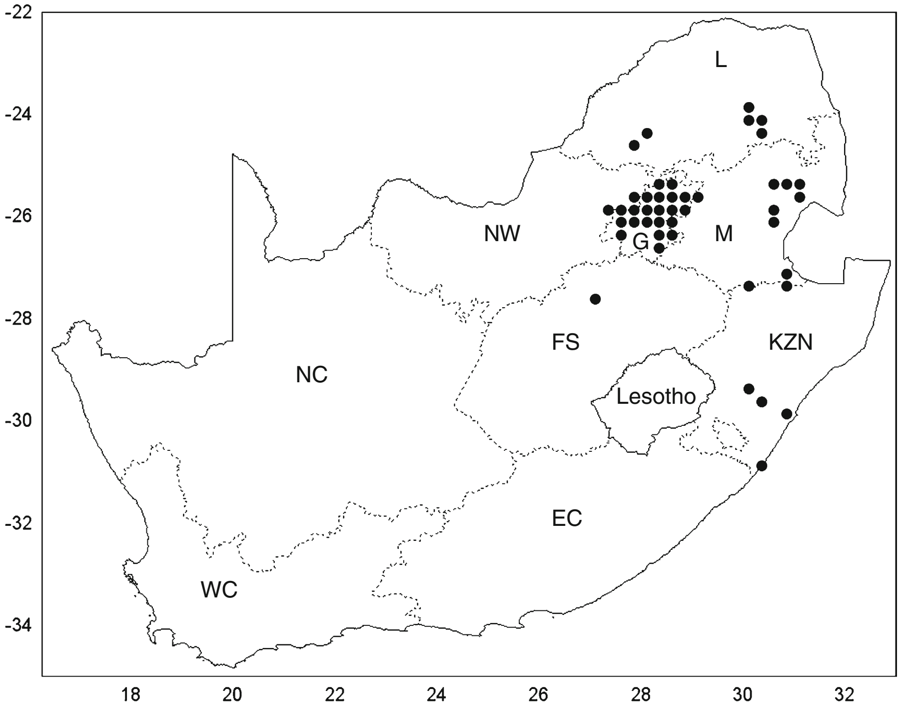

Fig. 1 The distribution of pompom weed (*Campuloclinium macrocephalum*) in South Africa occurs in provinces (*dotted lines*) with summer rainfall and dry winters. Each *black circle* represents weed presence in quarter degree squares (ca. 700 km^2). The weed is most abundant in Gauteng Province (*G*) but also occurs in North–West (*NW*), Limpopo (*L*), Mpumalanga (*M*), KwaZulu-Natal (*KZN*) and Free State (*FS*) provinces. The weed presently does not occur in the arid Northern Cape (*NC*), Eastern Cape (*EC*) and Western Cape (*WC*), a winter rainfall region

densities; (2) compare factors facilitating pompom weed invasion with those driving vegetation pattern, and (3) compare vegetation condition against invasion susceptibility. Field work was completed in April 2004, around the same time that insect and pathogen natural enemies were being investigated in quarantine as biological control agents (McConnachie 2007). A disease on pompom weed was discovered at one study site in February 2006 and later identified as a rust fungus *Puccinia eupatorii* Dietel, identical to the one in quarantine. The wild *P. eupatorii* strain has proved to be extremely virulent and is now found on plants throughout the country. An additional aim was to provide baseline data on pompom weed distribution and density across Gauteng with which to evaluate the impact of the rust fungus in future.

Materials and methods

A survey was conducted in 2002 to determine the extent of the pompom weed infestation on the Highveld of South Africa, a high plateau region ranging from 1,200 to 1,800 m above sea level in the central interior of the country. The Highveld incorporates the whole of Gauteng Province and parts of other provinces including Mpumalanga, North West, Limpopo and Free State (Fig. 1). The region receives the bulk of its rainfall (600–800 mm) during summer (September to March), with dry winters that experience frequent frosts and wild fires. Highveld vegetation is predominantly grassland, with savanna in the warmer and drier northern to western parts of the region. Much of the Highveld falls into the grassland biome of southern Africa. Landuse can be divided into completely transformed areas (metropolitan and mining), modified agricultural land and conserved natural areas.

Survey routes were randomly chosen in the 252 5 min squares covering Gauteng Province, with some overlap into adjacent provinces (Fig. 1). Pompom weed was recorded in 112 (44%) of the 5 min squares sampled. A total of 1,337 sites were recorded where pompom weed was present, both on roadsides (n = 1,228) and in rangelands away from roadside vegetation (n = 109). The coordinates of the sites were recorded using a GPS. The proportion of pompom weed in rangeland was considerably less than anticipated (8.2% of recorded infestations). Of the 109 non-roadside sites, 80 were selected as study sites with varying densities of pompom weed. Forty sites were sampled in March/April 2003 in northern-Gauteng and the remaining 40 in March/April 2004 across central and southern Gauteng. Savanna vegetation occurred in 14 sites, 42 were grasslands and the remaining 24 sites were wetland communities in both savanna and grassland areas. Annual rainfall in 2003 for sites in northern-Gauteng was 480 mm (20% below average) and 742 mm (normal) in 2004 for sites in central and southern Gauteng.

Species composition and basal cover

At each site a 100 m long line transect was laid out in homogenous vegetation containing pompom weed. Proportional species composition was determined by the spike-point method (Everson and Clarke 1987). The plant nearest to the spike at 0.5 m intervals along each transect was identified and recorded as being present (value = 1, n = 200 points). Nomenclature follows Germishuizen and Meyer (2003) (Appendix). Grass basal cover was also determined by the spike-point method at 1 m intervals along each transect. The distance and diameter of the grass tuft nearest to the spike was measured with a 100 paired measurements (cm) per transect. Tuft diameters were an average of the longest and shortest axis (cm), with a minimum diameter of 1 cm for small (annual) and creeping grass species. Basal cover was calculated according to the equation of Hardy and Tainton (1993).

Pompom weed variables

Pompom weed density was determined by counting the numbers of rooted plants in rectangular quadrats of 0.5 by 2 m (1 m^2). Quadrats were positioned so that the line transect bisected the quadrat on its longest side and were arranged every alternate 0.5 m along the tape so that they were independent, with 100 quadrats per transect. The average height of pompom weed (cm) in each quadrat was also recorded. Pompom weed condition was assessed under three categories: healthy (plants were mostly green), yellowing (early senescence or moisture stress) and browning-off or dying back (advanced senescence or drought effect). An assessment was made of damage by polyphagous insects, disease and/or browsers using a rating scale similar to that described by Olckers and Hill (1999). Damage categories were: zero damage, rare-exploratory feeding or browsing (no impact), trivial damage (negligible impact), moderate damage (growth and seed set reduced by up to 50%) and extensive damage (>50% reduction in growth and seed set).

Environmental variables

Vegetation was classified as grassland, savanna or wetland. Wetlands were classified as areas where the water table was near or periodically above the soil surface, with poorly drained hydric soils such as Vertic A and G horizons and the graminoid hydrophyte *Imperata cylindrica* (L.) Raeuschel was present. Mean annual rainfall for Gauteng Province was derived from the South African Rain Atlas (Zucchini and Nenadić 2006).

Soil erosion was classified according to Tongway and Hindley (1995), with the following erosion types: crust brokenness, terracettes, pedestals, rills, gullies and deposits. An eight point scale described by Walker (1976) (Walker-scale hereafter) was used in the estimation of the degree of shading, sward canopy cover, rockiness and stoniness of the soil. Terrain morphology units (TMUs) were ascribed to each site based on catenal position, namely crest, midslope, footslope and bottom land (valley bottom); with aspect and slope angle also recorded. Samples of topsoil to a depth of 20 cm were collected from cores taken at three random points along each transect. Soil samples were submitted to a certified soil laboratory for analysis of texture, fertility and total C, N and S.

Landuse assessments were based on the general condition and physiognomy of the vegetation using ecological attributes of the dominant species as indicators of past management. Landuse classes included cattle ranches, mixed farming (crops and livestock and/or game), nature reserves, smallholdings and unutilised/abandoned land. Grazing animal categories included mixed game (small to large ungulates), cattle, mixed livestock and horses. The level of utilisation by livestock or game was rated under the categories of undergrazed, optimally grazed, selectively grazed or overgrazed. Grasses were the dominant life form by far and each grass species was assigned to an ERG (Tainton 1999), refer to the Appendix. Under this paradigm Decreaser species dominate under ideal/optimal grazing management. Dominance by Increaser-1 species indicates underutilised rangeland; Increaser-2 species overgrazing and Increaser-3 species selective grazing.

In addition some grasses are influenced more by environmental factors (e.g. water-logging) than grazing and were termed Non-response species. Native forbs were kept separate and alien grasses and forbs were grouped together. Ecological Response Groups (ERGs) were used to categorise the vegetation structure; when forbs were dominant these sites were regarded as ruderal communities.

A range condition score (RCS) was calculated for each site based on the species attributes from 200 records per site. This was achieved by summing the ecological values assigned to the recorded species multiplied by their abundance (Tainton 1999), where Decreaser species were allocated 10 points, Increaser-1 species = seven, Increaser-2 species = four, Increaser-3 species = one, Forbs = one, Non-response species = five and IAP = zero points. A hypothetical benchmark served as an index of best possible range condition for comparison against actual sites assessed. The maximum value of the benchmark was 2,000 points consisting of 200 decreaser species records. RCS was then converted to a percentage of the benchmark (actual score/2,000*100) to derive a convenient value for comparisons.

Fires were rated as not having occurred (fire-protected sites) or rarely in the past 10 years, occasional fires (every 5 years) and frequent fires (annual to triennial). Other information included incidences of mowing and abandoned agricultural fields (old field succession).

Data analysis

The effect that environmental variables (herbivory, rainfall, soil, terrain and landuse) had on pompom weed density and height were compared by one-way analysis of variance (ANOVA), followed by Unequal N HSD post hoc tests in runs where significant differences were determined. Multivariate predictors of pompom weed density were analysed using regression trees and multiple regressions (MR). Statistica version 6.1 was used for all of the above analyses (StatSoft Inc. 2004). Data transformations were performed where necessary to meet the assumptions of ANOVA concerning homogeneity of variance. Regression tree analyses were used to identify variables most likely to have influenced pompom weed density while MR was used for predictive modelling. The most parsimonious MR model of pompom weed density with all significant terms ($P < 0.05$) was determined by subset regressions subjected to Akaike's Information Criterion (AIC) (Whittingham et al. 2006). Quantile regressions (LAD) to identify and estimate the extent to which limiting factors constrain pompom weed density were performed with the statistical program Blossom version W2005.08.26 (Cade and Richards 2005).

The relationship between species composition and environmental data was determined by multivariate analysis using Canoco 4.5 (ter Braak and Šmilauer 2002). A total of 224 species were recorded in 80 transects or sites, with 200 species records per site (16,000 records). Outlier species with only one record were removed from the species matrix to reduce noise. This reduced the number of species to 125 (15,730 records). Species relative abundances were log-transformed because the data were skewed, with many rare species and few dominant species. Down-weighting (value = 0.1) was applied to rare species as their functional importance in distinguishing differences between communities comprising a relatively large pool of species was minor. All alien species were deleted (24 species, 2,299 records of which 59% was pompom weed) because the objective was to fit environmental factors to native species compositions. Ordination is a tool that summarises ecological redundancy and exposes the key differences between communities in relation to constraining environmental factors. In this regard native species that occurred at many sites were made passive as they had a dampening effect on species turnover between samples. Passive species do not influence the ordination and their relation to other species can still be judged if required as they are added after the analysis (ter Braak and Šmilauer 2002). This left a residual 97 active species.

Detrended Correspondence Analysis (DCA) (ter Braak and Šmilauer 2002) of the plant community data revealed a unimodal response with a gradient length on the first and second axes of 5.784 ($\lambda_1 = 0.559$) and 5.286 ($\lambda_2 = 0.394$) SD units of species turnover, rendering Canonical Correspondence Analysis (CCA) the appropriate test for direct gradient analysis. In CCA scaling focused on inter-sample distances using Hill's scaling. Environmental variables were fitted to community data using the Monte Carlo Permutation Test with 499 permutations ($\alpha = 0.05$). In the ensuing ordination diagram sites were classified according to pompom weed density into three categories, namely sparse ($\leq$0.1 plants m^{-2} or $\leq$1,000 ha^{-1}), medium (0.1–0.5 plants m^{-2} or 1,000–5,000 ha^{-1}) and dense ($\geq$0.5 m^{-2} or >5,000 ha^{-1}).

Results

Pompom weed physical condition and herbivory

Surveys were carried out at a time of seasonal transition from summer to autumn. The annual stems of pompom weed die-back to the rootstock in late autumn (May). Plant condition had no effect on density or height (Table 1), but condition in April was affected by the type of vegetation it occurred in. The incidence of healthy green plants was lowest in the drier, warmer savanna, increasing in grassland, with wetland supporting the greatest frequency of healthy plants ($F_{(2,77)} = 3.195$, $P = 0.046$). Senescence was not significant but appeared highest in savanna and lowest in wetland. Soil moisture availability plays a role in the timing of senescence. Pompom weed was largely unaffected by herbivory (76 sites) with the worst damage scored as trivial (two sites), while two sites displayed some exploratory feeding damage. Herbivory (feeding damage) did not have a significant effect on pompom weed density but did affect plant height (Table 1). Grasshoppers were the most conspicuous herbivores on pompom weed. Antelope were occasionally responsible for light browsing. Plants affected by trivial damage and exploratory feeding had the same average height of 54 cm compared to sites with zero damage of 82 cm. The impact of feeding damage on pompom weed height should not be taken literally due to the large discrepancies in sample sizes.

Predictors of botanical composition

In CCA 9 environmental variables (three nominal) were included under the reduced Monte Carlo model (499 permutations) at the 95% confidence level. The eigenvalues for the first and second axes (Fig. 2) were 0.348 ($F = 3.704$, $P = 0.002$) and 0.244, with a cumulative variance of 27.3 and 46.5% species-environment relation, respectively. The sum of all canonical eigenvalues explained 72.1% of the total

Table 1 Summary of one way ANOVAs showing the significance of environmental factors on pompom weed density (plants m^{-2}) and height (cm)

Environmental factor (categories)	Density (plants m^{-2})			Height (cm)		
	df, err	F	*P*	*df*, err	F	*P*
Pompom condition	2, 77	0.917	0.404	2, 77	0.133	0.876
Feeding damage	2, 77	0.663	0.518	2, 77	6.217	0.003
Mean annual rainfall	2, 77	1.889	0.158	2, 77	0.454	0.637
Vegetation type	2, 77	0.844	0.434	2, 77	2.619	0.079
Soil texture class	7, 72	1.714	0.119	7, 72	1.345	0.242
Soil erosion type	8, 71	3.232	0.003	8, 71	3.002	0.006
Rocks	4, 75	4.787	0.002	4, 75	2.573	0.044
Stones	6, 73	1.733	0.126	6, 73	1.060	0.394
Terrain morphology	3, 76	1.481	0.226	3, 76	1.284	0.286
Aspect	5, 74	0.912	0.478	5, 74	1.635	0.161
Sward canopy cover (reciprocal = ground cover)	4, 75	1.644	0.172	4, 75	2.697	0.037
Grazing animal category	3, 76	1.741	0.166	3, 76	0.349	0.790
Mowing	1, 78	0.391	0.533	1, 78	11.702	0.001
Fire*	2, 77	3.280	0.043	2, 77	1.096	0.339
Landuse	4, 75	1.143	0.343	4, 75	0.653	0.626
Range management	4, 75	0.611	0.656	3, 73	2.031	0.099

Significant effects ($P < 0.05$) on weed density are in bold

* Data heteroscedastic, square root transformed

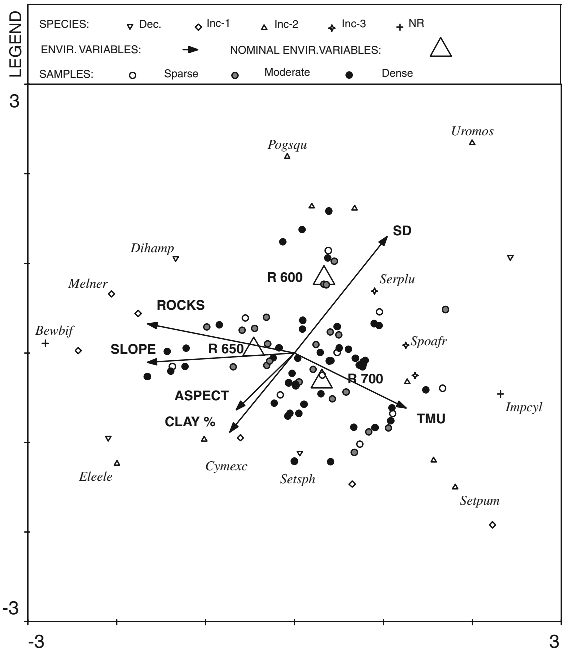

Fig. 2 Canonical Correspondence Analysis (*CCA*) showing the differences in species composition in 80 Highveld rangelands in association with three density classes of pompom weed (*Campuloclinium macrocephalum*) (sparse, medium and dense) after being constrained by mean annual rainfall (R600–R700 mm p.a.), topography and soil texture. Abbreviations: *TMU* terrain morphology unit (crest to bottomland) and *SD* Sample density. Species Ecological Response Groups (*ERGs*) are indicated by symbols for Decreaser (*Dec.*), Increaser-1 (*Inc-1*), Increaser-2 (*Inc-2*), Increaser-3 (*Inc-3*) and Non-response species (*NR*). Acronyms for indicator species: Bewbif, *Bewsia biflora*; Cymexc, *Cymbopogon excavatus*; Dihamp, *Diheteropogon amplectens*; Eleele, *Elephantorrhiza elephantina*; Impcyl, *Imperata cylindrica*; Melner, *Melinis nerviglumis*; Pogsqu, *Pogonarthria squarrosa*; Serplu, *Seriphium plumosum* (=*Stoebe vulgaris*); Setpum, *Setaria pumila* (=*S. pallide-fusca*); Setsph, *Setaria sphacelata*; Spoafr, *Sporobolus africanus*; Uromos, *Urochloa mosambicensis*

variance in the species data (Trace = 1.273, F = 1.971, $P = 0.002$). Mean annual rainfall, topography and soil texture were the dominant variables controlling species composition (Fig. 2). Sample density (SD) increased in the direction of sandy soils and was negatively correlated with clay % and aspect (maximum trajectory = north). Orthogonal to these gradients were catenal position (TMU) increasing in the direction of bottomlands in contrast to increasing slope steepness and rocky ground. Mean annual rainfall is depicted in a similar pattern to rainfall distribution in Gauteng. The savanna areas in the north and west receive 600–650 mm p.a. (R600 and R650 in Fig. 2) and the grasslands in the centre, south and east of the province receive 700 mm p.a. (R700).

Over 40% of the species data comprised occurrences of a few common species, the dominant ones being the grasses *Eragrostis curvula* (Schrad.) Nees, *Cynodon dactylon* (L.) Pers and *Hyparrhenia hirta* (L.) Stapf (Appendix). Species depicted in the CCA (Fig. 2) have limited ranges and serve as indicators of the compositional differences amongst sites. Grasses (Poaceae) were the dominant life form with 77 native species, of which only 12 (16%) were Decreaser species with a median transect frequency of 4%. Species on the right side of the diagram are characterised by hardy native encroacher species in overgrazed vegetation, exacerbated by sandy acidic soils. The wetland grass *Imperata cylindrica* (Appendix) was associated with bottomlands. Species depicted on the left side of the diagram have characteristics of degraded and underutilised vegetation. Pompom weed infestations ranged from 100 to 64,000 plants ha^{-1}. Based on the attributes of indicator species we conclude that pompom weed occurred across a wide range of degraded vegetation states. The three density categories of pompom weed were uniformly distributed across both axes (Fig. 2). Pompom weed appears to have a broad ecological niche with regard to the vegetation it establishes in and the variables that govern them.

Univariate predictors of pompom weed density

Pompom weed ranged in height from 33 to 116 cm (mean = 82 cm) (Fig. 3a) and density from 0.01 to 6.36 plants m^{-2} (mean = 1.4 plants m^{-2}) (Fig. 3b). No relationship was found between density and height ($r^2 < 0.001$, $t = 0.034$, $P = 0.973$). Both were affected to some extent by environmental factors (Table 1). Feeding damage and mowing had a significant effect on height but not on density. Fire on the other hand affected density but not height. Soil erosion and rockiness affected both variables. The identification of factors that promoted and/or reduced weed density was considered to be of paramount importance. Sites with multiple erosion categories had the greatest and most significant densities of pompom weed with terracettes and pedestals being the best combination for optimum weed dominance (Table 1). Rocky ground supported the densest pompom weed stands. Sites that experienced frequent

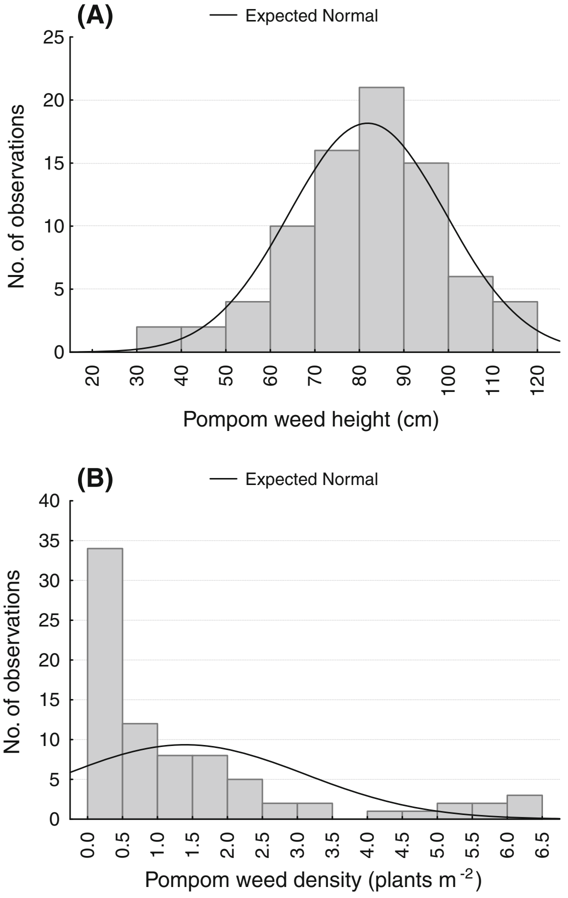

Fig. 3 Frequency histograms of height (**a**), and density (**b**) of pompom weed (*Campuloclinium macrocephalum*) showing the range and distribution of recorded values. The normal curve of expected frequencies has been superimposed over the observed frequencies

fires supported denser stands of pompom weed than sites where fire was absent or rare. In the grassland biome winter is the driest, most fire-prone time of year, when pompom weed is dormant. Enlarged perennating buds on the rootstock just below the soil surface were stimulated by fire and resulted in premature mass emergence of shoots in burnt areas before native grasses. Increasing fire frequency in the winter months therefore, not only extended the growth period of shoots (late winter growth through to the following autumn) but also facilitated densification because pompom weed was growing under conditions where native vegetation was still dormant.

Pompom weed density did not differ significantly across landuse and range management categories. Range condition was poor on average, 43 ± 13% of the benchmark, and its relation with pompom weed density was not significant ($r^2 = 0.028$, $F_{(1,78)} = 2.209$, $P = 0.141$). Analysis of ERGs was more informative (Fig. 4). More sites were dominated by Increaser-2 species (50%), but the density of pompom weed was greatest in sites dominated by Increaser-1 species (undergrazed). The weighted ratio between pompom weed density and ERG was however, not significant, indicating that multiple degradation states are all prone to invasion. Although RCS was greatest in sites dominated by Decreasers (by calculation), there were no differences in the percentage abundance (11.5 ± 1.7%) or in the number of Decreaser species (1.8 ± 0.2) between ERGs. Basal cover was also not statistically different between ERGs, being very low on average (9.8 ± 0.5%, $P = 0.345$) (data not presented). The low incidence of Decreaser species is indicative of a long history of selective grazing, overgrazing and site transformation (old field successions) that may have resulted in the local extinction of many palatable species prior to current practices, which now vary from abandoned or underutilised grasslands to areas of continued heavy grazing. This lag-phase following

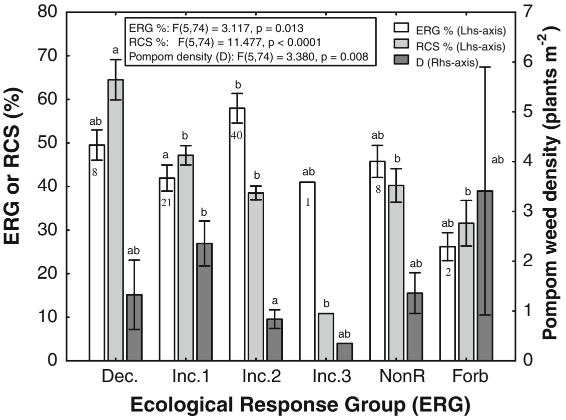

Fig. 4 The proportional abundance (ERG%, *Lhs*-axis) of Ecological Response Groups (*ERG*) in 80 Highveld rangelands, and their effect on range condition score (RCS%, *Lhs*-axis) and pompom weed density (D, *Rhs*-axis). The group codes Dec., Inc.1, Inc.2, Inc.3, NonR and Forb on the *x*-axis refer to Decreaser, Increaser-1, Increaser-2, Increaser-3, Non-response and Forb groups, respectively. Numbers in *white bars* refer to the number of sites in which these groups are dominant. *Bars* represent the mean ± SE with letters denoting significant differences ($P < 0.05$, one way ANOVA, within factor HSD test for unequal N). Raw data in ERG% and D categories were heteroscedastic (see Fig. 3b) and were corrected by log and square root transformations, respectively

Table 2 Summary of the significant environmental factors (n = 80, $P < 0.05$) with constraining effects on pompom weed density using quantile regression

Limiting factor	LAD summary (n = 80)		Maximum limiting function[b] (plants m^{-2})	Maximum limiting function[c] (%)
	Quantile	P ARSS[a]		
Silt	0.99	0.017	6.1	4.3
Avail. P	0.92	0.038	4.1	35.8
Rocks	0.90	0.006	3.0	52.4
Slope	0.94	0.039	2.2	65.1
Fire frequency	0.90	0.026	2.1	67.8
Total carbon	0.92	0.008	2.0	68.3
pH	0.89	0.047	1.9	70.1
Calcium	0.89	0.026	1.9	70.2
Total nitrogen	0.92	0.011	1.8	71.5
Sand	0.90	0.027	1.7	73.0
Total sulphur	0.90	0.039	1.5	76.8
Sample density	0.92	0.030	1.5	77.1
Basal cover %	0.99	0.016	0.1	98.2

LAD = Least Absolute Deviation or Quantile Regression

[a] P-value of the Asymptotic Rank Score Statistic (Cade and Richards 2005)

[b,c] Threshold pompom weed density ([b] no. m^{-2}, [c] %) based on minimum and maximum field densities of 0.01 and 6.36 m^{-2}

the decline of extinction-prone species may have allowed for the development of alternative vegetation assemblages characterised by particular species such as *H. hirta* on land now underutilised and *E. curvula* in areas of sustained heavy grazing, both compositions are susceptible to pompom weed invasion.

None of the factors subjected to ANOVA had a strong limiting effect on the weed's density. Factors that constrained the density of pompom weed were identified by quantile regression (Table 2). Grass basal cover was the only variable that had a strong direct influence on pompom weed density, with cover >19% restricting infestation densities by up to 99% (0.04 plants m^{-2}) of the maximum population density (6.36 plants m^{-1}) (Fig. 5). From the recurring pattern of poor range condition in association with a scarcity of Decreaser species and low basal cover (<19%), we conclude that injudicious management is one of the underlying causes of invasion susceptibility.

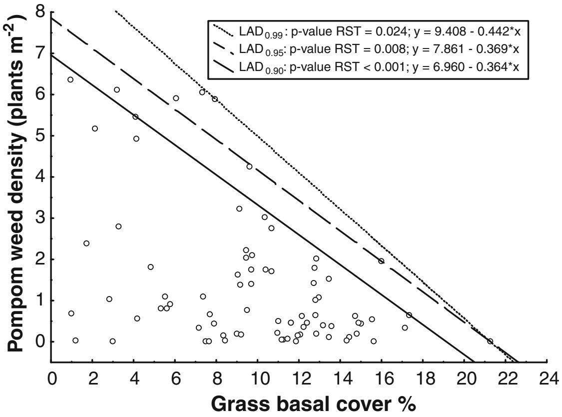

Fig. 5 The limiting relationship between grass basal cover (%) and *Campuloclinium macrocephalum* (pompom weed) density in 80 Highveld grassland communities. The lines depict different quantile regressions (*LAD*) at 99, 95 and 90th quantiles with P-values for the Rank Score Test Statistic (*RST*)

Multivariate predictors of pompom weed density

Regression tree analysis to identify the factors that affect the density of pompom weed was applied to soil, vegetation and rainfall data separately as no optimal tree could be produced from all environmental variables together. Soil texture, chemistry or state (erosion) did not constrain the density of pompom weed. Basal cover was the most important variable in the vegetation and landscape data. A dichotomy in weed density was projected at 10.824% basal cover, with >10.8% (36 sites) having an average density of 0.54 ± 0.55 (STD) plants m^{-2}. Sites where basal cover was <10.8% (44 sites) were further subdivided by fire frequency. Sites with occasional to frequent fires with basal cover >8.2% (10 sites) had 1.71 ± 0.85 pompom weeds m^{-2}, while <8.2% (10 sites) favoured stands of 4.68 ± 1.6 plants m^{-2}. Sites with zero burning (n = 24) had an average density of 1.73 ± 1.44 plants m^{-2} without further subdivisions in basal cover. Rainfall was less effective as a predictor of pompom weed density than basal cover and fire. A dichotomy occurred at 625 mm rainfall per annum; below this amount average density was 0.79 ± 0.54 plants m^{-2} (11 sites) and sites receiving more than 625 mm p.a. ($n = 69$) had a density of 1.49 ± 1.8 plants m^{-2}. Pompom weed density could possibly be restricted in xeric grasslands that receive less than 600 mm per annum, but wetlands in these semiarid areas would still be vulnerable to invasion.

Multiple regressions produced four significant predictive models for pompom weed density, of which sward basal cover % and fire frequency were common to all. No generic rangeland model could be generated for pompom weed density because the weed was not found in rangelands in good condition. In hindsight rangelands without pompom weed should have been sampled to validate the assumption that biotic resistance is greatest in vegetation in good condition. The most parsimonious range degradation model for pompom weed density with the lowest AIC value included the extra variable "Increaser-2 species", summarised as $R^2 = 40.3\%$, $F_{(3,76)} = 17.13$, $P < 0.0001$, estimated SE = 1.34:

$$\begin{aligned}\text{Pompom weed density} = 2.938 &- (0.146 * \text{BC}) \\ &+ (0.833 * \text{FF}) \\ &- (0.021 * \text{Inc2})\end{aligned}$$

where: Pompom weed density (no. plants m^{-2}) applies only to disturbed/degraded rangelands, BC = basal cover %, FF = fire frequency, Inc2 = Increaser-2 species frequency

The invasiveness of pompom weed may be due to the absence of natural enemies but factors external to biological control operating at the site level have the ability to facilitate or constrain invasion. Raw data showed that pompom weed declined strongly as basal cover increased, with only one pompom weed occurrence above 18% (Fig. 5). Basal cover and Increaser-2 species had a moderating influence on the effects of fire frequency. The density model predicted that pompom weed invasion could be prevented with basal cover of ≥16% coupled with zero burning and Increaser-2 frequency of ≥25%. The same model but with occasional fires resulted in weed populations of >7,000 plants ha^{-1} and with frequent fires this figure jumped to >16 000 plants ha^{-1}. Realistically, these are fire-maintained ecosystems and therefore, biotic resistance will be low and unable to prevent invasion if basal cover is <19%. From a management perspective, pompom weed density is constrained with a basal cover of 21% with occasional burning, providing Increaser-2 frequency is at least 40%. Under frequent burning basal cover would have to be at least 23% with 60% Increaser-2 incidence.

Discussion

Pompom weed is currently utilised by generalist herbivores that cause trivial damage at best. Natural enemies from the native geographical range are still in quarantine (McConnachie 2007). The most significant stress factor for pompom weed was moisture availability caused by the onset of the dry season (winter). Plants responded rapidly to moisture-stress by senescing, a phenomenon typical of herbaceous plants in South African grasslands. Insufficient herbivory in South Africa could contribute to the adaptation of a dormant period that is in contrast with the compensatory growth observed in its native range arising from herbivory (McNaughton 1983). Climate continues to be an unknown factor, particularly at what point rainfall becomes critical in preventing establishment and further spread. The weed is presently found in areas that receive >600 mm of rainfall per annum.

Environmental factors governing rangelands on the South African Highveld only showed partial overlap with factors facilitating pompom weed invasion. Rainfall, topography and soil texture were the primary drivers of vegetation pattern, while pompom weed exhibited disturbance-mediated invasion strategies. The fact that most of the infestations in the region (92%) occurred along roadsides further emphasises the ruderal attributes of pompom weed. Vehicles are likely to be important vectors of seed-dispersal (Von Der Lippe and Kowarik 2007). Invasion opportunity into rangelands is probably largely through seed dispersal by wind but establishment appears to be constrained by the quality of the sward and basal cover. Principal drivers of degradation were unsustainable commercial grazing strategies and habitat transformation in the past, namely draining of wetlands and old field successions on abandoned cultivated land.

The pattern of declining pompom weed density under increasing abundance of Increaser-2 species is ecologically meaningful in the context of rangeland rehabilitation. It appears that interspecific competition between grasses and pompom weed is optimal in rangelands dominated by Increaser-2 species, presumably from a history of unsustainable grazing management resulting in the elimination/extinction of Decreaser species. Decreasers are usually palatable species with K-selection traits, for example obligate reproducers of large seeds but with low seed production potential and poorly adapted long-distance dispersal mechanisms, e.g. *Themeda triandra* Forssk. (O'Connor 1991) (Appendix). Initial surveys in 2002 noted that pompom weed was not a significant invader on commonage or communal grazing areas, which are traditionally under continuous grazing pressure from an assortment of livestock, but chiefly cattle and goats. O'Connor (2005) found that communal rangelands at high altitude (1,200–1,600 m a.s.l.), although poor in desirable species composition for livestock production, did not support many alien plant species. O'Connor et al. (2003) found basal cover in KwaZulu-Natal province (Fig. 1) to be linearly related to altitude for both commercial and communal rangelands at elevations from 700 to 2,100 m above sea level and a rainfall gradient from 670 to 1,130 mm p.a. Mean basal cover for commercial rangelands increased from 20% at 1,400 m a.s.l. to 23% at 1,800 m a.s.l., an altitudinal range similar to Highveld rangelands in this study, suggesting that sites with <20% basal cover had become severely degraded and susceptible to pompom weed invasion. Introducing grazing strategies that promote Increaser-2 dominance would be more effective at constraining pompom weed in underutilised rangelands dominated by Increaser-1 species.

Biotic resistance to pompom weed invasion will be greatest in rangelands in good condition with a relative abundance of Decreaser species of at least 50% and/or a sward basal cover >19%. We concur with the findings of Lake and Leishman (2004) who found that IAPs did not invade undisturbed rangelands. These authors also discovered that herbivory of native species was greater than exotic species, suggesting that the absence of natural enemies was not a universal attribute for successful invasion by IAPs. Rather specific plant attributes such as greater leaf area compared to native species in the invaded sites and the types of disturbance were important for facilitating the establishment of IAPs. Similarly, pompom weed invaded degraded rangelands that had been abandoned or still grazed, but rangelands in good condition were conspicuously absent in sites that were strategically sampled along a pompom weed infestation gradient. The impact that pompom weed has on species composition is subtle rather than one that completely transforms the communities it invades. For example, *E. curvula* and pompom weed were the most dominant species across sites (Appendix), yet in a separate study both species were found to be excellent competitors that coexisted rather than being competitively exclusive under controlled conditions and in the field (Goodall et al. 2010). Three theories could contribute to this phenomenon. (1) The ability to source limiting resources not utilised by the other species (Tilman 1985) and may be due to differences in rooting depths of grasses and pompom weed. (2) Pompom weed, being free of natural enemies in its introduced range could have an advantage over native communities to withstand higher degrees of interspecific competition (Eppinga et al. 2006; Catford et al. 2008). (3) Certain Increaser-2 species, like pompom, may have r-selection traits such as rapid growth, early fecundity and the copious production of small seeds with effective dispersal mechanisms (Elton 1958; Sutherland 2004).

It is proposed that pompom weed does not invade and replace climax species. Rather factors mentioned in the preceding paragraph and niche opportunity may have provided suitable conditions for pompom weed to establish and exert a greater competitive ability over other pioneer cohorts that colonise disturbed ecosystems. The most frequently encountered native forb in rangelands invaded by pompom weed was *Helichrysum rugulosum* Less. (Appendix). Most pioneer forbs were alien species such as *Tagetes minuta* L. and *Verbena bonariensis* L. (Appendix) and served as indicators for potential pompom weed invasion into uninvaded rangeland areas. The dominant exotic grasses were *Paspalum* species (Appendix), of which four occurred in 38 sites (228 records). With only 8% of the locality records of pompom weed in Gauteng being in rangelands away from roadsides one could conclude that Highveld rangelands are largely resilient to invasion. A more likely scenario is that because the indicator weeds mentioned above are already widespread in the grassland and savanna biomes, the potential for further invasion is considerable.

The absence of specialist natural enemies in providing pompom weed with an opportunity for increased niche width in the introduced range

(Jeffries and Lawton 2008) can only be assessed sometime after biocontrol agents have been released. In addition to enemy release, other invasion hypotheses relevant to pompom weed include propagule pressure, ideal weed (r-selection traits), environmental heterogeneity, increased resource availability, disturbance, empty niche, adaptation and naturalisation (Catford et al. 2008). Unsustainable grazing strategies (Barnes 1992) and soil disturbance are believed to be the indirect drivers of pompom weed invasion in rangelands, which the weed can capitalise on in the absence of natural enemies. In other words, an enemy-free state is secondary to invasion opportunity created though injudicious ecosystem management.

Rangelands not at equilibrium are difficult to restore once they have been damaged (Suding et al. 2004). Alternative vegetation states resulting from bad range management practices are prone to invasion by native and invasive alien species (Grobler et al. 2006; Fensham et al. 2009). Resting and burning will not result in restoration and could be counter-productive by facilitating the invasion of pompom weed. It is proposed that grazing systems that sustain swards comprising a suite of Increaser-2 species coupled with integrated weed management utilising biological and herbicidal control methods is the most pragmatic manner to sustain the natural resource of degraded, low production rangelands on the Highveld and to maintain them free of pompom weed. If pompom weed primarily invades road reserves, then perhaps of greatest interest now are those habitats along the side of the road that have the least pompom weed, on condition that propagule pressure is the same in both. This would reveal factors limiting its distribution in potentially disturbed habitats and fence-line contrasts along road reserves with and without pompom weed warrant further investigation.

The status of the biological control of pompom weed is that all agents being tested are still in the quarantine phase. Host specificity testing is being carried out on two insect species and one pathogen (McConnachie et al. 2011). The insects are *Liothrips tractabilis* Mound & Pereyra (Thripidae) and *Cochylis campuloclinium* Brown (Tortricidae). The pathogen is the rust fungus *P. eupatorii* mentioned in the introduction. All agents appear to be host-specific but their success as biocontrol agents can only be determined once they have established in the field, pending approval for release, with the exception of the rust. The rust fungus has established at most infestations across the country and is clearly very damaging to adult plants in late summer. An impact assessment of the pathogen has been concluded and the findings will be published in due course. In the context of invasion biology it is hoped that these studies contribute to improved understanding of factors governing rangeland invasions, or rendering them susceptible to invasion, and the properties of the IAPs that establish and spread in them.

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Appendix

See Table 3.

Table 3 List of plants which collectively contributed to more than 85% of the 16,000 species records, including nomenclature, family, number of individual species records, number of sites in which they occurred and respective Ecological Response Group (ERG)

Nomenclature (Germishuizen and Meyer 2003)	Family	Records n (%)	Sites n (%)	ERG[a]
*Bidens pilosa** L.	ASTERACEAE	51 (0.3)	10 (12.5)	Alien
*Cirsium vulgare** (Savi) Ten.	ASTERACEAE	10 (0.1)	5 (6.3)	Alien
*Campuloclinium macrocephalum** (Less.) DC.	ASTERACEAE	1,357 (8.5)	72 (90)	Alien
*Conyza canadensis** (L.) Cronquist	ASTERACEAE	59 (0.4)	8 (10)	Alien
Helichrysum rugulosum Less.	ASTERACEAE	149 (0.9)	26 (32.5)	Forb

Table 3 continued

Nomenclature (Germishuizen and Meyer 2003)	Family	Records n (%)	Sites n (%)	ERG[a]
Seriphium plumosum L. (=*Stoebe vulgaris* Levyns)	ASTERACEAE	136 (0.9)	18 (22.5)	Forb
*Tagetes minuta** L.	ASTERACEAE	178 (1.1)	18 (22.5)	Alien
Commelina benghalensis L.	COMMELINACEAE	16 (0.1)	10 (12)	Forb
Elephantorrhiza elephantina (Burch.) Skeels	FABACEAE	56 (0.4)	9 (11.3)	Forb
*Plantago lanceolata** L.	PLANTAGINACEAE	46 (0.3)	13 (16.3)	Alien
Andropogon schirensis A.Rich.	POACEAE	48 (0.3)	8 (10.0)	I1
Aristida congesta Roem. & Schult.	POACEAE	193 (1.2)	22 (27.5)	I2
Aristida junciformis Trin. & Rupr.	POACEAE	265 (1.7)	11 (13.8)	I3
Bewsia biflora (Hack.) Gooss.	POACEAE	89 (0.6)	7 (8.8)	NR
Brachiaria brizantha (A.Rich.) Stapf	POACEAE	121 (0.8)	4 (5.0)	I1
Brachiaria serrata (Thunb.) Stapf	POACEAE	286 (1.8)	17 (21.3)	D
Cymbopogon excavatus (Hochst.) Stapf ex Burtt Davy	POACEAE	90 (0.6)	12 (15)	I1
Cynodon dactylon (L.) Pers.	POACEAE	1,400 (8.8)	55 (68.8)	I2
Digitaria eriantha Steud.	POACEAE	136 (0.9)	13 (16.3)	D
Diheteropogon amplectens (Nees) Clayton	POACEAE	138 (0.9)	19 (23.8)	D
Elionurus muticus (Spreng.) Kuntze	POACEAE	99 (0.6)	17 (21.3)	I3
Eragrostis chloromelas Steud.	POACEAE	76 (0.5)	5 (6.3)	I2
Eragrostis curvula (Schrad.) Nees	POACEAE	2,361 (14.8)	67 (83.8)	I2
Eragrostis gummiflua Nees	POACEAE	232 (1.5)	18 (22.5)	I2
Eragrostis nindensis Ficalho & Hiern	POACEAE	112 (0.7)	12 (15)	I2
Eragrostis plana Nees	POACEAE	189 (1.2)	24 (30)	I2
Eragrostis racemosa (Thunb.) Steud.	POACEAE	68 (0.4)	11 (13.8)	I2
Eragrostis rigidior Pilg.	POACEAE	77 (0.5)	6 (7.5)	I2
Heteropogon contortus (L.) Roem. & Schult.	POACEAE	204 (1.3)	37 (46.3)	I2
Hyparrhenia hirta (L.) Stapf	POACEAE	1,071 (6.7)	51 (63.8)	I1
Hyparrhenia tamba (Steud.) Stapf	POACEAE	210 (1.3)	5 (6.3)	I1
Imperata cylindrica (L.) Raeuschel	POACEAE	792 (5.0)	15 (18.8)	NR
Melinis nerviglumis (Franch.) Zizka	POACEAE	78 (0.5)	14 (17.5)	I1
Melinis repens (Willd.) Zizka	POACEAE	278 (1.7)	29 (36.3)	I2
Panicum coloratum L.	POACEAE	122 (0.8)	2 (2.5)	D
Panicum natalense Hochst.	POACEAE	82 (0.5)	13 (16.3)	D
Panicum schinzii Hack.	POACEAE	130 (0.8)	11 (13.8)	I2
*Paspalum dilatatum** Poir.	POACEAE	23 (0.1)	9 (11.3)	Alien
*Paspalum distichum** L.	POACEAE	101 (0.6)	14 (17.5)	Alien
*Paspalum urvillei** Steud.	POACEAE	95 (0.6)	13 (16.3)	Alien
Pogonarthria squarrosa (Roem. & Schult.) Pilg.	POACEAE	78 (0.5)	10 (12.5)	I2
Schizachyrium sanguineum (Retz.) Alston	POACEAE	248 (1.6)	20 (25.0)	I1
Setaria incrassata (Hochst.) Hack.	POACEAE	74 (0.5)	4 (5.0)	D
Setaria nigrirostris (Nees) T.Durand & Schinz	POACEAE	94 (0.6)	11 (13.8)	I1
Setaria pumila (Poir.) Roem. & Schult.	POACEAE	229 (1.4)	18 (22.5)	I2
Setaria sphacelata (Schumach.) Moss	POACEAE	447 (2.8)	21 (26.3)	D
Sporobolus africanus (Poir.) Robyns & Tournay	POACEAE	52 (0.3)	15 (18.8)	I3
Themeda triandra Forssk.	POACEAE	442 (2.8)	37 (46.3)	D
Trachypogon spicatus (L. f.) Kuntze	POACEAE	325 (2.0)	23 (28.8)	I1

Table 3 continued

Nomenclature (Germishuizen and Meyer 2003)	Family	Records n (%)	Sites n (%)	ERG[a]
Trichoneura grandiglumis (Nees) Ekman	POACEAE	40 (0.3)	5 (6.3)	I2
Triraphis andropogonoides (Steud.) E.Phillips	POACEAE	50 (0.3)	9 (11.3)	I1
Tristachya leucothrix Nees	POACEAE	151 (0.9)	3 (3.8)	I1
Urochloa mosambicensis (Hack.) Dandy	POACEAE	15 (0.1)	5 (6.3)	I2
*Verbena bonariensis** L.	VERBENACEAE	114 (0.7)	23 (28.8)	Alien
*Verbena officinalis** L.	VERBENACEAE	57 (0.4)	12 (15.0)	Alien

[a] *D* Decreaser, *I1* Increaser-1, *I2* Increaser-2, *I3* Increaser-3, *NR* Non response species (refer to "Materials and methods")

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ORIGINAL PAPER

3. Does allelopathy explain the invasiveness of *Campuloclinium macrocephalum* (pompom weed) in the South African grassland biome?

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Abstract *Campuloclinium macrocephalum* is an Asteraceous alien weed that invades roadside vegetation and grassland in South Africa. The role of allelopathy and competition in its invasiveness was investigated using *Eragrostis curvula* (weeping lovegrass, an indigenous grass), *E. tef* and *Lactuca sativa* (lettuce) as test species. Trials were conducted in Petri-dishes, pots and in the field. Root and shoot extracts of adult *C. macrocephalum* plants did not inhibit seed germination in any test species. The greatest effect was radicle stunting produced by leaf extracts at 10 and 25% w/v. *Eragrostis curvula* was less tolerant of the extracts than *E. tef*. Allelopathic effects could however not be confirmed in pot trials evaluating the interference potential of the weed or weed residue effects against *E. curvula*. *E. curvula* growth and biomass was not affected by plant densities of one or five *C. macrocephalum* per pot, whereas *C. macrocephalum* suffered a 17% mortality and density-dependant trade-offs of size and biomass for survival. Under field conditions *C. macrocephalum* had a broader ecological niche than *E. curvula*, invading hygrophilous and undisturbed grasslands not amenable for *E. curvula* establishment, this included well drained disturbed soils on which the latter proliferated. Evidence of competitive exclusion of *E. curvula* by *C. macrocephalum* or vice versa was not detected. The coexistence of both species irrespective of relative density suggested these species have different resource requirements. Allelopathy was not an adequate causal mechanism to explain invasiveness in *Campuloclinium macrocephalum*. A more traditional hypothesis such as the absence of natural enemies, at this stage, better justifies the weed's invasion success.

Keywords Interference · Density dependence · In vitro assays · Pot trials · Grassland belt transects · *Eragrostis* species · Lettuce · Grass ecological response groups

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Introduction

Campuloclinium macrocephalum (Less.) DC (=*Eupatorium macrocephalum* Less.), family Asteraceae tribe Eupatorieae, originates from South America (Williams 1976; Cabrera 1978; Breedlove 1986) and has recently become a major invader of roadsides, grassland, savanna and wetland ecosystems in South Africa (Fig. 1a). The plant is a perennial herb that produces several annual stems up to 1.3 m in height, developing from perennating buds on a woody rootstock below the soil surface. The plant's total biomass places considerable investment in underground structures and during winter all of the living biomass occurs below the soil surface (rootstock and roots), the stems having died off. The root system consists of clusters of thickened tuber-like roots. The common name "pompom weed" is attributed to the puffy pink blossoms borne throughout summer (Fig. 1b). According to the South African Plant Invader Atlas (Henderson 2007), pompom weed may be considered a pioneer species favouring disturbed habitats, with most of the locality records in Gauteng Province ($n = 1{,}337$) at the time of the study occurring along roadsides. Only 107 (8%) records were of infestations in adjacent grassland ($n = 88$) or savanna ($n = 19$).

The earliest herbarium record is of a specimen from Johannesburg collected in 1962. More frequent occurrences around Pretoria appeared from the mid 1960s and since then the plant has become conspicuous in grasslands in Gauteng Province, with outbreaks also occurring in surrounding provinces (Henderson 2001). The plant is a declared weed under the South African Conservation of Agricultural Resources Act of 1983, Act 43 of 1983. It remains a mystery as to why, how and when the plant arrived in South Africa. It was never propagated commercially by nurseries; however, there are anecdotal accounts of cut-flowers being sold around Pretoria, presumably from naturalised populations in the wild. We speculate the plant was introduced as a garden ornamental on a small scale or accidentally, e.g. as a contaminant of clothing or cargo. To our knowledge the species has not been cultivated in other countries outside of its native range, nor has it been reported invading other parts of the world.

As with other members of the tribe Eupatorieae that have spread exponentially after naturalisation in South Africa, e.g. *Chromolaena odorata* (L.) R. M. King & Robinson (chromolaena) (Goodall and Erasmus 1996), pompom weed is rapidly expanding its range in grassland and savanna regions since its introduction (Henderson et al. 2003; Henderson 2007). A key difference in the invasion potential of the two species is that chromolaena is frost-sensitive and is restricted to altitudes below 1,000 m, whereas pompom weed occurs from the KwaZulu-Natal coast to the central interior of the Highveld, an altitudinal gradient of ca. 2,000 m. Pompom weed is thus a serious threat to grassland and savanna ecosystems in southern Africa.

Campuloclinium macrocephalum is a densely bristly plant (Fig. 1c). Its trichomes consist of rigid unbranched multicellular hairs and sessile glandular trichomes. Both types occur on the stems, leaves and involucral bracts (Retief 2002). Under magnification both glands and hairs present an unidentified brownish substance and thus, trichomes secreting phototoxic substances may play a role in its invasion success. Such incidences of allelopathy have been cited as being an important trait for successful invasion, the so-called "novel weapons hypothesis" (NWH) (Callaway and Ridenour 2004; Bais et al. 2003). While acknowledging the importance of the absence of natural enemies in invasion biology, those authors proposed that certain invader species are able to transform vegetation because they possess allelochemicals. Under this hypothesis the processes of adaptation allows natural neighbours to cohabit an area with an allelopathic species without suffering negative side effects, but in new environments native species may be intolerant to the same chemical compounds responsible for the allelopathic effect. There could also be neighbourhood effects on overall community composition and structure with weed density expansion.

The "novel weapons hypothesis" is not without contestation. Strong evidence exists refuting the role of (−)-catechin, the phytotoxin principally used in building a case for the NWH as an allelochemical promoting invasiveness of spotted knapweed (*Centaurea maculosa* Lam.). Blair et al. (2005) found no measurable catechin in soils of spotted knapweed sites using new methods to quantify catechin. Duke et al. (2009a, b) refuted the mode of action of catechin causing damage by oxidation, claiming it to be a strong antioxidant that is rapidly denatured by

Fig. 1 *Campuloclinium macrocephalum* (pompom weed) showing (**a**) an infestation in grassland, and close-ups of (**b**) flowers and (**c**) bristly stems and leaves, yellowing due to autumn senescence

extracellular root enzymes. Furthermore these authors showed catechin to be poorly phytotoxic to several plants species in bioassays without soil and in a dose–response experiment in soils from areas where spotted knapweed is found, the lowest dose for a growth reduction was still much higher than that observed in nature.

Harper (1977) refuted the role of allelopathy in plant interactions, calling experiments that testify to allelopathic effects "laboratory artefacts" stating categorically that "any species can, by appropriate digestion, extraction, and concentration, be persuaded to yield a product that is toxic to one species or another." It is now well established that allelopathic effects from laboratory studies should only be accepted if they are supported by field experiments (Stowe 1979; Foy 1999). Under field conditions organic toxins produced by an allelopathic species could be rendered unavailable to neighbouring species or broken down by the combined interactions of soil texture, organic matter, temperature, irradiance and microbial breakdown. Most allelopathy experiments fail to take this crucial step of in-field validation. Any studies that claim allelopathy under laboratory conditions are potentially rejecting a true null hypothesis. Hypotheses such as "novel weapons" (Callaway and Ridenour 2004) cannot be supported if the basis for allelopathy hinges on in vitro experimentation involving extracts or leachates alone.

The factors or mechanisms causing or facilitating the invasion of pompom weed into grassland are not yet understood. In this paper the role that allelopathy and competition play in the weed's ability to invade grassland is examined from an ecological perspective. We hypothesize that compounds in pompom weed and/or the weed's competitive ability may affect the germination, growth, survival and recruitment of native grasses and forbs in invaded communities. The findings are presented in three experimental approaches summarised below in the determination of the relative importance of allelopathy and competition in pompom weed.

1. Bioassays (two tests): seeds of test species exposed to pompom weed extracts in vitro in Petri dishes to establish the allelopathic potential of pompom weed on the seed germination and early seedling development of (a) a perennial and an annual grass species and (b) a representative of sensitive broadleaf species.
2. Pot studies (two tests): (a) establish the role of interference in pots by growing pompom weed with the most sensitive species from point one above, and (b) establish the role of crop residues by growing the most sensitive species from point one above in pots containing plant remains.
3. Field investigation: Observe the relation, association and interaction between the frequency and density of pompom weed with the perennial grass species from point one above and grass ecological response groups in Highveld grassland.

Materials and methods

We chose three test plants for this study. *Eragrostis* is a dominant genus in grasslands throughout South Africa, with *Eragrostis curvula* (Schrad.) Nees (weeping lovegrass) being one of the most common perennial indigenous grass species (Acocks 1953). Seed of a naturally occurring strain (cv. Ermelo) (Rhind 1973) is commercially available making it a popular species for site rehabilitation, reclamation and as a pasture species (Campbell 2000). Both *E. curvula* and pompom weed favour disturbed roadsides and grasslands (Gibbs Russel et al. 1990; Henderson et al. 2003). Both species are strongly rooted; the fibrous root system of *E. curvula* is extensive, exploiting soil depths of up to 5 m (Gomes and Asaeda 2009). In our study *E. curvula* and pompom weed were found in grassland sites ($n = 80$) with a range of soil types (7–62% clay), soil pH-KCl (3.78–6.51), acid saturation (1–68%) and soil depths, including sites where plants were growing in exposed subsoil resulting from soil erosion. *Eragrostis curvula* seed also germinates under high levels of soil sodium (Ryan et al. 1975), making it amenable to a wide variety soil conditions (Foy et al. 1980). *Eragrostis curvula* was the most sensitive indicator species for interference in biomass production in-field trials involving *Parthenium hysterophorus* L. (van der Laan et al. 2008).

We propose that *E. curvula* is a good candidate to test for allelopathy in pompom weed as both species have the potential to be equal competitors under disturbed conditions, but having the ability to use allelochemicals could shift the balance in favour of the phytotoxic species, leading to a reduction in the co-competitor species. This knowledge is not only important in the field of invasion biology but also has financial and ecological implications on commercial rangelands and conservation areas in South Africa. To compliment this we also included an annual grass, *E. tef* (Zucc.) Trotter, a commercially available non-invasive native of East Africa. Lettuce (*Lactuca sativa* L.) was selected to represent a sensitive broadleaf species as it is widely used as a test plant in allelopathy experiments (Satoh et al. 1989; Viles and Reese 1996; Junttila 2006).

Bioassays

Test One: Extracts applied to seeds of Eragrostis species

Aqueous extracts from the roots and shoots of pompom weed were assayed at 1, 10 and 25% w/v (fresh weight) for their effects on germination and early seedling development (radicles and coleoptiles) of *E. curvula* and *E. tef*. Adult pompom weed plants with roots and shoots intact were harvested from an infestation in Highveld grassland. Roots and shoots of pompom weed were separated and chopped into 10 mm sections. Root and leafy shoots were weighed separately into 2.5, 25 and 62.5 g portions and put into flasks containing 250 ml of distilled water to obtain extract concentrations of 1, 10 and 25% w/v. Flasks were placed in a growth chamber and left to infuse in the dark for 48 h at 20°C, then filtered through Whatman No. 1 filter paper (particle retention 11 μm) directly before applying the treatments.

Extracts were tested on certified seed of *E. curvula* (cv. "Ermelo") and *E. tef* (cv. "Rooiberg"). Test One comprised 14 treatments consisting of three concentrations (1, 10 and 25% w/v) of root and shoot extracts and a distilled water control on the two *Eragrostis* species. Seeds were germinated according to the International Seed Testing Association (ISTA) standards. *Eragrostis curvula* and *E. tef* were germinated in separate growth chambers at alternating temperatures of 20/35°C and 20/30°C, respectively, each with an 8 h day length. "Cool white" fluorescent tubes provided light with a spectral irradiance of 11 Wm^{-2}.

Seeds were placed onto two layers of Anchor's seed germination paper circles moistened with extract in Petri dishes (9 cm diameter). Petri dishes were sealed in clear Ziploc plastic bags to maintain optimal moisture conditions and checked every alternate day for signs of desiccation. Germination paper was watered with extract by eyesight and not by volume if visual wetness was below the standard wetness after watering. Standard wetness was obtained by first moistening the filter paper and then holding the Petri dish in a slanting position in order to drain out the excess fluid. The approximate volume to obtain standard wetness on unsoiled (dry) germination paper is about 2–3 ml. Extracts were refrigerated at 5°C for the 10 day duration of the experiment after the initial treatment applications.

Each treatment was replicated three times with 50 seeds per replicate. Treatments and replicates were randomised. Radicle and coleoptile lengths of developing seedlings were measured (mm) on two occasions for each species, after 5 and 10 days for *E. tef* and 7 and 10 days for *E. curvula*. Measured seeds were discarded. The trial was concluded on the 10th day with the second measurement of the remaining seeds of both species. Germinating seeds were also rated for abnormalities such as missing or defective radicles and coleoptiles. Seeds that had not germinated were accepted as dormant, dead or infertile, but lumped into the infertile category due to the difficultly of evaluating viability of small seeds. Data from the 10-day test period was pooled according to species and organs (coleoptiles and radicles).

Test Two: Extracts applied to lettuce seeds (Lactuca sativa)

Fresh pompom weed material collected in the wild was separated into roots, leaves and flowers. Plant parts were milled fresh and an extraction matrix was prepared with two solvents; water and hexane. Water has a strong polarity, while hexane is non-polar. These widely differing polar properties should result in a wide range of chemicals extracted from the plant material immersed in them. Hexane was used to determine if extract solubility is a factor in the potential allelopathy of pompom weed. The extraction matrix was made up of plant parts and solvents at a standard concentration of 50 g plant material: 200 ml solvent and allowed to soak for 72 h. Extracts were then centrifuged, filtered through Advantec No. 2 filter paper and diluted 1:10 with ultrapure water in order to ameliorate any inherent toxicity in the solvent. The experiment also included solvent controls, viz. ultrapure water and a 1:10 concentration of hexane in ultrapure water. Seeds were germinated according to the ISTA standards. Extracts were applied to lettuce seeds (cv. Commander) in Petri dishes in the same manner as Test One and germinated in a growth chamber at 15/23°C with a day length of 12 h. Treatments were replicated three times with 50 seeds per replicate. Treatments and replicates were arranged in a randomised block design. The trial was terminated after 3 days with the measurement of radicle lengths.

Pot studies

We report on two pot trials, i.e. interference (Test Three) and crop residue (Test Four) studies, following an account of pot preparation and fertigation procedures. The potting medium was a mixture with equal quantities by volume of palm peat, vermiculite and potting soil in pots with a diameter and depth of 20 cm. Both experiments were conducted in a tunnel with overhead fertigation. Pots received equal amounts of moisture and nutrients. Pots were watered for 10 min four times a day between 08H00 and 15H00. Overhead jets were calibrated to deliver 3 ml s^{-1} m^{-2} nutrient enriched solution at a pressure of 200 kPa. Calcium nitrate (N 15.6%, Ca 21.0%) was applied twice a week at a rate of 1 g l^{-1} with an electrical conductivity (EC) of approximately 120 mS/m. A complete fertilizer (N 5%, P 4%, K 20% plus micro nutrients) (National Plant Foods) especially developed for plants grown in a pre-enriched composted growing medium was applied thrice weekly at the same rate and EC as calcium nitrate. Calcium and phosphorus were applied separately to avoid a calcium phosphate precipitate. An agricultural disinfectant/plant sanitiser with didecyl dimethyl ammonium chloride 120 g l^{-1} was applied twice a week at a rate of 2 ml per 100 l of water to prevent disease. The moisture content of the growing medium could not be determined but plants were healthy and did not show signs of moisture stress or nutrient deficiencies.

Test Three: Interference study

Eragrostis curvula and pompom weed were grown together in pots to measure the effects of interference (competition + allelopathy) on the dry matter yield of both species. Achenes were collected from *C. macrocephalum* infestations and refrigerated at 5°C for 1 week to break a potential physiological dormancy (Baskin and Baskin 2004). Seeds were then stored under dry ambient conditions at room temperature for 6 months. Achenes with pappus attached were germinated in Petri dishes in a growth chamber at 12 h dark/light (spectral irradiance 11 Wm^{-2}) and 15/28°C, respectively. Germination was first observed after 6 days. Pompom weed seedlings were then transplanted into pots at the two-leaf stage at two densities, one or five plants. *Eragrostis curvula* cv. Ermelo was sown at a fixed rate of 0.05 g/pot (15 kg/ha) as the companion species once the pompom weed seedlings had developed to the four-leaf stage (±1 cm in height). Five treatments were tested in a simple additive design:

- *E. curvula* with one or 5 pompom weed (Cm1+Ec; Cm5+Ec): mixtures.
- *E. curvula* control (Ec): monoculture.
- Pompom weed controls with one or 5 plants (Cm1; Cm5): monoculture.

Test Three was completely randomised with six replicates per treatment. Plants were harvested after 5 months. Roots of whole plants were placed on a coarse sieve and rinsed under running water to remove growth medium. Plant material was then oven-dried at 80°C for 4 days and the dry mass (g; DM) of shoots and roots were recorded separately.

Test Four: Crop residue study

Eragrostis curvula was grown in potting medium in which pompom weed or *E. curvula* monocultures had previously been grown to measure the effects of plant remains of both species on the dry matter yield of *E. curvula*. For this purpose the pompom weed and *E. curvula* control pots from the interference study (Test Three) were used once these plants had been harvested. *Eragrostis curvula* cv. Ermelo was the only species tested and was sown at the same rate. The five treatments were:

- *E. curvula* in medium containing the remains of one and 5 pompom weeds (1R; 5R).
- *E. curvula* in medium containing the remains of one and 5 pompom weeds plus milled material of one and 5 *C. macrocephalum* plants (1RM; 5RM).
- *E. curvula* in medium containing the remains of *E. curvula* (Control, C).

Upon conclusion of Test Three, oven-dried pompom weed was milled into 1 mm particles and bagged. Milled material was applied as a layer 10 mm below the surface of the growing medium at dosages of 15 g/pot for the single (1RM), and 30 g/pot for the five weed (5RM) treatments. A delay of approximately 1 week occurred between harvesting plants from Test Three and the start of Test Four. *Eragrostis curvula* was sown in mid-summer, 1 day after the addition of the milled pompom weed material, and harvested after 4 months when growth had ceased in winter. *Eragrostis curvula* was oven-dried, weighed and analysed in the same manner as Test Three.

Field investigation

Vegetation assessments were carried out in 80 invaded grassland sites in Gauteng province. Belt transects of 100 m were laid out in infestations with a weed density ranging from 1 pompom weed per transect to several plants m^{-2}. The spike-point method was used to determine proportional species composition from 200 nearest-to-point plant identifications collected at 0.5 m intervals along the transect. Pompom weed density per transect was determined by counting rooted plants in 100 contiguous 1 × 1 m quadrats.

Native grasses were grouped into ecological response groups according to their response to the intensity and frequency of defoliation (Dyksterhuis 1949; Camp and Hardy 1999), primarily through grazing. The five groups identified consisted of non-response (NR), Decreaser (D), Increaser 1 (i1), Increaser 2 (i2) and Increaser 3 (i3) species. Dominant species are parenthesised. Non-response species in this case were grasses that are adapted to growing in soils that are permanently or seasonally waterlogged and are not strongly influenced by grazing (*Leersia hexandra* Swartz and *Imperata cylindrica* (L.) Raeuschel). Decreaser species were those that are abundant in grassland in good condition, but decrease when the sward is over or undergrazed (*Brachiaria serrata* (Thunb.) Stapf, *Diheteropogon amplectens* (Nees) Clayton and *Themeda triandra* Forssk.). Increaser 1 dominance is indicative of underutilisation (*Hyparrhenia hirta* (L.) Stapf, *Schizachyrium sanguineum* (Retz.) Alst. and *Trachypogon spicatus* (L. f.) Kuntze). Increaser 2 dominance is caused by sustained overgrazing (*Cynodon dactylon* (L.) Pers., *E. curvula* and *Heteropogon contortus* (L.) Roem. & Schult.) and Increaser 3 species dominate under sustained selective grazing pressure (*Aristida junciformis* Trin. & Rupr., *Elionurus muticus* (Spreng.) Kunth and *Sporobolus africanus* (Poir.) Robyns & Tournay). We report on the interactions between pompom weed, *Eragrostis curvula* and grass ecological response groups.

Data analysis

Trials involving Petri dishes (Bioassays) and pots (Pot Studies) had randomised block designs. Data from these experiments were subjected to analysis of variance (ANOVA) followed by Tukey post hoc tests for means ($\alpha = 0.05$). Data from vegetation assessments were summarised using regressions and simple charting. The computer software used for the analyses were Statistica 6.1 (StatSoft Inc. 2004) and Blossom version W2005 for the quantile regressions (Cade and Richards 2005).

Results

Bioassays

Test One: Extracts applied to seeds of Eragrostis species

Differences in germination percentages occurred between extracts in the *E. curvula* test; however, none of the treatments were significantly different to the control ($P > 0.207$). Germination inhibition did not occur in *E. tef* ($P = 0.105$). Both species produced germination rates above 80% in all treatments, well above the 70% minimum permissible for commercial seed (ISTA rules). Radicles of both *E. curvula* and *E. tef* were more sensitive than coleoptiles to extracts; the latter were not significantly different from the control treatments. Root extracts had a negligible effect on radicle size and appeared to have stimulated their growth (Fig. 2), but this effect was not significant. Shoot extracts had a detrimental effect on radicle size, aggravated by increasing extract concentrations. In *E. curvula* significant radicle-stunting was produced by both 10% and 25% shoot extracts ($P < 0.01$). Radicle stunting only occurred in the 25% shoot extract treatment in *E. tef* ($P = 0.017$).

Relative tolerances of *E. curvula* and *E. tef* to extracts were compared after transforming radicle and coleoptile data into incremental values (treatment–control); where *E. curvula* and *E. tef* controls both equal zero for their respective treatments (Fig. 3). Treatment by species interactions were significant for radicles ($F_{(6,28)} = 4.23$, $P = 0.004$) but not for coleoptiles ($P = 0.051$). The 25% shoot extract was the only treatment that produced differences between the two species, with *E. curvula* producing smaller radicles ($P < 0.001$) than *E. tef.*

Seedling abnormalities were detected but there was no significant difference between species. Main

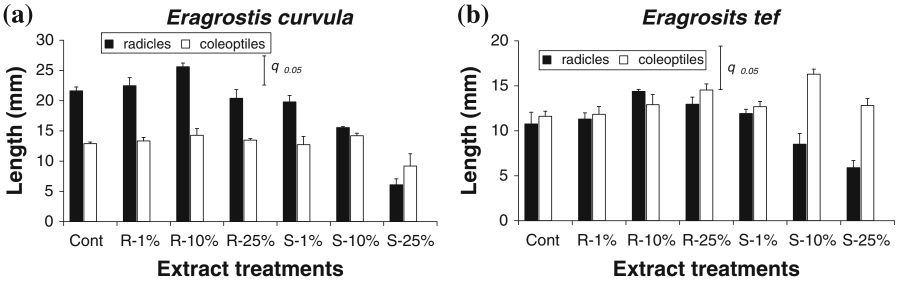

Fig. 2 Growth of radicles and coleoptiles of (**a**) *Eragrostis curvula* and (**b**) *E. tef* after 10 days exposure to three aqueous extract concentrations (1, 10 and 25%) from the roots (R) and leafy shoots (S) of *Campuloclinium macrocephalum*. *Eragrostis* species are presented separately because (**a**) *E. curvula* was grown under temperatures of 20/35°C while (**b**) *E. tef* was grown at 20/30°C. Means are presented with their respective standard errors and the critical range *q* of the Tukey post hoc test ($\alpha = 0.05$, $v = 14$, $k = 7$) (Zar 2003)

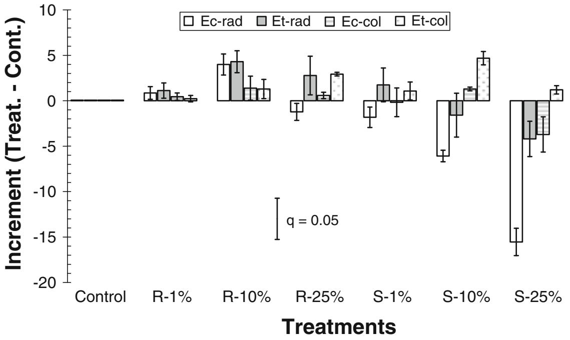

Fig. 3 Relative tolerance (mean ± SE) of germinating radicles (rad) and coleoptiles (col) of *Eragrostis curvula* (Ec) and *E. tef* (Et) seeds that were exposed to three concentrations (1, 10 and 25%) of aqueous extracts from the roots (R) and shoots (S) of *Campuloclinium macrocephalum*. Increment was used to measure and compare relative tolerance by subtracting the mean control size (mm) from mean treatment size (mm) in radicle and shoot categories in each replicate. The critical range *q* ($\alpha = 0.05$, $v = 28$, $k = 7$) is applicable for all groups in the legend

abnormalities included missing radicles, defective radicles and coleoptiles and spindly seedlings. The greatest incidence of abnormalities, 49.4 and 75%, occurred in the 10 and 25% w/v shoot extracts, respectively ($F_{(6,28)} = 12.34$, $P < 0.001$), while the remaining treatments were not significantly different from the distilled water control.

Test Two: Extracts applied to lettuce seeds (Lactuca sativa)

Interactions between solvents (hexane, water) and plant tissues were not responsible for eliciting significant differences in the germination or radicle development of lettuce seeds. The average germination percentage was 90.4%, with the distilled water control achieving a maximum of 96.6% and the hexane control a minimum of 80.6%. The largest mean radicle size was observed in root extract in water but was not significantly larger than the smallest radicles emerging from the leaf extract in water. Flower, root, and to a lesser extent leaf extracts, in hexane and water had little effect on germination and root elongation of lettuce seeds at the concentrations tested. Water soluble and insoluble extracts from the below and/or ground structures of pompom weed do not appear to be inhibitory to the germination and early seedling development of lettuce.

At this juncture it is not certain what extract concentration of pompom weed may be realistic to reflect an allelopathic effect in nature. Pot trials using the most sensitive indicator species, in this instance *E. curvula*, were carried out to determine whether potential allelopathy from the pompom weed extracts was repeated under more natural conditions by growing both species together with nutrient supplementation.

Pot studies

Test Three: Interference study

Pompom weed grown at one or five plants per pot and planted with *E. curvula* at a fixed sowing rate did not affect the DM yield of roots or shoots of the latter (Fig. 4). The yield of pompom weed roots ($F_{(3,20)} = 19.45$, $P < 0.001$) and shoots ($F = 6.66$,

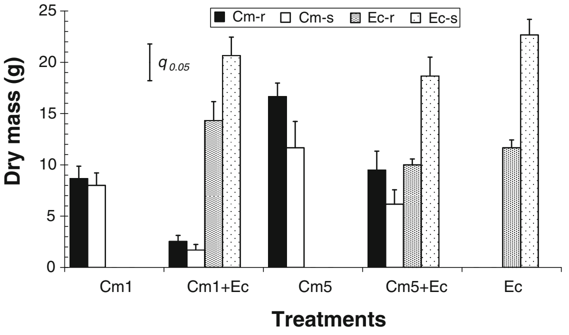

Fig. 4 The effect of intra and interspecific interference on the dry matter yields (mean ± SE) of roots (-r) and shoots (-s) of *Campuloclinium macrocephalum* (Cm) and *Eragrostis curvula* (Ec) in pots at 5 months after planting. *Campuloclinium macrocephalum* was planted one (Cm1) and five plants (Cm5) per pot, with and without *E. curvula* sown at a rate of 0.05 g per pot. The treatments Cm1, Cm5 and Ec are pure stands of weed and grass, respectively. The critical range q ($\alpha = 0.05$, $v = 20$, $k = 4$) is only applicable for weed root (Cm-r) and shoot (Cm-s) groups. *Eragrostis curvula* root and shoot yields were not significantly different ($\alpha = 0.05$, $v = 20$, $k = 3$)

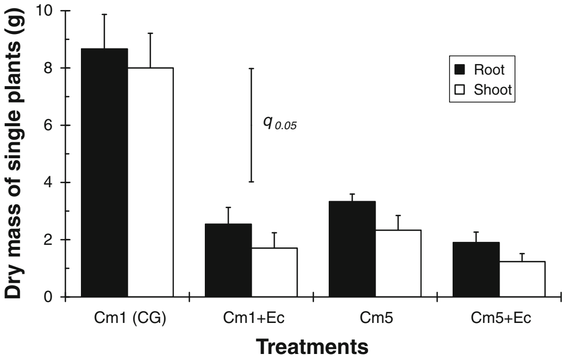

Fig. 5 The effect of intra and interspecific interference on the dry matter yields of individual *Campuloclinium macrocephalum* grown at one (Cm1) or 5 (Cm5) plants per pot, with or without *Eragrostis curvula* (Ec). Values are represented as means with their respective standard errors and the critical range q ($\alpha = 0.05$, $v = 20$, $k = 4$). The control group (CG) is significantly different to all the other treatments

$P = 0.003$) when planted with *E. curvula* differed significantly. Grass interference reduced root and shoot production of pompom weed by 75% in Cm1+Ec and 45% in Cm5+Ec. The 5-plant control (Cm5) collectively produced almost double the root DM of the single plant treatment (Cm1) ($P = 0.002$). Shoot yields between the two pompom weed controls was not significantly different ($P = 0.392$) but total DM was 70% greater in the 5-plant treatment ($P = 0.010$). Mixtures followed a similar pattern. Pompom weed died in one replicate of the Cm1+Ec treatment (17% mortality) and its remains at the time of harvesting were one-tenth the size of the other samples in treatment. Variation in the type (intra vs. interspecific) and intensity (plant density) of interference had little affect on resource allocation, i.e. root-shoot ratios, in either pompom weed ($P = 0.513$) or *E. curvula* ($P = 0.133$). This suggests a history of independent adaptation of both species to plant community competition on separate continents. The ability of both species to coexist in a confined growing space offers little evidence to support phytotoxicity in either species.

Relative interference on net pompom weed production from intraspecific, interspecific and absolute competition (total biomass = pompom weed + *E. curvula*) was determined by comparing the mean weights of pompom weed individuals (Fig. 5). Intraspecific interference (Cm5) reduced root and shoot dry matter yields by 62 and 71% respectively. Combined intra- and interspecific (with *E. curvula*) interference caused a 78–85% reduction in pompom weed root and shoot DM. Significant differences were detected between root ($F_{(3,20)} = 19.22$, $P < 0.001$) and shoot yields ($F_{(3,20)} = 19.0$, $P < 0.001$). Roots and shoots of Cm1 (control group) were significantly heavier than those of individual plants in all the other treatments with pompom weed.

Total biomass DM (roots + shoots), i.e. all plant species per pot, varied significantly ($F_{(4,25)} = 14.28$, $P < 0.001$) between treatments (Fig. 6). A difference of 70% in weed biomass between single and the 5-plant controls (Cm1, Cm5) was considered statistically not significant ($P = 0.052$), however, Cm1 had significantly less biomass than the remaining treatments ($P \leq 0.01$). The grass control (Ec) only produced significantly more dry mass than Cm1 ($P = 0.002$). These results indicate that young pompom weed plants are more sensitive to inter- and intraspecific interference than is *E. curvula*, which showed negligible modulation at differing pompom weed densities (zero, one and 5 plants/pot). The potential of increased amounts of phytotoxins onto the companion species with increasing pompom weed density was not apparent. Pompom weed densities of one and 5 plants/pot is equivalent to 32 and 159 plants m^{-2} which is 5 and 25 times larger than the observed

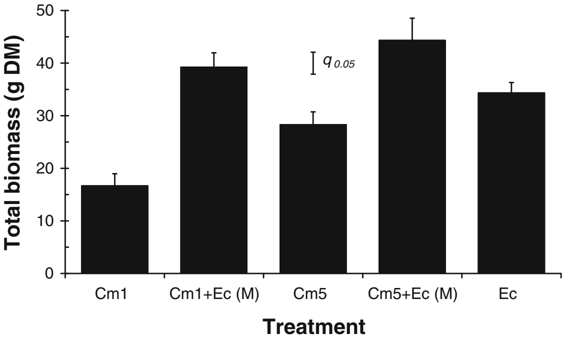

Fig. 6 The effect of intra and interspecific interference on the total biomass for all plant species per pot, with or without, *Campuloclinium macrocephalum* at one (Cm1) or 5 (Cm5) plants per pot and the test species *Eragrostis curvula* (Ec) sowed at a rate of 0.05 g per pot. Mixtures are denoted by the *letter M* in *brackets*. Means are presented with their respective standard errors and the critical range q ($\alpha = 0.05$, $v = 25$, $k = 5$)

maximum density in the field. Thus the question of what extract concentration may be realistic to reflect an allelopathic effect in nature may now not be relevant. Pompom weed was not allelopathic to *E. curvula*, the most sensitive bioassay indicator species, when both species occupied the same growing space and shared root and shoot contact; unless allelochemicals increase in quantity and/or potency with age.

Test Four: Crop residue study

Pots containing plant remains from monocultures of pompom weed and *E. curvula* grown in Test Three did not have a significant effect on yield of *E. curvula* roots, shoots and plant numbers (Fig. 7). Pompom weed density (1R and 5R) did not have a significant impact on *E. curvula* roots ($P = 0.071$) and shoots ($P = 0.077$). The incorporation of milled pompom weed (1RM and 5RM) appeared to have mitigated the effects of the pompom weed remains on *E. curvula* production in the high density pompom weed treatment (5R), but the effect was not significant ($P = 0.078$). Pompom weed remains were therefore no more allelopathic than those of *E. curvula*.

Field investigation

Pompom weed was present at variable densities in all of the 80 grassland sites sampled but *E. curvula* was present in only 67 sites. The relationship between

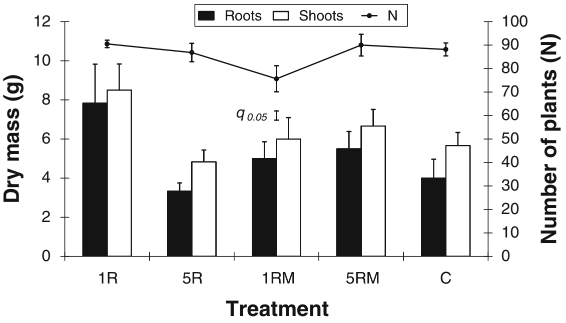

Fig. 7 The effect of *Campuloclinium macrocephalum* residues (R), *C. macrocephalum* remains plus dried milled plants (RM) at planting densities of one or five plants per pot and *Eragrostis curvula* remains (Control—C) on the biomass production of *Eragrostis curvula* four months after sowing at a fixed rate of 0.05 g/pot. Dry matter yields of *E. curvula* (*bars*) roots and shoots are presented on the first y-axis. The number of *E. curvula* plants (*line*) in pots is presented on the second y-axis. Means are presented with their respective standard errors. The critical range q ($\alpha = 0.05$, $v = 25$, $k = 4$) applies to all groups in the legend (Wilks $\lambda = 0.514$, $F_{(12,61)} = 1.457$, $P = 0.166$)

pompom weed frequency (%) and pompom weed density (m^2) based on sites with *E. curvula* was significant (Fig. 8). Pompom weed density increased at a rate of 1.5 plants m^{-2} with each 10% increase in pompom weed frequency. Maximum pompom weed frequency and density at sites where *E. curvula* was present were 46% and 6.4 plants m^{-2}, with a mean pompom weed frequency and density of 7.5% and 1.3 plants m^{-2} respectively. These data pertain to pompom weed only.

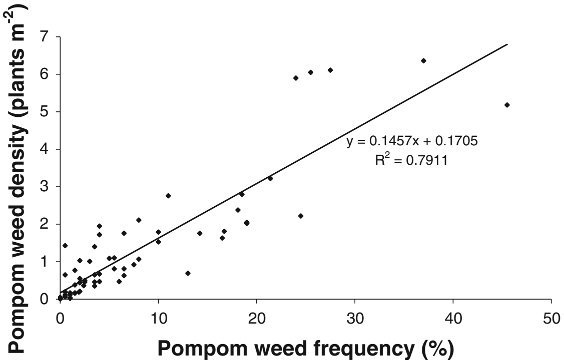

Fig. 8 The relationship between pompom weed (*Campuloclinium macrocephalum*) frequency (%) and density (m^{-2}) in 67 belt transects at sites with *Eragrostis curvula* in Gauteng Province, South Africa (regression summary: $r = 0.889$, $r^2 = 0.791$, $t = 15.69$, $P < 0.001$)

The linear association between the percentage frequencies of *E. curvula* (x-axis) and pompom weed (y-axis) was judged to be significant ($P = 0.015$) (Fig. 9) but the correlation coefficients were weak ($r = -0.297$, $r^2 = 0.088$) due to heteroscedasticity. Least absolute deviation (LAD) or quantile regression was more appropriate at explaining the response of pompom weed to the limiting function of *E. curvula* frequency at the 90th quantile (Fig. 9). *Eragrostis curvula* at low frequencies of 0.5–5% appeared to reduce pompom weed frequency by halving it from 40% (6 plants m^{-2}) to 20% (3.1 plants m^{-2}). An abrupt change in the pattern of the relationship occurred above 5% *E. curvula* frequency and the trend flattened, resulting in the next halving of pompom weed from 20 to 10% at *E. curvula* frequencies of 40–50%, an increase of 45% in the occurrence of *E. curvula*. At the highest observed frequency of 67% *E. curvula* seemingly imposed a maximum limitation on pompom weed frequency of 9% (1.5 plants m^{-2}), which was not much different to the weed's frequency at *E. curvula* frequencies above 30%, and still a considerable weed density when extrapolated to 15,000 plants ha^{-1}. This indicated a high degree of coexistence between *E. curvula* and pompom weed.

The notion of low competing plant abundance of one species (*E. curvula*) causing fundamental reductions in the populations of a second species (pompom weed), but not when the first species is at higher levels of abundance (Fig. 9) is somewhat contrary to the concept of density-dependant interspecific competition. In the LAD model 90% of the pompom weed frequency values (y-axis) were less than or equal to the function of *E. curvula*. In essence *E. curvula* was a surrogate for the unmeasured factors limiting at many sites (Cade and Noon 2003). Furthermore, no association was detected between pompom weed and *E. curvula* using contingency tables ($\chi^2 = 1.37$, $df = 1$, $P = 0.242$).

Landuse management at the 80 grassland sites was summarised into three categories, unutilised land (54%), rangeland for grazing (41%) and abandoned fields or old-field successions (5%). The majority of sites were not grazed (59%) and 39% experienced under or selective grazing pressure. Overgrazing did not occur at sites with zero to 0.5% *E. curvula*, based on the indicator species present (Fig. 10). Sites where *E. curvula* was virtually absent had the greatest incidence of seasonal flooding or inundation (54%).

Rather than being suppressed by pompom weed, sites with zero *E. curvula* (Fig. 10), an i2 species, were dominated by the grass ecological groups NR (dominant in wetlands), D (well managed grassland) and i1 (moribund grassland). Those groups would have offered little niche opportunity for *E. curvula* to establish. Pompom weed (CM*) and contributions of the NR–D–i1 group mosaic declined proportionately with increasing *E. curvula*. Wetland grasses (NR

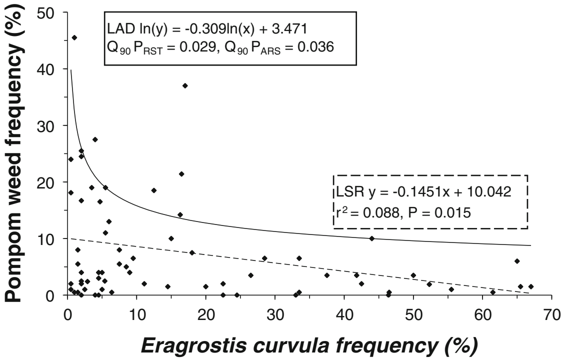

Fig. 9 The relationship between *Eragrostis curvula* and pompom weed frequencies in belt transects from 67 grassland sites in Gauteng Province. The lines depict different regression models applied to frequency data; viz. least squares regression (*LSR*) *dashed line* and quantile regression (LAD) *solid line* where Q_{90} is the 90th quantile of log-transformed data (P-values: P_{RST} = Rank Score Test Statistic, P_{ARS} = Asymptotic Rank Score Statistic)

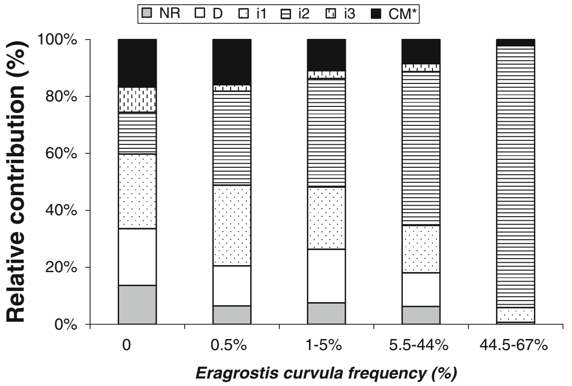

Fig. 10 The relative contribution of grass ecological groups and pompom weed to grassland composition in sites along a gradient of increasing *Eragrostis curvula* dominance (an Increaser 2 species), as predicted by quantile regression at the 90th quantile ($P = 0.03$; Fig. 9). Group acronyms stand for: non-responsive (NR), decreaser (D), increaser (i1, i2, i3) and pompom weed (CM*). See "Materials and methods" for more details

species) and *E. curvula* had opposite soil moisture and drainage requirements, the former being absent in sites with more than 34% *E. curvula*, the latter only achieving dominance in sites without NR species. Decreaser species were under represented in all *E. curvula* frequency categories suggesting selective grazing or poor management in the past was largely responsible for their reduction. These rangelands could have been abandoned due to deteriorating carrying capacity, based on the evidence that most sites are presently not utilised. Poor drainage, high soil moisture and to a lesser extent grazing management had more to do with limiting the abundance of *E. curvula* in some sites than the abundance of pompom weed.

Discussion

Asteraceous weeds are notorious at producing compounds that kill or suppress neighbouring species under artificial conditions. For example, *P. hysterophorus* (parthenium) contains sesquiterpene lactones, an important group of compounds involved in allelopathy (Reinhardt et al. 2004). Leaf extracts of this weed are apparently strongly phytotoxic to plants, inhibiting germination and affecting seedling development of several broadleaf and grass species (Adkins and Sowerby 1996; Tefera 2002) at concentrations ranging from 6 to 25% w/v. Reinhardt et al. (2004) in sequestering parthenin from parthenium found trichomes harvested on both leaf surfaces contained almost 100% parthenin but stems and roots contained only trace amounts of the substance. The water solubility of parthenin was reported as being low and in this regard, active compounds accumulated in trichomes of *C. macrocephalum* may have remained insoluble in the aqueous extracts, thereby reducing their allelopathic effect on *Eragrostis* (Test One). In our study, extracts of *C. macrocephalum* exhibited mild allelopathy on *Eragrostis* species at 10 and 25% concentrations by stunting radicles, but produced no detectable phytotoxic activity causing germination inhibition and coleoptile abnormalities. At similar concentrations parthenium had a great impact on seed germination and plant growth, regardless of the solubility of parthenin. On the other hand, water insoluble compounds from pompom weed tissues in hexane did not cause a reaction in the germination of lettuce either.

Significant variation in the sesquiterpene chemistry of *P. hysterophorus* has been reported from populations sampled from various regions of its worldwide distribution (Picman and Towers 1982), but this should not account for the discrepancies in magnitude of allelopathy between laboratory and field studies involving parthenium extracts. Pure parthenin was considered a weak germination inhibitor (Belz et al. 2007) of several African grass species under natural conditions, including *E. curvula* (van der Laan et al. 2008). In spite of parthenium displaying strong laboratory allelopathy (Adkins and Sowerby 1996; Tefera 2002), Belz et al. (2009) discovered that parthenin is not sufficiently persistent, phytotoxic, or bioavailable in natural soils “to cause an allelopathic effect that could contribute to the invasiveness of parthenium.” Given the invasiveness of parthenium under soil and environmental conditions favouring the degradation of parthenin, Belz et al. (2009) speculated that self-stimulatory allelopathy rather than inhibitory allelopathic interactions may assist the species in forming dense monotypic stands.

In our study *E. curvula* was a more sensitive indicator species of allelopathy than *E. tef*, confirmed by studies on parthenium on a range of native perennial grass species (van der Laan et al. 2008). Results from in vitro experiments were not convincing when one considers extracts did not inhibit germination in any of the test species and root-stunting of *E. curvula* was not repeated in the pot trials. This would imply that the extracts tested were more concentrated in the in vitro assays than natural levels of allelochemicals in the weed. In addition to parthenium, sufficient evidence exists in the Asteraceae alone of species that produce plant–chemical interactions in laboratory experiments failing to do so under natural conditions. In a similar vein to the rejection of the NWH based on the properties of spotted knapweed (Duke et al. 2009a, b); Ito et al. (1998) found that the allelochemical dehydromatricaria ester (DME) produced by *Solidago altissima* L. inhibited seedling development in rice in both agar and soil cultures. The inability to detect DME in soil water in natural stands; however, led them to conclude that S. *altissimo* has little allelopathic activity in the field. The allelopathic effects of chromolaena in laboratory studies (Hoque et al. 2003) is not repeated under natural conditions, where it is an important species in fallow-cropping systems in Africa and Asia (Roder et al. 1995; Koutika et al. 2002; Ikuenobe and Anoliefo 2003) and is perceived amongst farmers to be a beneficial plant rather than a noxious weed.

The relative importance of plant age, phytotoxicity and competition could not be determined because neither pompom weed density nor allelopathy had a strong impact on companion species in the interference study (Test Three). The only positive allelopathic result involved Petri-dish bioassays of leaf extracts on *E. tef* and *E. curvula* from adult pompom weed (Test One). In the interference and crop residue experiments (Tests Three and Four), conducted under more natural conditions in pots, young pompom weed plants or remains were not allelopathic to *E. curvula* at densities far higher than the maximum observed field density of 6.4 plants m^2 (=0.2 plants/pot). In a field trial investigating interference between parthenium and three common grass species in South Africa, *E. curvula* consistently produced the lowest biomass over two growing seasons at parthenium densities of 5 and 7.5 plants m^{-2} (van der Laan et al. 2008). More importantly, parthenium density did not influence the biomass of *E. curvula* significantly. In our study there was no indication of phytotoxicity, neither was the growth of *E. curvula* affected by pompom weed density. Nevertheless, having survived at populations (plants m^{-2}) 25 times the observed maximum naturalised density (Fig. 8), pompom weed has exhibited an extraordinary tolerance to intense competition at early establishment. This provides an indication of coexistence but the relative importance of above and below ground organs on interference requires additional investigation.

The degree of allelopathy increasing with age could simply be a function of increasing biomass (Koricheva 1999) and in the case of pompom weed, the quantity of glandular trichomes responsible for producing the toxin. In contrast, the ability to exert an allelopathic influence soon after germination would be an advantage in aiding early establishment and release from neighbourhood competition (Kruse et al. 2000). For example, Wardle et al. (2006) discovered that aqueous extracts and leachates of the Asteraceous biennial *Carduus nutans* L. (nodding thistle) were more allelopathic on grasses and legumes at the rosette stage (young plants) than adults. An association between pompom weed and *E. curvula* was not apparent in the field investigation, confirming the "not allelopathic" outcome of the interference experiment (Test Three) and making it unlikely that plant age is a factor in the potential allelopathy of pompom weed.

Symbiosis was not apparent between pompom weed and *Eragrostis curvula*. Pompom weed's ecological niche is broader than that of *E. curvula* but the niche overlap between these species appears to be quite wide, both species preferring disturbed conditions. Pompom weed exhibits a greater degree of adaptive plasticity by invading a wider range of soil types, soil moisture gradients and disturbance regimes. *Eragrostis curvula* is adapted to disturbed sites on well drained soils (van Oudtshoorn 2006), being largely absent in wetlands (Kotze and O'Connor 2004) and grasslands that are moribund or underutilised (Goodall 2000). Where one of these species was more dominant it usually implied site conditions precluded the other and had no bearing on the competitive superiority of one over the other. Interference studies in pots and in-field studies measuring intraspecific interference were mutually conclusive; *E. curvula* at high densities limits dominance of *C. macrocephalum*, but does not exclude pompom weed establishment. Coexistence might involve a trade-off of size for survival and reproduction in pompom weed where landuse favours the dominance of *E. curvula*. Two hypotheses could provide plausible alternatives for its invasion ecology. The ability of pompom weed to survive and reproduce under intense *E. curvula* competition may involve sourcing unidentified limiting resources not utilised by the grass (Tilman 1985). Alternatively, being predator-free and disease-free, could provide pompom weed the advantage of being able to withstand degrees of interspecific competition from native communities in the introduced range (Huffaker et al. 1976). In this study the pot trials and field investigation collectively support the conclusion that both species are excellent competitors which are able to coexist rather than be competitively exclusive in vegetation they both occupy.

Thorpe et al. (2009) discovered from field experiments on two continents that some species in the introduced range of the controversial forb *Centaurea maculosa* Lam. (Callaway and Ridenour 2004; Bais et al. 2003; Blair et al. 2005; Duke et al. 2009a, b) had reduced growth compared with co-occurring species in its native range which did not. This implied a level of adaptation in plant communities to phytotoxic compounds in the alleged allelopathic species through shared evolutionary history, which was absent in the introduced range. In this context adaptation and coexistence could be possible if pompom weed was allelopathic and *E. curvula* had evolved defence mechanisms against them. Laboratory studies on *Eragrostis* and lettuce revealed "evolved" defences were weakest in *E. curvula* but interference experiments refuted the role of allelopathy in *C. macrocephalum*. The novel weapons hypothesis in this case did not apply to pompom weed.

We acknowledge the difficulties of separating allelopathic interference from other forms of interference. Under laboratory conditions parthenium may be considered far more allelopathic than pompom weed, and yet under field conditions parthenium has been proven to not be allelopathic (van der Laan et al. 2008; Belz et al. 2009). Stowe (1979) remarked that bioassays do not simulate natural conditions, and if studied in isolation the investigator is left unable to accept or reject allelopathy from a positive or negative result. Under field conditions phytotoxic compounds produced by plants are rapidly degraded in the soil to the extent where they are biologically unavailable to produce plant–plant interactions (Harper 1977; Schmidt and Ley 1999). The identification of chemicals produced by plants aiding in their defence against neighbourhood competition, predation and disease is a crucial step in proving the existence of "novel weapons". The compounds produced by pompom weed trichomes have not been described and so the question what constitutes a natural concentration that cause an allelopathic effect cannot be answered until we know more about the chemistry of this species. Proving allelopathy is a daunting endeavour (Romeo 2000; Duke et al. 2009b) because the onus is on researches not only to identify the chemical structures but to also use ecologically relevant species, and demonstrate the production and movement of phytotoxin from the donor plant to the receiving plant growing in the same soil, which in itself is a complex substrate.

We conclude that allelopathy does not appear to be a major factor for the invasiveness of pompom weed in the grassland biome based on ecological evidence. Other site-based factors, multivariate in nature, are likely to govern the density of pompom weed and its association with, or impact on, other species. Secretions from glandular trichomes on the leaves may have alternative properties such as anti-fungal or as a defence against herbivory (antifeedant). Biochemical studies on pompom weed are an urgent priority. This raises the question as to which hypothesis best fits the success of *C. macrocephalum* as an invasive alien species. Based on the evidence presented here, the absence of natural enemies and or niche differentiation along resource gradients carries more weight than *C. macrocephalum* being a phytotoxic species.

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ORIGINAL PAPER

4. Altered growth, population structure and realised niche of the weed *Campuloclinium macrocephalum* (Asteraceae) after exposure to the naturalised rust *Puccinia eupatorii* (Pucciniaceae)

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Abstract The alien forb *Campuloclinium macrocephalum* (Less.) DC. (Asteraceae, pompom weed) invades disturbed rangelands in South Africa. After >40 years of naturalisation, infestations around Pretoria were severely damaged in 2006 by the biotrophic rust *Puccinia eupatorii* Dietel (Pucciniaceae). The disease spread rapidly and has now established at most pompom weed infestations in the country. This study reports on the efficacy of the pathogen as a biocontrol agent of pompom weed, its effect on the retention of the weed's realised niche and tolerance to the disease. Eighty rangeland infestations originally assessed over two years in 2003 and 2004 were re-surveyed in 2009. Data from untreated plots at two pompom weed experimental sites where the rust was first detected were also used. *Puccinia eupatorii* induced premature senescence in late summer and stimulated the production of compensatory growth in autumn. Plants without disease symptoms senesced in late autumn without compensatory growth and remained dormant throughout winter. Pompom weed density did not change significantly over time in both the rangeland study and untreated plots. Disease incidence in rangelands in 2009 was most severe in dense infestations, with sparse infestations having the highest incidence of natural senescence and fewer pustules. The rust did not reduce the realised niche; infestations in grasslands, savanna and wetlands persisted despite significant damage by the disease. Persistence, despite high annual stem mortality, suggests that pompom weed remains adapted to the fungus after prolonged separation from the disease. This study supports earlier findings that disturbance remains key to pompom weed invasion even under biological control. The rust may enhance the biological control of pompom weed with augmentation of additional insect agents, but is unlikely to inflict a significant reduction in the weed's population on its own. This may be an important consideration for the biological control of weeds involving biotrophic fungi.

Keywords Biological control · Climatic conditions · Compensatory growth · Realised niche · Premature senescence · Tolerance

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Introduction

The rangeland weed *Campuloclinium macrocephalum* (Less.) DC (= *Eupatorium macrocephalum* Less.) from South America, has come under attack by the biotrophic rust fungus *Puccinia eupatorii* Dietel in South Africa, where the plant is known colloquially as pompom weed (pompom hereafter). The rust fungus (rust hereafter) was collected on pompom in Argentina and imported into quarantine laboratories in Stellenbosch, South Africa, in December 2003 (McConnachie et al. 2011). In April 2006 pompom stems in untreated plots at an experimental site near Pretoria, close to where the invasion originally started, had been killed by the rust. The rust spread rapidly and most infestations of pompom now have the disease.

Pompom has a perennial rootstock that produces annual stems in spring (September/October) that grow up to 1.3 m in height. Stems senesce in autumn (May) and the plant survives as a rootstock during winter. Winter is the dry season and thus pompom is not adversely affected by grass fires, frost and negligible rainfall at this time of year. Pompom has been in South Africa since the 1960s and prior to 2006 was free of close-association natural enemies. *Puccinia eupatorii* is an autoecious macrocyclic rust (Cummins 1978) having several spore stages on one host. In quarantine the optimum temperature for urediniospore germination and infection was 18 °C with a minimum dew period of 6 h (McConnachie et al. 2011). Physical symptoms appear 11–14 days after urediniospores penetrate the stomata, with pustules appearing on both leaf surfaces. Infections cause leaf necrosis and abscission, leading to premature death of the annual stem. Biotrophic fungi feed on nutrients in healthy host plant tissue (Deacon 1997) and thus function as nutrient sinks.

The unexpected appearance of *P. eupatorii* at one of the experimental sites initially provided an opportunity to measure the effects of the disease on pompom density and height in the first three years it was present in the country. Based on the visual magnitude of the damage caused by the disease, it was anticipated that pompom populations would provide indications of adaptation or decline by the end of the three year monitoring period. In a two year study on *Cirsium arvense* L. (Asteraceae) involving cutting stems and inoculating plants with the pathogen *Puccinia punctiformis* (Str.) Röhl, it was found that the rust reduced plant biomass in the first year and repeated cutting reduced both above- and below-ground biomass by the second year (Kluth et al. 2003).

In 2003 and 2004 (2003 survey hereafter), and prior to the appearance of the rust, a study of 80 pompom infestations in rangelands across Gauteng Province revealed that rainfall, topography and soil texture were the primary drivers of the natural vegetation pattern while pompom exhibited disturbance-mediated invasion strategies attributed to the invader's traits as a ruderal weed (Goodall et al. 2011). Selective grazing and over-grazing were the primary cause for rangeland invasions. Pompom was absent in rangelands in good condition but invaded both xeric and mesic grassland in a range of degradation states without exhibiting significant differences in population densities or size of plants.

The establishment of the rust in the introduced range of pompom offers an opportunity to address two ecological questions and to evaluate the efficacy of the pathogen as a biocontrol agent. The pompom population in South Africa has been isolated from its natural enemies and the pathogen in particular for over 40 years. The plant has been a declared weed for over a decade. The first question is do naturalised plants remain adapted to host-specific predation even after prolonged separation in years and/or generations from such agencies? Several invasion theories apply to pompom (Catford et al. 2008; Goodall et al. 2010; Henderson et al. 2010), namely prolific seed production (propagule pressure), resource availability, land-use management, episodic disturbance, invader traits, competition and the ability to recover rapidly from mechanical damage. Allelopathy is not part of pompom weed's invasive arsenal (Goodall et al. 2010) hence the Novel Weapons hypothesis does not apply in this instance (Callaway and Ridenour 2004). Furthermore, endophytes play a critical yet cryptic role in plant defences and the presence or absence of mutualistic bacteria or fungi in naturalised plants could explain inconsistencies in weed biocontrol introductions (Evans 2008).

Hypothetically, if weed populations crash after re-introduction of natural enemies, then it could be speculated that the adaptation to close-association predation is weakened with ensuing generations under enemy-release conditions, lending support to hypotheses supporting trade-offs in plant defence mechanisms for increased growth and reproduction (Keane and Crawley 2002; Colautti et al. 2004; Joshi and Vrieling 2005) and/or the absence of endophytes (Evans 2008). If populations remain stable and/or continue to increase after successful natural enemy re-introduction, the ability to persist under predation would imply a genetic trait that is not rapidly lost and possibly those plants were introduced with mutualistic endophytes.

The second question raised is; does the rust reduce the realised niche of pompom weed? Pathogens can act as nutrient sinks by taking up some nutrients which would otherwise be available for the host plant's metabolism. Also, depending on disease severity, plant-stress is further exacerbated by reduced photosynthesis resulting from disease-related chlorosis and necrosis (Berger et al. 2007). If *P. eupatorii* affects carbohydrate metabolism, pompom will be gradually weakened over the growing season and subsequent years, affecting its ability to persist and proliferate. The realised niche is the actual distribution and range of habitats that a species has established in and is most adapted to after accounting for superior competitors and limiting factors. Weed biocontrol agents, even if they do not kill their hosts directly, could reduce weed niche width if survival is no longer possible at sites that were more favourable for colonisation under enemy-free conditions. For example, disease-free pompom has not established in grasslands with a sward basal cover > 21 %, but densities from the 2003 survey ranged from 1,000 plants ha^{-1} at 18 % basal cover to >60,000 ha^{-1} at 6 % basal cover (Goodall et al. 2011). In other words natural enemies that affect the host's invasive traits will have a greater influence than other biotic or abiotic characteristics that initially rendered sites amenable to pompom invasion. If the pathogen on its own has no effect on weed density or the extent of its realised niche, it does not invalidate the concept, as the success of weed biocontrol has been shown to increase, globally, with the choice and the number of agents released (Denoth et al. 2002), and more recently the absence of close-association endophytes (Evans 2008).

To answer these questions, the 80 rangelands in the 2003 survey were revisited in April 2009, three years after the initial rust outbreak, with the aim of determining any effect the pathogen has on field population densities. In addition, use was also made of time series data from the untreated plots at experimental sites monitored from 2005 to 2010 where the rust was first detected on pompom in 2006.

Materials and methods

Two unrelated data sets are presented, namely (a) untreated (control) plots at two experimental sites comparing mechanical and herbicidal control methods and (b) 80 rangeland sites across Gauteng. The terms 'primary stem', 'seedlings' and 'compensatory stems' are used to separate the life stages and responses of pompom stems to damage. The primary stem emerges in spring (September–October) and dies back to the rootstock in late autumn (May), if it has not been damaged, and regrows annually. Compensatory or replacement stems emerge when primary stems have been damaged, but the ability to complete their annual life cycle would be limited by the amount of time remaining before frost, fire or moisture stress kills them during the dry winter months. The term regrowth applies to both primary and compensatory stems in contrast to seedlings.

Plot studies

The experiment comparing methods of controlling pompom was initiated in December 2004 on two properties near the epicentre of invasion in Gauteng Province, approximately 15 months prior to the initial outbreak of the rust. One trial site was in disturbed xeric grassland with a high forb component and dense pompom weed (xeric site hereafter). The other trial site was in a highly disturbed wetland (mesic site hereafter) that had been mined for peat, indiscriminately burnt and further degraded by numerous boreholes which had dropped the water table (Marais 2004). Species composition at the mesic site had been altered by human activities and the major contributors of biomass were two graminoid species (Poaceae), namely the wetland grass *Imperata cylindrica* (L.) Raeuschel, and the terrestrial grass *Hyparrhenia hirta* (L.) Stapf., together with dense pompom. Soils at the xeric site were shallow (<25 cm) and well drained while soils at the mesic site were deep (>1 m) and poorly drained.

Data on the manual and herbicide treatments are not presented. Only data from the untreated plots are presented. Both trials conformed to a randomised complete block design with each treatment replicated in each of the three blocks. Plot size was 5 m × 5 m with a 3 m buffer zone between plots. Five parallel permanent transects, each 5 m long, were established 0.75 m apart in every plot, with the first transect being randomly assigned between 0.50 and 0.75 m in from the edge of the plot. Line intercept measurements (end-start distance) were recorded for all species encountered along each transect, with every species having a plot total intercept distance (cm) from which percentage cover could be calculated ($\mathrm{sp}_i/2{,}500 \times 100$, where $\mathrm{sp}_i =$ total intercept distance (cm) of the ith species). Basal cover (width of tuft) was used for grasses and canopy cover for forbs (forb basal area is often too small to measure). Pompom numbers were counted in each plot (n) along with height of each plant (cm). Chlorophyll content of 10 sample plants per plot was determined using a SPAD chlorophyll meter (Konika Minolta SPAD-502). Enumerations were repeated in February each year, until and including February 2010.

In addition, pompom regrowth and seedling development were monitored in sub-plots over the study period. Ten random points were staked in each untreated plot as permanent sub-plots. The stake formed the centre pin of circular ¼ m^2 sub-plots with a radius of 28.2 cm. In each sub-plot, counts (n) and heights (cm) of seedlings and regrowth were measured. Sub-plot surveys were carried out three to four times a year from August 2005 until January 2010. Both line intercept and sub-plot data include periods before and after the appearance of the rust. Turnover in basal cover of native species was also investigated to determine how these responded to the disease on pompom and hypothetically reduced competitiveness in the weed.

The third replicate block at the xeric site was unfortunately destroyed by a third party sometime in 2008 and measurements and analyses after this date were from two replicates.

Rangeland study

Pompom infestations in the 2003 survey were re-evaluated in April 2009 and additionally included rust damage assessments. Not all sites were recovered—66 of the original 80 sites (82.5 %) were sampled, 11 sites were no longer accessible and three sites had been destroyed. During the original surveys, line transects of 100 m were laid out in homogeneous vegetation containing pompom (Goodall et al. 2011). Pompom was counted in quadrats (1 m^2) arranged at alternating 0.5 m intervals along transects, from which the density and frequency of occurrence (% quadrats with pompom present) was calculated. It was not possible to position transects in exactly the same place in 2009 due to limited accuracy of the GPS instrument to within a 5 m radius. To reduce this error two parallel transects of 100 m were laid out 2 m apart at each site using photographic records, the transect bearing and GPS coordinates. Pompom density was again based on quadrat counts m^{-2}; the average of each equidistant quadrat pair along the two transects was used.

The condition of adult stems in quadrats ranged from healthy, through diseased, to dead stems. Healthy plants were mostly green with some yellowing from natural autumn senescence and a low incidence of pustules. The leaves of diseased plants were generally yellow or brown with a high incidence of pustules and necrotic lesions and stem die-back (premature senescence). Dead stems were regarded as an effect of the rust because it was too early in the season for complete natural senescence. The condition of the plant populations at sites were rated according to three categories of disease incidence (DIC): namely (DIC-1) healthy and/or naturally senescing stems with a maximum disease incidence < 25 % (11 sites), (DIC-2) some adults still alive, but most stems diseased and dying back or dead (13 sites), and (DIC-3) only dead stems present (32 sites). Pompom was not found at 10 sites and so could not be allocated a DIC category. Some environmental factors were re-evaluated as they may have changed since the original study, namely shading by woody species (% shade), degree of topsoil erosion (% loss) and mowing (presence/absence), or were evaluated differently, namely soil moisture status (permanently dry or seasonally wet).

Since 2006, Gauteng's provincial government has embarked on campaigns to control pompom with herbicide in a systematic manner using the road network to treat infestations. This region overlapped with many of the study sites. Thus herbicide damage is likely to be an important cause for real population decline in certain areas. A probability of control factor was added to determine if the rust and/or herbicide could have influenced the results. Probability of herbicide control (PHC) was based on site proximity to known clearing initiatives targeting pompom with herbicide. The ratings used were (a) 'no'-pompom unlikely to have been sprayed during the intervening years between 2004 and 2009, or (b) 'yes'-spraying likely to have occurred at least once on the site between 2004 and 2009.

Data analysis

Data are presented according to biological year, which starts and ends in mid-winter (1 July–30 June). This coincides with the dry season in Gauteng (Fig. 1). The rangeland survey in April 2009 was conducted during a period when rainfall was 76 % above the long-term average rainfall (LTAR) and the incidence of rust damage to pompom populations was severe. Data from both plot and rangeland studies were subjected to repeated measures ANOVA. The Tukey post hoc test was used to identify and separate means with significant differences ($P < 0.05$) when the null hypothesis was rejected (F pr. <0.05). Density and frequency data from the rangeland survey were log transformed prior to analysis to correct for heteroscedasticity. The trial sites were not contrasted as soils and soil moisture were very different.

In addition, pompom density data from the 2009 rangeland survey were subjected to quantile regression analysis of the same limiting factors (Table 1) that were found to constrain density in the 2003 survey (Goodall et al. 2011). Samples of top soil to a depth of 20 cm were collected from three random points along each transect and submitted to a certified soil laboratory for analysis of texture, fertility and total C, N and S. The statistical program Blossom version W2005.08.26 (Cade and Richards 2005) was used for quantile regression analysis. Cade et al. (1999) describe a limiting factor as the active constraint, one that is least available among those factors affecting an organism's survival, growth and reproduction at any given point in time and space. Analysis of covariance (ANCOVA) was also performed with limiting soil factors as regressor variables on the effect of DIC (categorical variable) on weed density (dependent variable). Statistica 6.1 was used for ANOVA and ANCOVA.

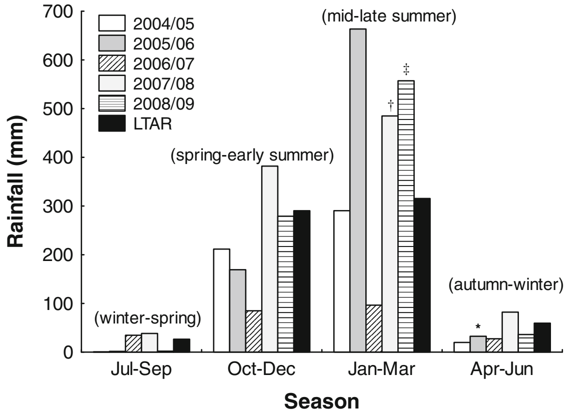


Fig. 1 Rainfall distribution according to seasons in given biological years (1 July–30 June, winter to winter) compared to the long term average seasonal rainfall (LTAR) for the area encompassing plot and rangeland studies on pompom weed in Gauteng. The symbols (*asterisk*, *dagger*, *double dagger*) denote when severe rust damage to most adult *Campuloclinium macrocephalum* (pompom weed) stems occurred, preceded by periods of above average rainfall. The symbol *asterisk* indicates the first appearance of the rust at the xeric herbicide trial site in April 2006, both mesic and xeric sites were affected in March 2008 (*dagger*) and February 2009 (*double dagger*)

Results

Plot study (line intercept)

Climatic conditions had a strong influence on the performance of pompom weed. Seasonal rainfall during the study was compared with the LTAR (Fig. 1). Normal rainfall patterns occurred in 2004/2005. In 2005/2006 climatic conditions became very wet and cool in the second half of the year, coinciding with the first appearance of the rust on pompom. The 2006/2007 year was characterised by drought with below average rainfall in each quarter, receiving a total of only 243 mm for the year or 35 % of the LTAR. Pompom was healthy in February 2007, but by April 2007 plant condition was very poor due to moisture stress, and hence rust damage could not be determined. Both the 2007/2008 and 2008/2009 years experienced above average rainfall. Early frost in April 2008 killed off pompom stems at both trial sites before the rust could exert its maximum effect. By February 2009 pompom condition had been severely affected by the rust.

Pompom populations remained stable in the untreated plots at the xeric site for the duration of the experiment (Fig. 2b), regardless of variable rainfall patterns or rust damage. Populations in mesic grassland declined significantly in 2006/2007 (Fig. 2a), but appear to be affected more by moisture stress than by rust, symptoms of which were not observed during the drought. The effects of the drought on the population at the mesic site persisted into 2007/2008, coupled with disease symptoms. The temporary decline in pompom numbers and grass basal cover over this period (Figs. 2a, 4a) is attributed

Table 1 Summary of the significant soil fertility factors with constraining effects on *Campuloclinium macrocephalum* (pompom weed) density in 2009 using quantile regression (LAD) compared with original data from the 2003 survey

Limiting factor	LAD summary 2003–2004 (n = 80)[a]			LAD summary 2009 (n = 56)		
	Quantile	*P* ARSS[b]	MLF (%)[c]	Quantile	*P* ARSS	MLF (%)
Sample density (g/mL)	0.92	0.030	77.1	0.60	0.033	102.2
Sand (%)	0.90	0.027	73.0	NS	NS	NS
Silt (%)	0.99	0.017	4.3	NS	NS	NS
Phosphate (mg/L)	0.92	0.038	35.8	0.98	0.011	11.6
pH (KCl)	0.89	0.047	70.1	NS	NS	NS
Calcium (mg/L)	0.89	0.026	70.2	NS	NS	NS
Total carbon (%)	0.92	0.008	68.3	0.85	0.035	85.5
Total nitrogen (%)	0.92	0.011	71.5	0.80	0.035	94.1
Total sulphur (%)	0.90	0.039	76.8	NS	NS	NS

[a] *Source*: Goodall et al. (2011)

[b] *P* value of the Asymptotic Rank Score Statistic (Cade and Richards 2005)

[c] Maximum limiting function of measured factor/s on pompom density, where minimum density = 100 % (0.01 plants m^{-2} in 2003–2004, 0.4 in 2009) and maximum density = 0 % (6.3 plants m^{-2} in both years)

to a lower than normal water table because of the boreholes, which under drought conditions caused the water table to drop even further to below the plant rooting zone. In addition, the high smectitic clay content at the mesic site imposes further restrictions on the availability of soil water for plant-uptake during dry periods. Adult plants were severely affected by the rust in February 2008 but populations were no different to those during the drought. In 2008/2009 a boom in pompom populations occurred at the mesic site, coinciding with abundant rainfall, but the rust had killed most of the annual stems at both sites by February 2009. Plant height could not be determined as a result.

The drought of 2006/2007 did not adversely affect plant height (Fig. 2c, d). Height was adversely affected by the interactions between the rust and high rainfall. Plant height rather than density was a more sensitive indicator of rust damage. The initial rust outbreak at the xeric site in February 2006 clearly had an impact on plant height although plants appeared healthy. First symptoms of the rust were noted in mid-March 2006, with leaves having a sickly grey-green appearance but without yellowing. A notable change in plant condition took place in mid-April 2006, early autumn of the 2005/2006 season (Fig. 3b), with mature stems mostly having died, as opposed to the pre-rust era (April 2005) when plants were at an early stage of senescence with only leaf yellowing. At this stage of the disease's history in the country, urediniospore infection appears to present symptoms later in the season when stems are mature. Pompom in mesic grassland in April 2006 was not affected by the rust (Fig. 3a) so the initial outbreak appears to have been reasonably localised. These trends were repeated in the high rainfall years of 2007/2008 and 2008/2009, but symptoms appeared earlier than the initial outbreak. By February 2008 the rust was more widespread with both sites being affected.

Average canopy cover of pompom did not change significantly at either site over the study period (Fig. 2e, f). Chlorophyll measurements (Fig. 3a, b) verify that the rust had minimal effect on plants in February in 2006 (present but no visible damage yet) and 2007 (drought, unsuitable for rust development). Differences within sites-by-time were significant. The effect of natural senescence (pre-rust) on chlorophyll content was observed in April 2005, with the main effect being leaf yellowing without stem die-back. Chlorophyll contents in rust damaged plants in April 2006 were not significantly lower than naturally senescing plants in April 2005. The effect of the 2006/2007 drought on chlorophyll content was only detectable at the end of the season in April 2007; with plants dying back rapidly as moisture availability became critical towards the dry season. The wet summers (February) of 2007/2008 and 2008/2009 resulted in rust outbreaks with significantly lower chlorophyll measurements than in February 2005 and 2006.

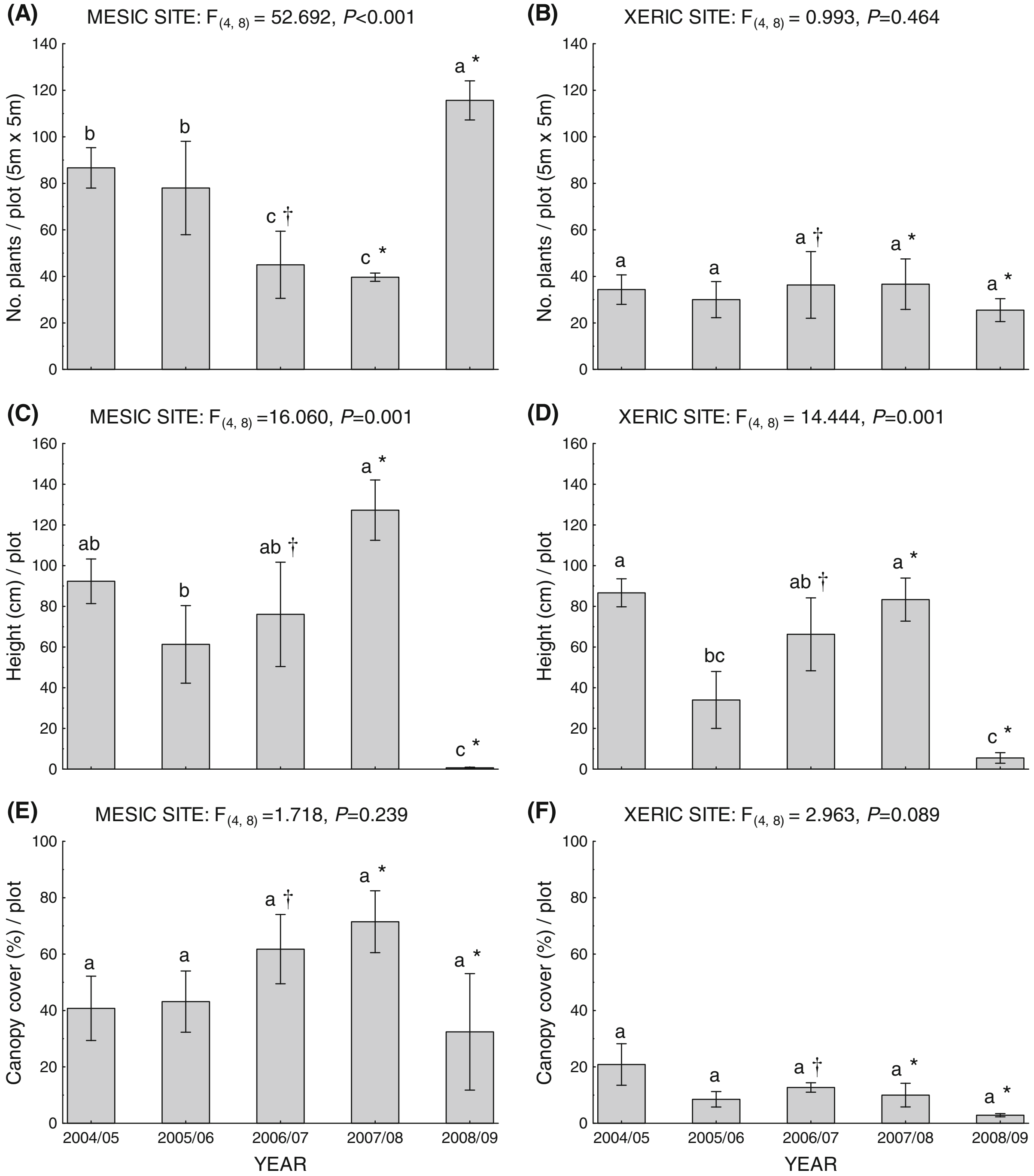


Fig. 2 Changes in *Campuloclinium macrocephalum* (pompom weed) numbers, height and canopy cover in untreated plots in mesic (**a**, **c**, **e**) and xeric grassland (**b**, **d**, **f**) in February from 2005 to 2009. The symbols *asterisk* and *dagger* denote damage by the rust fungus *Puccinia eupatorii* and drought conditions respectively. Means with the same letter are not significantly different ($P > 0.05$) according to within subjects repeated measures ANOVA, post hoc Tukey test

Dominant native species persisted at both sites for the duration of the study with the xeric site having a higher grass and forb richness (Fig. 4f, h) than the mesic site (Fig. 4e, g). Graminoid basal cover at the mesic site declined during the drought of 2006/2007 (Fig. 4a) and the effect persisted into 2007/2008,

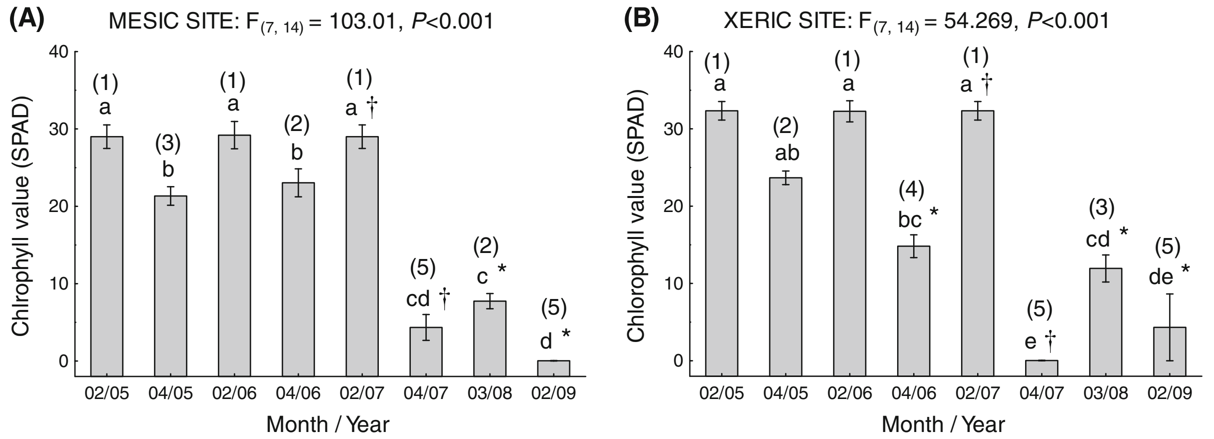


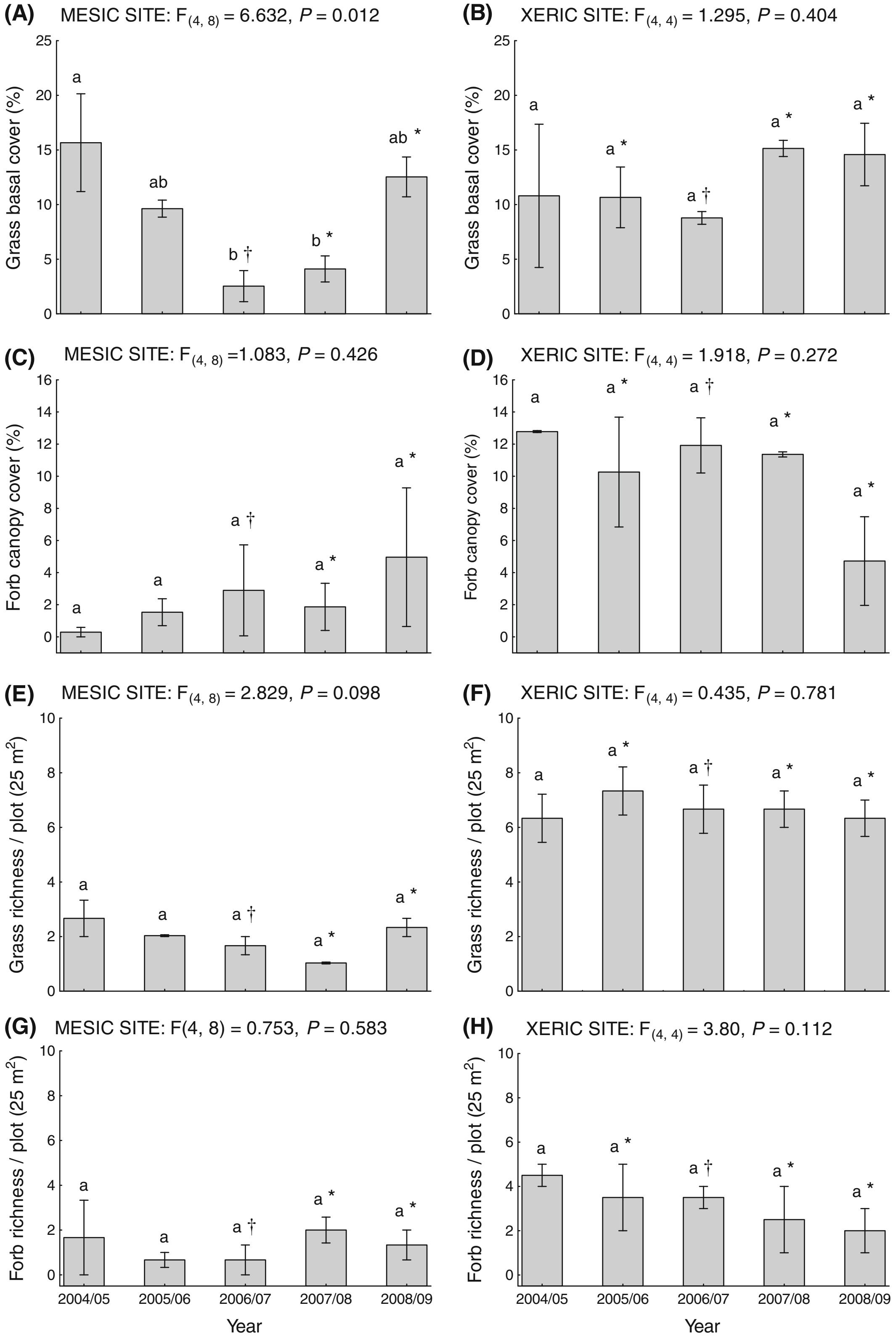
Fig. 3 Changes in *Campuloclinium macrocephalum* (pompom weed) chlorophyll content in untreated plots in mesic (**a**) and xeric grassland (**b**) in mid-summer (February) and autumn (April) 2005–2009. The symbols *asterisk* and *dagger* denote significant damage by the rust fungus *Puccinia eupatorii* and drought conditions respectively. Means with the same letter are not significantly different ($P > 0.05$) according to within subjects repeated measures ANOVA, post hoc Tukey test. Numbers in brackets refer to plant condition at time of measurement, where 1 = healthy with green leaves, 2 = leaves green/yellow, 3 = leaves yellow, 4 = leaves yellow/brown with stem dieback, 5 = stems dead

corresponding with temporary declines in pompom populations relating to soil type and moisture availability over the same period (Fig. 2a). Forbs, many of which were annuals, experienced a higher turnover than graminoid species at both sites. Species richness, however, did not change significantly over time for both grasses and forbs at either site (Fig. 3b). Species switches within growth forms, e.g. forb$_i$ replacing forb$_j$, were by native species and were not significantly affected by either pompom persistence, or lowered vigour of pompom through disease or climatic variability. These appear to be degraded stable communities and repeated measures analysis suggests that pompom was not a transformer species. Both sites have a history of anthropogenic disturbance, making it feasible for pompom to be an occupant of vacant niches. Pompom persisted in mesic and xeric grassland without experiencing declining population densities.

Plot study (quadrats)

Primary stems or regrowth emerged from the woody rootstocks from September (Fig. 5a, b) and grew rapidly, reaching maximum height (90–120 cm) in December (Fig. 5c, d). Peak flowering occurred from December to February, but flowers were seen throughout summer. Seed-set was completed shortly after florets senesced and achenes were available for wind-borne dissemination from early summer, lasting well into autumn. The initial outbreak of the rust in March/April 2006 at the xeric trial potentially curtailed the period available for seed production but did not affect population size or rate of growth of primary stems. Drought reduced the number of primary stems per unit area as moisture availability became more critical in late summer of 2007, but plant height was not affected (Fig. 5c, d). This was not the case in 2008 with the rust and frost killing stems, but not the subterranean rootstock. Rust damage in 2009 and 2010 occurred after December, and thus did not have a significant impact on growth and population development. Compensatory stems were measured for the first time in autumn and winter (April and July respectively) 2009 and were not affected by the rust. Compensatory growth did not occur on plants that senesced naturally prior to the rust establishment.

Pompom seedlings were found at the mesic site throughout the year over the study period (Fig. 5e) but seldom at the xeric site (Fig. 5f). The opportunity for seedling establishment in xeric grassland coincided with above average rainfall in early to mid-summer. The majority of the seedlings at both sites occurred in sheltered spots. Seedlings under living pompom were not as numerous as under grass tufts and pompom stems killed by the rust. Flooding occurred at the wetland site in February 2006, followed by a large number of seedlings, but these diminished as winter progressed. Possible causes of seedling mortality included rust disease, frost,



(A) MESIC SITE: $F_{(4,\ 8)} = 6.632$, $P = 0.012$

(B) XERIC SITE: $F_{(4,\ 4)} = 1.295$, $P = 0.404$

(C) MESIC SITE: $F_{(4,\ 8)} = 1.083$, $P = 0.426$

(D) XERIC SITE: $F_{(4,\ 4)} = 1.918$, $P = 0.272$

(E) MESIC SITE: $F_{(4,\ 8)} = 2.829$, $P = 0.098$

(F) XERIC SITE: $F_{(4,\ 4)} = 0.435$, $P = 0.781$

(G) MESIC SITE: $F(4, 8) = 0.753$, $P = 0.583$

(H) XERIC SITE: $F_{(4,\ 4)} = 3.80$, $P = 0.112$

◀**Fig. 4** Annual summer (February) cover and richness assessments of grasses (**a**, **b**, **e**, **f**) and forbs (**c**, **d**, **g**, **h**) from 2004 to 2009 (in biological years) in untreated plots (5 m × 5 m) at the mesic and xeric grassland sites invaded by *Campuloclinium macrocephalum* (pompom weed). The symbols (*asterisk*, *dagger*) denote *Puccinia eupatorii* damage to pompom weed and drought respectively. Sites are not compared. Means with the same letter are not significantly different ($P > 0.05$) according to repeated measures ANOVA, within subjects post hoc Tukey HSD test

dry-season moisture stress and intraspecific competition. There was no clear indication of how seedling survival contributed to adult population size with recruitment at the mesic site being high, contrasting with poor recruitment at the xeric site. The average number of primary stems per quadrat in December 2005 was not significantly different to numbers at the end of the study in January 2010 at both sites. This suggests equilibrium population density has already been achieved and recruitment of seedlings to the adult population only occurs after limiting factors such as moisture availability reduces pompom density. Fire occurred at both sites in August 2006 and stimulated primary stem production (Fig. 5a) but killed seedlings (Fig. 5e). Seedlings attained a maximum height of 30 cm at the mesic site in December 2005, before the appearance of the rust (Fig. 5g, h). The degree to which the rust affects seedling survival requires further investigation.

Plot study (compensatory stems)

Puccinia eupatorii induced premature senescence by March or even by February during severe outbreaks (Fig. 3a, b). The severity of outbreaks appears to be influenced by climate, especially abundant rainfall (Fig. 1). The rootstock produces compensatory stems when diseased stems die. Premature senescence of primary stems followed by compensatory growth indicates a rust effect (see dates 04/09 and 07/09 in a–d, Fig. 5) and premature senescence without replacement as a response to drought (see 06/07 in a–d, Fig. 5). When the rust is absent, natural senescence occurs from late autumn without a compensatory effect. Compensatory stems appear to be less affected by the rust than primary stems.

Rangeland study

Pompom infestations were present at 56 of the 66 sites assessed (84.8 %). The absence of pompom at 10 sites was not exclusively rust-related. One site adjacent to a dam was under water and no longer supported terrestrial vegetation. In addition, four sites in the 2003 survey had supported low weed densities of <5 % quadrat frequency (<0.03 plants m^{-2}), thus observer or transect error in the 2009 survey could not be ruled out. The remaining five sites had frequency and density values ranging from 6 to 36 % and 0.17–1.53 plants m^{-2}. It was not possible to determine the cause of the disappearance but the probability of the rust or herbicidal control could not be excluded. Analysis of the range data was therefore based on the 56 sites that still had pompom.

Pompom density from the 2003 survey had decreased in 2009 at 25 of the 56 sites (45 %), increased at 26 sites (46 %) and remained relatively static at five sites (9 %). Within subjects (time effect) repeated measures ANOVA of pompom density was not significant ($F_{(1,55)} = 0.109$, $P = 0.742$), with a mean of 1.5 plants m^{-2} in the 2003 survey to 1.4 in 2009. Both negative and positive changes in density occurred in all three DICs. A within subjects repeated measures ANOVA of pompom frequency (% presence in quadrats) was significant ($F_{(1,55)} = 8.25$, $P = 0.006$), with a mean of 36.1 % in 2003, dropping to 29.4 % in 2009. The effect of DIC on pompom frequency over time was not significant ($P = 0.095$), implying these changes were not likely to be rust-related. This was confirmed by analysing only those sites with dead adult stems ($n = 32$, $P = 0.386$). Frequency data were then subjected to a two-factor repeated measures ANOVA using PHC ($n = 2$) and DIC ($n = 3$) as predictor variables. Probability of control by herbicide (PHC) was a better predictor of changes in pompom frequency over time ($F_{(1,50)} = 21.975$, $P < 0.001$) than DIC ($F_{(2,50)} = 1.223$, $P = 0.303$). The interaction between PHC and DIC was also not significant ($F_{(2,50)} = 0.072$, $P = 0.931$).

All adult stems presented symptoms of rust damage in DIC-2 and DIC-3 with a 1–10 % disease incidence in DIC-1. Dead stems were the most severe symptom of infection with DIC-2 = 70 % and DIC-3 = 100 %. Adult stems at the 11 sites in DIC-1 were not killed by the low incidence of the rust. Compensatory stems were not found at DIC-1 sites, 8 of 13 sites in DIC-2 and 24 of 32 sites in DIC-3 had regrowth in April 2009. Two sites in DIC-3 with healthy regrowth had been partially mowed; however, this might be an artifact of the timing of mowing. Replacement stems were produced soon after the primary stem had died suggesting that pompom

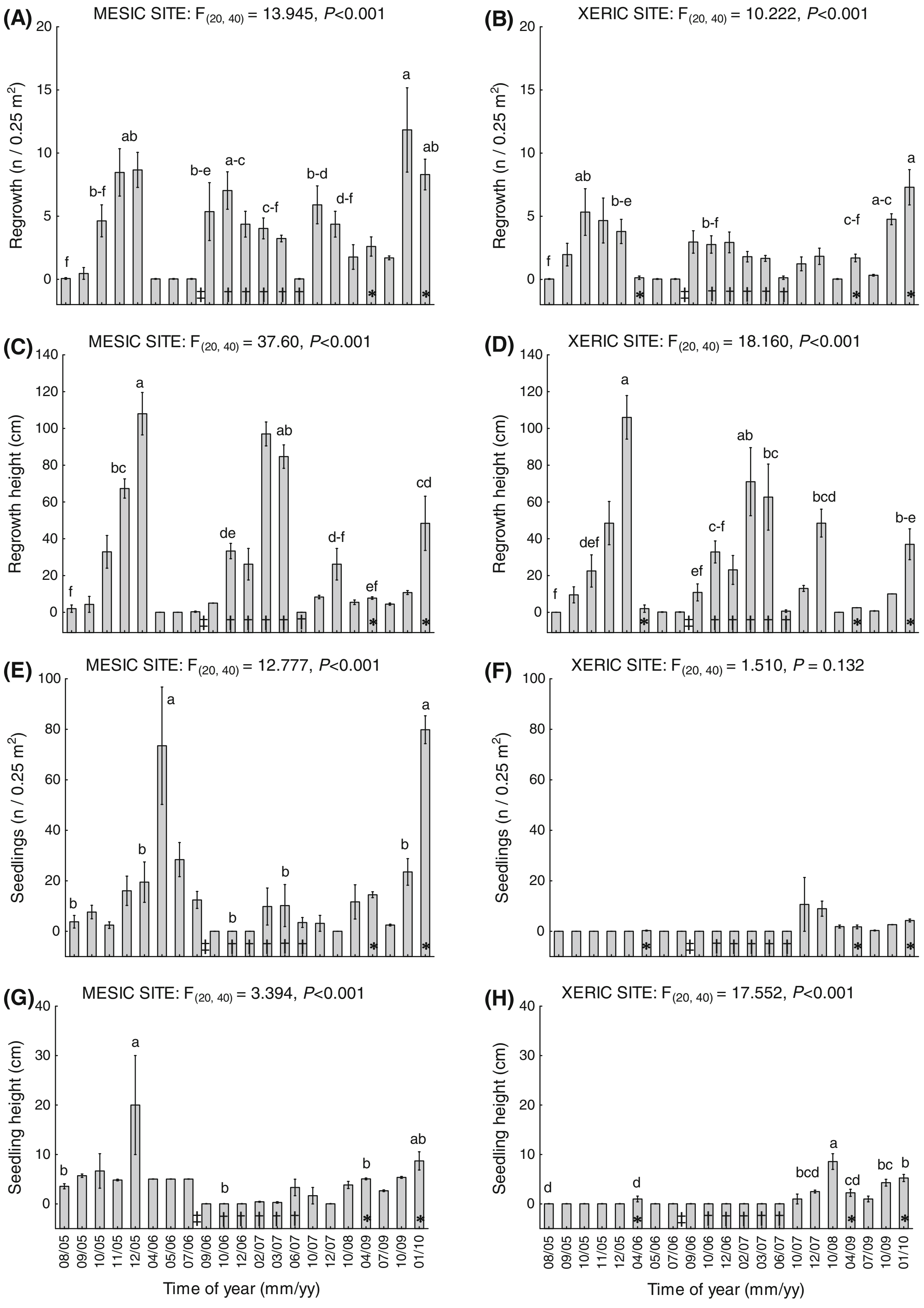

(A)
MESIC SITE: F(20, 40) = 13.945, P<0.001
Regrowth (n / 0.25 m2)
f
b-f
ab
b-e
a-c
c-f
b-d
d-f
a
ab
(B)
XERIC SITE: F(20, 40) = 10.222, P<0.001
Regrowth (n / 0.25 m2)
f
ab
b-e
b-f
c-f
a-c
a
(C)
MESIC SITE: F(20, 40) = 37.60, P<0.001
Regrowth height (cm)
f
bc
a
de
ab
d-f
ef
cd
(D)
XERIC SITE: F(20, 40) = 18.160, P<0.001
Regrowth height (cm)
f
def
a
ef
c-f
ab
bc
bcd
b-e
(E)
MESIC SITE: F(20, 40) = 12.777, P<0.001
Seedlings (n / 0.25 m2)
b
b
a
b
b
b
a
(F)
XERIC SITE: F(20, 40) = 1.510, P = 0.132
Seedlings (n / 0.25 m2)
(G)
MESIC SITE: F(20, 40) = 3.394, P<0.001
Seedling height (cm)
b
a
b
b
ab
(H)
XERIC SITE: F(20, 40) = 17.552, P<0.001
Seedling height (cm)
d
d
bcd
a
cd
bc
b
‡
†
*
08/05 09/05 10/05 11/05 12/05 04/06 05/06 07/06 09/06 10/06 12/06 02/07 03/07 06/07 10/07 12/07 10/08 04/09 07/09 10/09 01/10
Time of year (mm/yy)

Fig. 5 Mean numbers (n) and heights (cm) of *Campuloclinium macrocephalum* (pompom weed) regrowth (**a**–**d**) and seedlings (**e**–**h**) in permanent subplots (0.25 m^2) at the mesic and xeric grassland sites respectively, from August 2005 to January 2010. Symbols: *asterisk* denotes shoot damage by the rust *Puccinia eupatorii*, *double dagger* denotes a wildfire at both sites in late August 2006, and *dagger* denotes drought throughout the course of the 2006/2007 biological year. Sites are not compared. Means with the same letter are not significantly different ($P > 0.05$) according to repeated measures ANOVA, post hoc within subjects Tukey HSD test

readily aborts stems once they become severely damaged by the rust. The condition of the compensatory growth worsened with increasing disease severity, with a 1–10 % reinfection incidence in DIC-2 and 26–50 % in DIC-3 sites. Dead compensatory stems were not observed at any of the sites in early autumn (April 2009), but it is likely that a high proportion of these will succumb to the disease before reaching maturity. The surviving late regrowth will have additional hardships, contending with the first frosts in May and moisture-stress and grass fires from June until September.

Adult stems in all DICs produced seed. Seed dispersal was 76–90 % complete in DIC-1 and DIC-2 sites, with complete seed dispersal in DIC-3. These differences do not imply statistical significance. Flowering of compensatory stems varied across DICs. Compensatory flowering occurred at all sites with the exception of DIC-1 sites, but the incidence was <10 % of plants per site. Although most adult stems produced seed, the impact of the rust on seed production could not be determined from this census because seed-bearing capitula were not counted, nor were seeds harvested for viability testing. Seed production potential, however, is unlikely to increase from compensatory growth due to moderately high pathogen reinfection rates and the relatively low incidence of flowering.

Testing the effect of the rust on pompom's realised niche was done by repeated measures ANOVA using frequency data. Disease interaction with vegetation type (savanna, grassland and wetlands) was not significant ($F_{(4,47)} = 0.748$, $P = 0.564$), as were the interactions with soil moisture ($F_{(2,50)} = 0.554$, $P = 0.578$), shading ($F_{(2,50)} = 0.058$, $P = 0.943$) and soil erosion ($F_{(4,42)} = 0.971$, $P = 0.433$). The differences in pompom's temporal frequency were therefore not explained by the interactions of the rust and environmental heterogeneity.

The exercise repeated with PHC instead of DIC had essentially the same outcome with the exception of the interaction between soil erosion and PHC ($F_{(3,43)} = 9.278$, $P < 0.001$). Areas of zero or low soil erosion (e.g. crust brokenness, 16 sites) in association with PHC in the intervening years had a lower pompom frequency in 2009 (18 %) compared to these same sites in 2003 (49 %, $P = 0.017$). Sites with identical erosion categories but without PHC ($n = 20$) did not change significantly. By implication, herbicide spraying was ineffective at reducing pompom frequency in areas that were affected by soil erosion. Ad hoc herbicidal control is therefore only likely to be effective in areas that experience minimal topsoil loss.

Pompom density from the surveys of 2003 and 2009 were compared in relation to significant limiting factors (Table 1). Published data from the 2003 survey (Goodall et al. 2011) have been included for comparison. A fundamental difference between these times is the number of sites, 80 in the 2003 survey and 56 in 2009, which reduced the effectiveness of the regressions on the smaller data set. Regression quantiles in 2009 failed to produce limiting relationships in 5 out of the 9 limiting soil factors of 2003; namely % sand, % silt, pH, calcium and % sulphur. Available soil phosphate (soil P) was the only variable where a constraining effect was still detected at the maxima of the response distribution. Sample density and total carbon and nitrogen % produced significant constraining effects at intermediate stages of the response distribution, meaning their role as limiting factors only applied to a subset of sites.

One way ANOVA determined that pompom density in 2009 differed significantly between DICs ($F_{(2,53)} = 4.585$, $P = 0.015$), with DIC-3 supporting more stems m^{-2} on average than DIC-1 (1.9 vs. 0.6 m^{-2}); DIC-2 had an average of 1.1 stems m^{-2} and was not different to DIC-1 or DIC-3. Analysis of covariance (Table 2) was then conducted using the soil fertility data as regressor variables to determine if these played a role in the interactions between disease severity (categorical variable) and weed density (dependent variable). Most of the soil covariables did not independently yield a significant effect on pompom density, with the exception of total carbon. The multiple regression models were all significant at predicting mean population density adjusted for covariance (Table 2). Soil fertility provides convincing evidence of relationships, although weak (adjusted

Table 2 ANCOVA summary of the effect of disease severity (DIC) on *Campuloclinium macrocephalum* (pompom weed) density in 2009 using soil fertility factors as covariates

Covariate	Univariate results				Covariance regression model						
	Covariable		Independent var.		adj R^2	Model		Residual		F	*P*
	$F_{(1,52)}$	*P*	$F_{(2,52)}$	*P*		$SS_{(df=3)}$	MS	$SS_{(df=52)}$	MS		
Sample density	1.94	0.17	3.96	0.025	0.131	18.10	6.00	83.60	1.60	3.756	0.016
Sand (%)	0.51	0.479	4.39	0.017	0.107	15.85	5.28	85.93	1.65	3.197	0.031
Silt (%)	0.64	0.427	3.892	0.027	0.109	16.07	5.36	85.71	1.65	3.249	0.029
Phosphate (mg/L)	2.06	0.157	4.817	0.012	0.133	18.32	6.11	83.45	1.60	3.806	0.015
pH (KCl)	0.963	0.331	3.933	0.026	0.155	16.59	5.53	85.19	1.64	3.375	0.025
Calcium (mg/L)	2.08	0.155	4.972	0.011	0.133	18.35	6.12	83.42	1.60	3.813	0.015
Total carbon (%)	**4.728**	**0.034**	3.749	0.030	0.173	22.24	7.41	79.53	1.53	4.848	0.005
Total nitrogen (%)	3.312	0.075	3.767	0.030	0.152	20.21	6.74	81.57	1.57	4.294	0.009
Total sulphur (%)	0.797	0.376	4.442	0.017	0.112	16.32	5.44	85.46	1.64	3.31	0.027

One-way ANOVA determined ($F_{(2,53)} = 4.585$, $P = 0.015$) that healthiest populations (DIC-1) had the lowest density (1,600–10,300 plants ha^{-1}) and the most severe disease symptoms (DIC-3) occurred in the densest stands (13,000–24,000 plants ha^{-1})

Numbers in bold highlight covariates having a significant univariate effect on the response variable

$R^2 < 0.2$), with disease severity and weed density. In ANCOVA DIC always exerted a significant univariate effect on density and was therefore primarily a function of weed density, with the greatest impact in the densest stands. The poor accountability for variance in the model suggests pompom density may be regulated by numerous covariate interactions, after accounting for multicollinearity (total organic carbon was correlated with several of the covariates). In addition to soil fertility, for example disturbance frequency, disturbance magnitude and competition with the grass layer, may also be important determinates of pompom density.

Discussion

Pompom has been actively spreading in South Africa since the 1970s (Goodall et al. 2011) and for the most part, in the absence of biological control and organised eradication programmes involving herbicides. Had these conditions prevailed subsequent to 2004 one would have expected the weed to increase its distribution and abundance at a predicable rate, given that the potential for further invasion is still considerable. Large scale clearing initiatives in Gauteng were implemented around the same time as the appearance of rust in early 2006. In spite of these factors the weed continued to spread in South Africa (see Fig. 1 of Goodall et al. 2011 vs. Fig. 3 of McConnachie et al. 2011), but the effect of the rust on the rate of spread is unknown. In this study fitness costs were incurred with 45 % of the rangelands surveyed in 2009 having fewer pompom plants than in 2003, while an additional 9 % of sites remained similar to original densities. The cause of variation in the pompom population remains tentative. The rust disease may not be exclusively responsible for reductions in pompom density or the number of infestations, however, it is still considered to be an important contributing factor. The rust would affect the future rate of spread if diseased plants produce less seed than uninfected plants.

Resistance or tolerance to pathogens are strategies that hosts utilise to manage disease. The distinction is resistance involves traits that prevent or limit infection through barriers (e.g. glandular trichomes) and/or mechanisms/processes (e.g. formation of cork layers, production of toxins, contained cell death); whereas tolerance refers to traits that do not reduce infection but instead reduce fitness consequences to the plant (e.g. vegetative reproduction) (Roy and Kirchner 2000). Under this definition pompom weed has no resistance strategies against *P. eupatorii*. The estimation of tolerance to the rust was not straightforward due to the ambiguous nature of the observed biological responses. Changes in weed density and reproduction were not directly related to damage levels caused by the rust. In contrast adult stem mortality increased with disease severity, as did compensatory growth with increasing stem mortality. In general, diseased stems grew to normal size and completed their reproductive cycle before dying back approximately one month prior to complete senescence in May.

Pompom weed has an abundance of enlarged perennating buds on the rootstock, not all of which grow in spring as primary stems. These buds also function as a form of investment for both biotic causes of damage and abiotic causes, e.g. mowing (Clay and van der Putten 1999, Henderson et al. 2010). Juenger and Bergelson (2000) showed that early flowering and compensatory regrowth function as tolerance characteristics in the case of herbivory and mechanical damage. Differences in survival versus growth are not necessarily implicit in the frequency or intensity of defoliation as many species overcome high levels of phytomass destruction simply because of their regrowth capacity (van der Meijden et al. 1988). The dual function of regrowth/resprouting in geophytic herbs is for the production of new shoots after winter and as a response to a loss in apical dominance after damage. This is evident in perennial herbaceous weeds (Klimešová et al. 2008) and as an adaptation to herbivore and pathogen damage (McNaughton 1983; Hatcher and Paul 2000, 2001).

The principle effect of the rust on pompom appears to be premature senescence of annual stems after the completion of flowering and seed dispersal. The rust performs in a manner typical of biotrophic fungi with a long-term parasitic relationship with the host plant, rather than one that kills the host as part of the infection process (Deacon 1997). In this case pompom stems complete their annual life cycle before the onset of severe leaf necrosis leading to shoot death. The effect of early senescence on reallocation in equilibrium rootstock size and density was not measured but is vital in the assessment of the rust as a biological control agent against pompom weed. The drain on stored reserves resulting from severe damage to above ground parts, and the energy expended on replacing photosynthetic tissue, might not be as severe for species with strong resprouting traits (Weiher et al. 1999). It is conceded that there is a distinctive difference between rapid damage to resprouting species from cutting for example, than the slow progressive and degenerative damage resulting from disease. From a pathological perspective the changes in phenology, as witnessed by compensatory growth in diseased plants as opposed to the lack of compensatory growth in healthy plants, demonstrate some degree of tolerance. Premature senescence prompted compensatory growth but the combined effects of both on fitness, i.e. subterranean plant biomass, in time and space was not measured. The incidence of regrowth was always less than the incidence of stem mortality (62 vs. 70 % in DIC-2 and 75 vs. 100 % in DIC-3 sites), of which a significant portion of these were diseased (26–50 %). Of interest will be a comparison of the tolerance response to rust infection with other forms of stem damage including cutting and the introduction of insect biocontrol agents.

Data from the rangeland sites revealed that although adult stems had the highest mortalities, compensatory growth was also affected by the rust. Disease symptoms on young regrowth in autumn were influenced by the magnitude of damage to the primary stems on the same rootstock and high rainfall. In the plot study the rust performed particularly well in the untreated plots under conditions of above average rainfall, but under drought conditions the rust was ineffectual. The rusts *P. xanthii* var. *parthenii-hysterophorae* and *P. abrupta* var. *partheniicola* have both had limited success on the biological control of *Parthenium hysterophorus* L. (Asteraceae) in Australia due to erratic rainfall and high incidence of drought (Dhileepan et al. 2006). The importance of rainfall variability on the success of biocontrol agents has not been adequately addressed and only a few studies have measured its impact (Norris et al. 2002). The long-term impact of climate change with increasing drought frequencies in southern Africa (Fauchereau et al. 2003) might hamper the pompom pathogen, which under favourable moist conditions only becomes evident from mid-summer.

At this point it is not known if the rust is affecting pompom seed production, but data from the quadrat assessments suggest seedlings are killed to some extent. In the rangeland study most of the primary stems affected by the rust had completed flowering and fruiting. With peak flowering usually occurring before leaves are completely infected, the impact of the rust on seed production and viability requires further investigation to truly appreciate the value of this biocontrol agent. The rust *P. lagenophorae* Cooke on *Senecio vulgaris* L. (Asteraceae) reduced capitula production by 43 % and seed production by >60 %

(Paul and Ayres 1986, 1987) but without incurring seedling mortality. This disease also initiated early in the season and caused more rapid senescence compared to the control. The time between pompom flower senescence to capitula bearing seeds ready for wind dispersal is quite short. A study testing the efficacy of a systemic herbicide on seed mortality applied to flowering pompom found that older flowers in the process of becoming seed-bearing capitula still produced viable seed even after plants had died (Thaphathi et al. 2007).

Numerous insect and pathogen associations regulating pompom density in its native range (McConnachie et al. 2011) implies that *P. eupatorii* on its own, being biotrophic, is unlikely to reduce pompom densities in South Africa to levels where it has minimal impact on the vegetation it invades. In the context of classical weed biocontrol the rust will most certainly have an additive effect on *C. macrocephalum* in conjunction with the insect agents once approved for release. Although it was responsible for considerable damage, additional agents, and/or other control methods (Olckers and Hill 1999), are still needed to reduce pompom to acceptable levels in areas where the rust has established. The imminent release of *Liothrips tractabilis* Mound & Pereyra (Thripidae), a specialist stem deformer that prefers feeding on new growth (McConnachie et al. 2011) might provide compatible and complimentary suppression of pompom weed in the near future. Mutualistic interactions between insect and fungal biocontrol agents against weeds are currently quite rare (Friedli and Bacher 2001) and contradictory in some cases (Kruess 2002), but with an increasing number of projects involving both, greater clarity regarding the compatibility and complementarity of these agents should improve the success rate of weed biocontrol.

With a long co-evolutionary history between the rust and pompom in the native range, it seems unlikely that there would be enough selection pressure to reduce disease-tolerance after a relatively short isolation period of 40 years. Pompom exhibits the life history attributes of a ruderal weed with a disturbance-mediated invasion strategy driven by environmental factors external to biocontrol (Goodall et al. 2011). Pompom has no defences against the rust other than its strong resprouting ability. It may be more cost-effective for the species to trade annual stems rather than having evolved resistance strategies. The ability to tolerate disease may also be due to endophyte presence (Evans 2008). Although damaging, the rust did not affect pompom's competitive ability or the retention of its realised niche in xeric and mesic rangelands that have been degraded by poor management practices. The reduction of pompom in this study was correlated to herbicide intervention. As a natural enemy on its own, the rust is not considered keystone to the reduction of pompom in rangelands. This study continues to support findings from the earlier study that weed characteristics and disturbance are the primary mechanisms for invasion and emphasises their importance in invasion biology, both before and after enemy release conditions in the naturalised region. The evaluation of biocontrol agents prior to testing should include cognisance of agent and weed attributes. Post-release evaluations should also focus on biotic and abiotic factors affecting native ecosystems at a local level to gain a balanced perspective on the causes of poor control by established agents. Hopefully synergy between the rust and insect biocontrol agents will provide an effective natural control measure against pompom weed in the future.

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5. Testing the performance of registered herbicides on the control of *Campuloclinium macrocephalum* (Asteraceae) in South African grasslands

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Summary

We report on the performance of the registered herbicides picloram and metsulfuron-methyl on the control of *Campuloclinium macrocephalum* (pompom weed) in grasslands. Herbicide trials in hydric and xeric grasslands were treated for three consecutive years in either summer (February) or autumn (April) and monitored for an additional 3 years after spraying ceased. Uncontrolled factors such as a host-specific rust, fire and drought were observed from the second year of the study. Metsulfuron-methyl and picloram did not differ in efficacy according to the timing of applications, but average mortality of marked plants was <80% expected of registered herbicides. Populations of *C. macrocephalum* in plots were reduced proportionately to the percentage mortality of marked plants. Picloram and metsulfuron-methyl applied at 252 g a.i. ha^{-1} and 45 g a.i. ha^{-1}, respectively, were not detectable by gas chromatography in the upper 25 cm of the soil profile during any of the sampling intervals from 0 to 56 days after treatment. Three annual applications of registered herbicide did not reduce *C. macrocephalum* successfully, and it is estimated that between five (summer) to seven (autumn) annual treatments are required to reduce weed density to <1 plant per plot (25 m^2). Future research should focus on rust–herbicide interactions, the role of fire in seedbank management and fire as a treatment that could be integrated with chemical control.

Keywords: pompom weed, disease, drought, fire, mortality, recruitment, regrowth, seedlings, environmental factors.

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Introduction

Campuloclinium macrocephalum (Less.) DC. (Asteraceae; pompom weed) is an alien geophytic herb that invades disturbed grasslands in the northern and eastern parts of South Africa (Henderson, 2001). Commonly known as pompom weed, it was introduced into South Africa from South America in the 1960s, but became naturalised shortly thereafter (Goodall *et al.*, 2011). The weed shows preference for degraded rangelands and invasions in pristine grassland are rare. Furthermore, *C. macrocephalum* density is also constrained by grass basal cover (gbc). The highest density of 6.4 plants m^{-2} was found in rangelands with <5% gbc, and conversely, rangelands with gbc >15% had the lowest *C. macrocephalum* densities. These results

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indicated that this weed would have difficulty in establishing in rangelands with >21% gbc (Goodall *et al.*, 2011).

Campuloclinium macrocephalum produces annual stems from a perennial rootstock just below the soil surface. Stems that emerge in spring (October) are called primary stems and grow rapidly, reaching an average height of 1.3 m by December (Henderson, 2001). Physical damage to primary stems results in copious regrowth (Goodall *et al.*, 2012). Primary stems senesce in mid-May in areas that receive frost, but can survive in winter (June–August) in warmer parts of the country. The weed over-winters as rootstocks.

In 2003, a biological control programme was initiated on *C. macrocephalum* (McConnachie *et al.*, 2011). Three promising agents, a thrips, a flower-feeding moth and the rust fungus, *Puccinia eupatorii* Dietel, have proven to be host specific, but remain in quarantine, pending approval for release. At this time, only one herbicide, metsulfuron-methyl 600 g kg^{-1} (Brush-Off®, DuPont De Nemours International), was registered for the control of *C. macrocephalum* in South Africa. Physical control involving cutting or mowing stems and removing inflorescences to prevent seed set is practised on a small scale, but neither of these methods is effective, as suppression is temporary and infinitesimal in relation to the scale of the problem (Henderson, 2007). Research into the chemical control of *C. macrocephalum* was implemented in 2005 and involved herbicide trials at two localities in Gauteng Province.

In 2006, *P. eupatorii* was discovered outside of quarantine and causing extensive damage to *C. macrocephalum* infestations around Pretoria. By 2008, the disease had spread to most infestations throughout the country. The pathogen causes severe damage to the leaves, resulting in premature death of primary stems in March, as opposed to natural senescence of uninfected plants in May–June. Rust damage stimulates the production of regrowth in late summer and autumn from dormant buds on the rootstock, a phenomenon that does not occur on uninfected plants after they senesce (Goodall *et al.*, 2012). To date, the disease has had negligible impact on field populations of *C. macrocephalum* (McConnachie *et al.*, 2011; Goodall *et al.*, 2012). *Campuloclinium macrocephalum* continues to spread and has the potential to invade a much larger area in southern Africa (Trethowan *et al.*, 2011).

The objectives of this study were to compare the efficacy of metsulfuron-methyl and picloram on the density of *C. macrocephalum* populations over 6 years at two study sites in South Africa. Key to these objectives is the principle that a registered herbicide should provide at least 80% mortality of rootstocks of plants treated under normal conditions. The primary question raised is as follows: does herbicide efficacy of targeted individuals translate into similar reductions in field populations? If not, could environmental factors have the potential to affect herbicide performance? The second question is a secondary objective because during the course of the study, uncontrolled variables, namely rainfall variability, the rust fungus on *C. macrocephalum* and wild fires, were observed at both study sites.

Materials and methods

Study sites

The study was conducted at two localities: open grassland (xeric site) and a wetland (hydric site) near the city of Pretoria (altitude ca. 1450–1500 m a.s.l.) in Gauteng Province. The xeric site has a shallow, well-drained soil of tillite-conglomerate origin. The hydric site has poorly drained soil with a deep (>1 m) vertic A horizon. The long-term average annual rainfall for both sites is 701 mm p.a. (Fig. 1), falling mostly during summer (October to March).

The xeric site has a higher forb component than the hydric site, which has few broadleaf species other than *C. macrocephalum*. Both sites are disturbed communities with dense *C. macrocephalum* infestations. The hydric site has been modified by peat mining, and the water table has been tapped by numerous boreholes

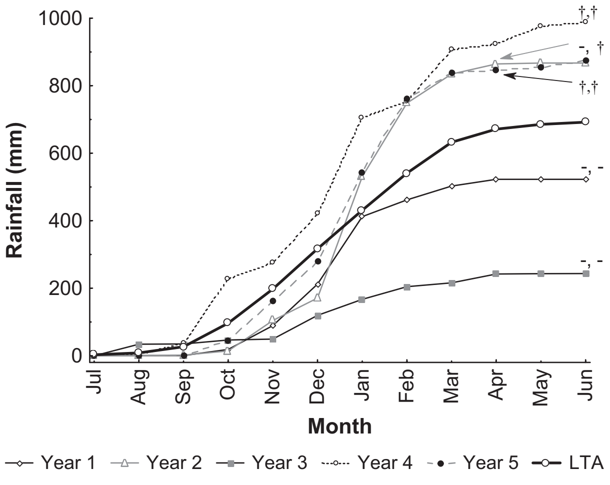

Fig. 1 Cumulative annual rainfall in biological years (July to June) as an average of the hydric and xeric study sites in Gauteng Province, South Africa, over the study period (LTA. long-term average) of 5 years (2004/2005 to 2008/2009); data for year six are absent. The incidence or absence of the rust *Puccinia eupatorii* between February and April at each site (hydric, xeric) is depicted as a dagger (†) or dash respectively.

5-2

that supplement water supply to the city. The hydric site is situated on a nature reserve with large game animals (ungulates). The xeric site is at an airport, and if herbivores are present, they are likely to be small mammals at low density.

Experimental design and treatments

The experiment used a randomised block design with a 3 × 2 factorial configuration with three treatments (two herbicides and an untreated control) and two times/application dates (either February or April). Picloram as potassium salt (Access® 240 SL, 240 g a.i. L^{-1}, Dow AgroSciences) was in the process of becoming registered and was included in the study at the proposed registered dosage, along with metsulfuron-methyl. Metsulfuron-methyl (Brush-Off®, 600 g a.i. kg^{-1}, DuPont) is registered for use at a concentration of 25 g formulated product (fp) per 100 L water (45 g a.i. ha^{-1}). Picloram as potassium salt 240 g L^{-1} (Access® 240 SL) became registered for use at a concentration of 350 mL fp per 100 L water (252 g a.i. ha^{-1}). Mineral oil (840 g a.i. L^{-1}) at a concentration of 500 mL fp per 100 L water was used as an adjuvant in both herbicide treatments. The study was repeated at two sites, a wetland (hydric site) and a xeric grassland site. Each treatment was replicated three times at each site.

Plots measured 5 × 5 m with a 3 m buffer zone between plots. Herbicide treatments involved broadcast sprays applied with a Holder knapsack sprayer fitted with an 8003 EVS even flat fan spray tip and a 50 μm mesh sieve. The sprayer was calibrated to deliver the spray mixture (herbicide, adjuvant and water carrier) at a per plot equivalent of 300 L ha^{-1} v/v by operating at a pressure of 1.8–2 kPa, a flow rate of 900 mL min^{-1}, a swath width of 0.5 m and a walking speed of 1 m sec^{-1}. Herbicides were applied to fully developed stems (± 1 m), which were at the flowering stage during the February applications and the seed-bearing stage in April.

In addition, herbicide residue plots for metsulfuron-methyl and picloram were laid out at each site. The tank mixtures used in the application of treatments were applied to the residue plots in the same manner. Soil samples from the top 25 cm of the soil profile were taken prior to treatment (time zero) and thereafter at 1, 7, 14, 28 and 56 days after treatment. Samples were analysed for herbicide persistence on a gas chromatograph (GC) fitted with an ECD detector.

The growing season in South Africa traverses calendar years (Fig. 1), so the results are presented according to the biological year starting and ending in mid-winter (1 July–30 June). The study was initiated in February 2004/2005 and terminated in April 2009/2010 (6 years). For simplicity, biological years are presented as numerical years: year one = 2004/2005 (start) and year six = 2009/2010 (end). Herbicides were applied once per annum over a course of 3 years. The study thus has a treatment phase, years one to three, and a post-treatment phase, years four to six, in which only monitoring occurred.

Sampling procedure

The number of *C. macrocephalum* plants in each plot (25 m^2) was counted prior to the application of treatments for the first 3 years, with a final count in year four, 1 year after the third and final application of herbicide. The vigour of *C. macrocephalum* during the treatment phase was determined using a chlorophyll meter (Konika Minolta SPAD-502, Konika Minolta, Osaka, Japan) and a visual assessment of verdancy under the categories: healthy (leaves and stems green), senescing (plants yellowing) or dying (plants browning off). The chlorophyll meter is generally used for monitoring the nutritional condition of crop species, with values being proportional to leaf nitrogen content. Values approaching 30 indicate healthy, actively growing plants (Konica Minolta, 2009).

Plant vigour before and during treatment is an important factor affecting herbicide efficacy (Riethmuller-Haage *et al.*, 2007). The efficacy of chemical control can also be compromised if target weeds are under moisture (Morrison *et al.*, 1995) or disease-related stress (Vitelli & Madigan, 1999). In this study, it was important to know whether chlorophyll values assisted in the detection of environmental stress. Chlorophyll readings were taken from the leaves at the apex, middle and base of 10 randomly selected *C. macrocephalum* stems in each plot.

Rootstock mortality of marked plants was assessed in the herbicide and control plots approximately 1 year after each treatment. For this purpose, 10 plants in each plot were randomly selected and marked prior to treatment each year during the treatment phase. Mortality was judged as the absence of regrowth on marked plants and converted to percentage mortality.

In addition, regrowth and seedlings were monitored in permanent circular subsample units, each having an area of 0.25 m^2 (radius = 28.2 cm) and fixed by a central pin. Subsample units were randomly arranged, with 10 units per plot in each of the three replicates. Metsulfuron-methyl and the untreated control were the only treatments monitored at this submetre level. Seedlings were easily identifiable by the presence of cotyledons and distinguishable from the stems of young regrowth, which are much thicker and grew more vigorously. Recruitment surveys (seedlings and regrowth) were carried out in each of the four seasons every year at both localities for the 6 year duration of the study, and included data from before treatments commenced, during the treatment phase and for 3 years post-treatment. Recruitment data were separated into emergence during the growing season ('summer' – September to March) versus emergence in autumn and winter ('winter' – April to August). The effect of metsulfuron-methyl applied in February on recruitment patterns over the study period was compared with the untreated control. The terms 'primary stem' (spring growth), 'seedlings' and 'regrowth' (response to damage of primary stem) are used to separate the life stages of *C. macrocephalum* stems. The term 'total growth' applies to both primary stems and regrowth, in contrast to seedlings.

Table 1 Main effects of treatments (T: two herbicides and the untreated control), applied twice a year (AT: summer or autumn), at two localities (S: hydric and xeric grassland) over three consecutive years (Y), on the mortality of *Campuloclinium macrocephalum* rootstocks; GLM repeated measures ANOVA

Effect	SS	DF	MS	*F*	*P*
Treatment (T)	74298	2	37149	63.762	<0.001
Application time (AT)	1515	1	1515	2.599	0.120
Site (S)	164	1	164	0.282	0.600
Error	13983	24	583		
Year (Y)	3754	2	1877	5.506	0.007
Y*T	4667	4	1167	3.422	0.015
Y*AT	6174	2	3087	9.055	<0.001
Y*S	3582	2	1791	5.254	0.009
Y*T*AT	5146	4	1287	3.774	0.01
Y*T*S	1777	4	444	1.303	0.282
Y*AT*S	1361	2	681	1.996	0.147
Y*T*AT*S	850	4	213	0.623	0.648
Error		48			

Statistical analysis

Data were subjected to repeated measures analysis of variance under the general linear model (GLM). The Tukey post hoc test was used to detect significant differences amongst means ($P < 0.05$). Analyses were performed on Statistica version 12 (StatSoft, 2013).

Results

Mortality and herbicide residues

The variables (a) 'treatment' and (b) 'year' produced significant individual effects (Table 1); (a) *C. macrocephalum* mortality in the control was significantly lower than picloram and metsulfuron-methyl ($P < 0.001$) and (b) *C. macrocephalum* mortality in year three was significantly higher than years one ($P = 0.01$) and two ($P = 0.031$). The variable 'site' did not play a significant role in the main effects.

The mortality of *C. macrocephalum* from picloram was not significantly different to metsulfuron-methyl at either application time (February vs. April) over the treatment period (Fig. 2). Average mortality from herbicides in the first year was higher for applications made in April compared with February (73% vs. 52%), but in the second and third years, mortality was higher for February applications (82% vs. 51%). The reasons for low mortality rates from February herbicide applications in year one are not clear as site influences were inconsistent; picloram achieved 37% (hydric site) vs. 60% mortality (xeric site) and metsulfuron-methyl achieved 73% (hydric site) vs. 37% mortality (xeric site). The average mortality over 3 years for picloram was 69% (Feb) vs. 57% (April)

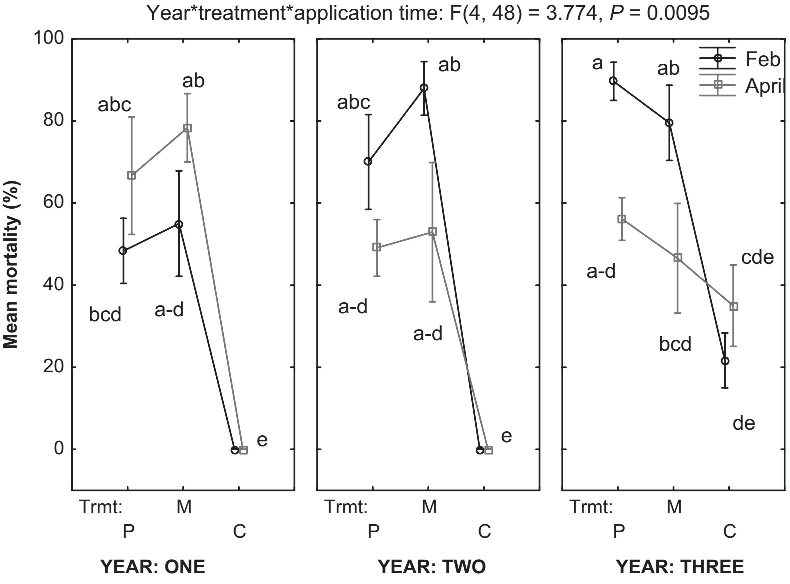

Fig. 2 Mean percentage mortality of marked *Campuloclinium macrocephalum* plants in plots over 3 years after the application of picloram (P) and metsulfuron-methyl (M) in February or April compared with the untreated control (C). In each year, mortality was determined a year after treatment. Means (± SE) with the same letter are not significantly different ($P > 0.05$) by the Tukey post hoc test for equal number of observations.

and metsulfuron-methyl was 74% (Feb) vs. 59% (April). These averages were below the accepted levels of control required from registered products.

Herbicides provided significantly higher mortality rates than the untreated control in the first and second year (natural death = 0%). Mortality in the control occurred only in the third year with a mean of 21.7% in February, increasing to 35% in April. Drought conditions also became progressively worse during the third year (Fig. 1).

Picloram and metsulfuron-methyl applied according to label rates, that is 252 and 45 g a.i. ha^{-1} respectively, were undetectable in soil samples from both sites at each sampling interval. The GC apparatus is accurate at detecting compounds <0.05 μg kg^{-1} soil. Hence, there was no detectable evidence of persistence of herbicides at the rates applied in this experiment.

Mature plant density (weed density)

Differences in the mean density of mature *C. macrocephalum* per plot between sites at the start of the study were not significant ($F_{(1,24)} = 1.670$, $P = 0.209$). However, site interactions became highly significant over time, and for this reason, sites were analysed separately. At the hydric site, temporal patterns in weed density were not affected significantly by the interactions of treatments, timing of applications and year under treatment (Fig 3A). February counts in the untreated control steadily declined from years one to four, but April counts in the same plots fluctuated, with autumn counts in year one being very close to those in the autumn of year four. This made it difficult to assess the net effect of mortality caused by herbicides applied in summer. Weed densities were

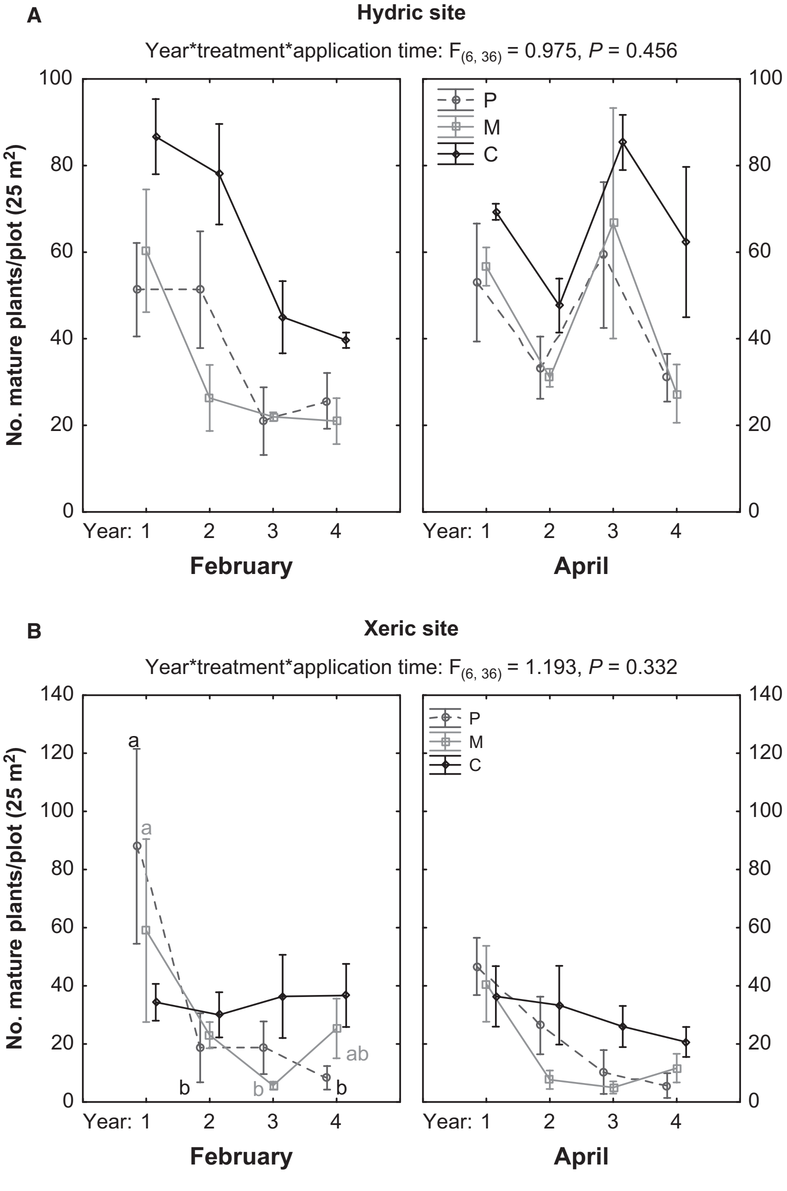

Fig. 3 Mean density (±SE) of mature *Campuloclinium macrocephalum* plants per plot at the hydric (Fig. 3A) and xeric site (Fig. 3B) during the treatment phase of the study. Treatments were picloram (P), metsulfuron-methyl (M) and the untreated control (C). Counts in February and April in the untreated control were from the same plots. There were no significant differences between treatments within a time ($P > 0.05$). Differences over time within each treatment are indicated by letters.

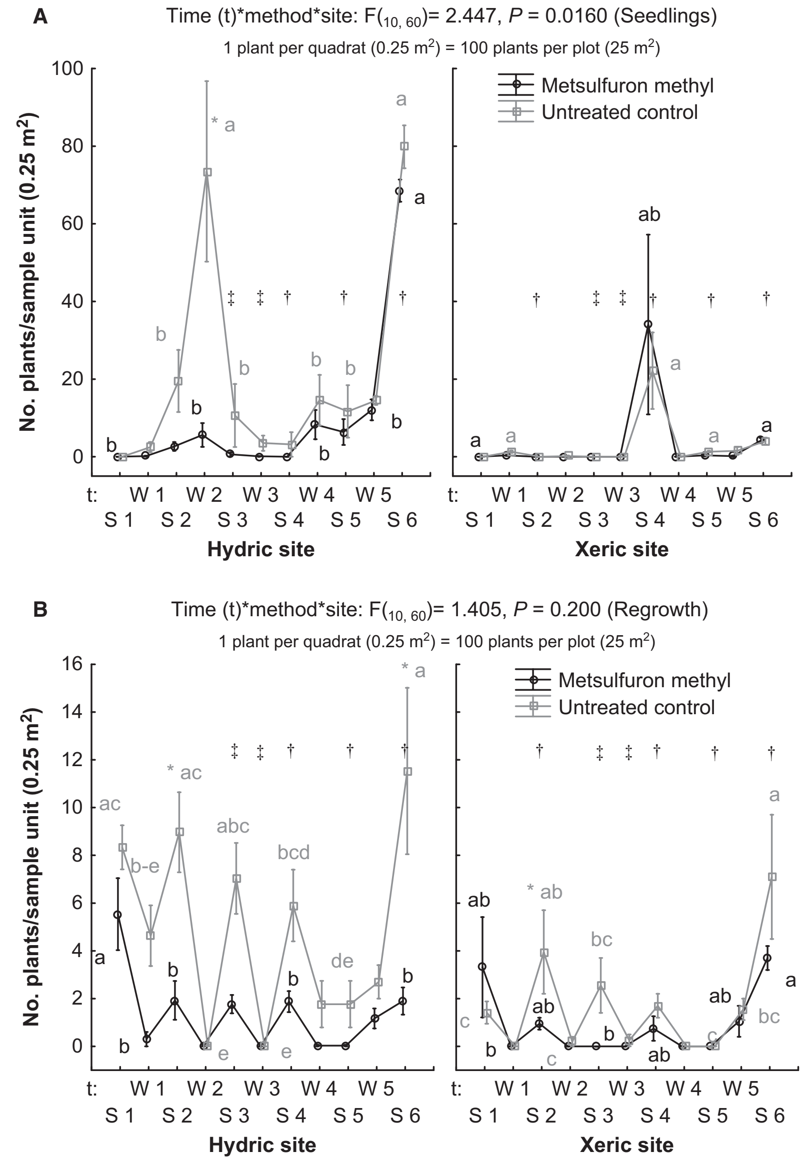

Fig. 4 Mean (±SE) number of *Campuloclinium macrocephalum* (pompom) seedlings (Fig. 4A) and regrowth (Fig. 4B) in sample units of 0.25 m^2 in hydric and xeric grasslands from 2004/2005 (year one) to 2009/2010 (year six). The untreated control is compared with the summer herbicide treatment of metsulfuron-methyl. The *x*-axis chronologically summarises repeated monitoring episodes from year one to year six for surveys conducted in summer (S1–S6: September to March) and winter (W1–W5: April to August). The last herbicide application was in February 2007 (S3). An asterisk depicts significant differences between treatments within a time ($P < 0.05$); differences over time within each treatment are indicated by letters; indicated differences not applicable across sites. Wildfire occurred at both sites in August 2006 (W2) followed by drought from October 2006 to September 2007 (S3 and W3) (see Fig. 1). Symbols are used to highlight outbreaks of the rust *Puccinia eupatorii* (†) and the drought duration (‡).

consistently lower in the picloram and metsulfuron-methyl treatments than the untreated control; both herbicides were significantly different to the untreated control when ‘treatment’ was the main effect ($F_{(2,12)} = 7.031$, $P = 0.01$). At the xeric site, weed density in the untreated control remained relatively constant in both summer and autumn (Fig 3B). The application of picloram or metsulfuron-methyl in summer caused significant reductions in weed density in the respective treatments, but not when applied in autumn. There were no significant differences between variates when ‘treatment’ was the main effect ($F_{(2,12)} = 0.507$, $P = 0.615$). There appeared to be no advantage in using either picloram or metsulfuron-methyl from an efficacy perspective. The timing of applications did not affect the outcome at the hydric site, but summer applications seemed to be more effective than spraying in autumn at the xeric site.

Recruitment

Seedlings were found every year in both summer (S) and winter (W) at the hydric site and infrequently at the xeric site (Fig. 4A). At the hydric site, seedlings were more numerous in control plots but were significantly more abundant than the herbicide treatment only in winter of year two. Herbicides suppressed seedling emergence to some extent during the treatment phase. In winter of year four, about 16 months after the final herbicide application, a mean of 830 seedlings per plot (332 000 ha^{-1}) was found in the herbicide treatment, similar to the untreated control, and this figure increased to 6850 seedlings (2 740 000 ha^{-1}) by the end of the study. Seedling recruitment at the xeric site (Fig. 4A) was low. Recruitment in the untreated control and herbicide treatments was not significantly different.

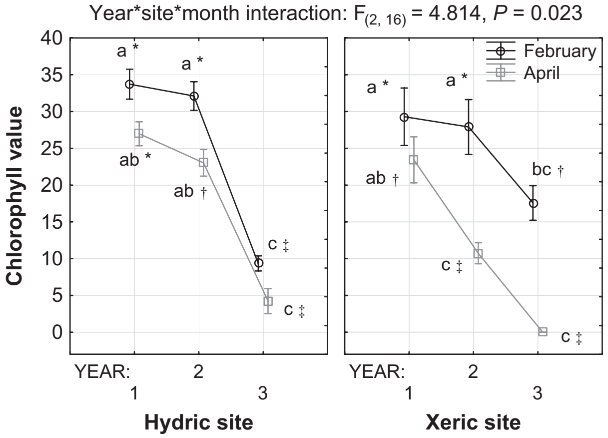

Fig. 5 Chlorophyll meter values from *Campuloclinium macrocephalum* immediately prior to the application of treatments in February and April at each site. Means (± SE) with the same letter are not significantly different ($P > 0.05$). Symbols summarise the majority (>67%) vigour rating from visual assessments, namely healthy (*), senescing (†) and dead or dying plants (‡). Disease was detected in April of the second year at the xeric site and drought occurred at both locations in year three.

In general, seedlings were more common under grass tufts and around the bases of diseased plants than in bare or exposed areas (nurse plant effect). Seedlings were absent under healthy pompom plants. Peaks in seedling abundance occurred in wet years. Herbicides did not offer lasting control of seedlings.

Primary stems were found only in the summer months (Fig. 4B), the exception being W1 (hydric site) when primary stems survived beyond autumn due to a mild climate in that year. Evidence of regrowth in winter occurred in years four and five at the hydric site and year five at the xeric site. Total growth fluctuated in the untreated control during summer at both sites; plant density at S6 (end) was higher than S1 (start) at both hydric (ns) and xeric sites. Plant density in the herbicide treatment was lower at the hydric site in S6 and similar at both times at the xeric site. Although herbicides reduced the amount of total growth significantly at the hydric site, survival of mature plants remained relatively high during both treatment and post-treatment phases.

Uncontrolled environmental factors

Rainfall

The treatment phase of the study was characterised by 2 years of below average rainfall (years one and three) and 2 years of above average rainfall (years two and four) (Fig. 1). Years of above average rainfall were characterised by disease (daggers in Fig. 1), while below average rainfall years did not incur any disease symptoms whatsoever.

Vigour

Chlorophyll contents in February and April were not significantly different in the first and second year at the hydric site and only in year one at the xeric site (Fig. 5). The rust was first discovered at the xeric study site in April of year two (Fig. 1) and may have contributed to the differences between summer and autumn chlorophyll values at that locality. Plants that received herbicide in February of years one and two at both sites were healthy according to chlorophyll meter readings and visual appraisals of plant vigour and verdancy. The health of plants in the autumn treatments deteriorated progressively from years one to three with disease and drought being contributing factors in years two and three respectively. This corresponded with lower autumn mortality rates over the same period (Fig. 2). The drought had a major impact on vigour, with plants dying at the hydric site and senescing prematurely at the xeric site in February of year three.

Disease

Disease outbreaks were always severe, killing most primary stems by March. Outbreaks occurred in year two (xeric site) and in years four to six at both sites.

Wildfire

Regrowth was significantly higher in September of year three, a month after wildfire, compared with other years in the control and herbicide plots at the hydric site (Fig. 6A). Winter wildfire had no effect on spring regrowth at the xeric site (Fig. 6B) and may be because shoots emerged earlier compared with the hydric site. Fire may have triggered earlier emergence of *C. macrocephalum* at the hydric site, but metsulfuron-methyl had a much greater effect on the suppression of regrowth. Fire killed seedlings in the untreated control (Fig. 7), and seedlings did not reappear for several months, implying the heat of the fire may also have killed some of the seeds in the soil. Drought conditions occurred from December of year three (Fig. 1), but moisture stress in early spring following the fire is unlikely to have been the cause of complete seedling mortality (Fig. 1). There were too few seedlings at the xeric site to warrant further analysis.

Discussion

Metsulfuron-methyl and picloram did not provide >80% average mortality of *C. macrocephalum* rootstocks over the treatment period. However, herbicide efficacy on marked individuals did translate into similar reductions in populations in plots. Herbicides applied in summer had an average rootstock mortality

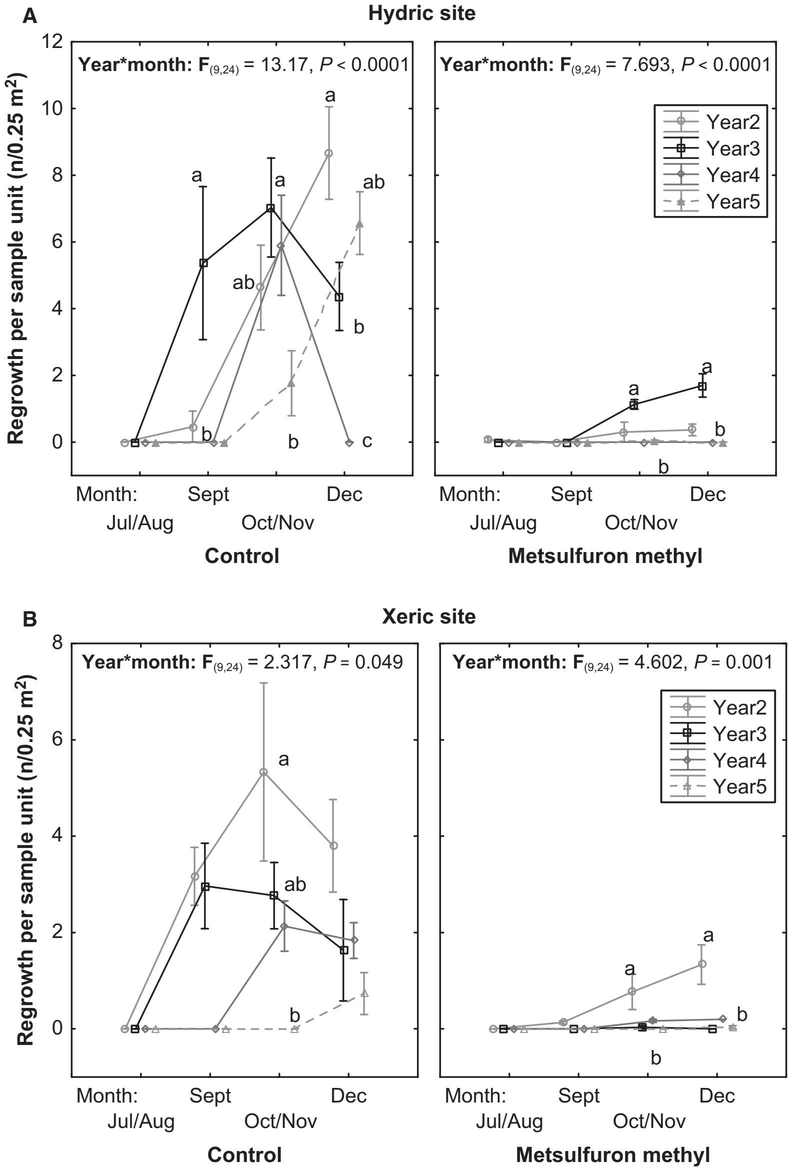

Fig. 6 Emergence and abundance of *Campuloclinium macrocephalum* regrowth (=primary stems) during spring and early summer (Sep–Dec) in control and herbicide (metsulfuron-methyl) plots from the second to the fifth year at both study sites. Wildfires occurred in August of year three at both localities. Letters are used to indicate significant differences in each month ($P < 0.05$).

of 72% with a 63% reduction in *C. macrocephalum* density per annum; autumn applications achieved 58% mortality with a 50% reduction in plot density. Three annual broadcast applications of registered herbicide were not sufficient to bring *C. macrocephalum* density to acceptable levels of control. Simple linear GLM regression estimated that at least 5 annual broadcast applications of registered herbicide in summer would be required to reduce total *C. macrocephalum* density to less than one plant per plot ($r = 0.667$, adjusted $r^2 = 0.433$, $F = 36.96$, $P < 0.0001$) using count data from both sites. It was estimated that at least seven annual applications in autumn would be required to achieve a similar result ($r = 0.416$, adjusted $r^2 = 0.155$, $F = 9.62$, $P = 0.003$). No relation was found in the untreated control ($r = 0.093$, adjusted $r^2 = -0.036$, $F = 0.19$, $P = 0.667$), and populations were considered to be stable over the long term (>10 years). These findings concur with other studies that herbicide experiments require a treatment period longer than 4 years to provide a more comprehensive assessment of costs vs. benefits (Kettenring & Adams, 2011).

Management strategies with long-term objectives, a variety of treatment options and routine follow-ups have been more successful at reducing invasive plants to a stage below the impact threshold than single-application strategies (Kettenring & Adams, 2011). A 3-year-control period utilising registered herbicides applied to *C. macrocephalum* foliage was only partially effective, and, in practice, the accrued expense and effort would have been wasted if follow-ups were discontinued at that point. This is particularly worrying because Asteraceae weeds are usually quite susceptible to herbicides and have relatively short-lived seedbanks,

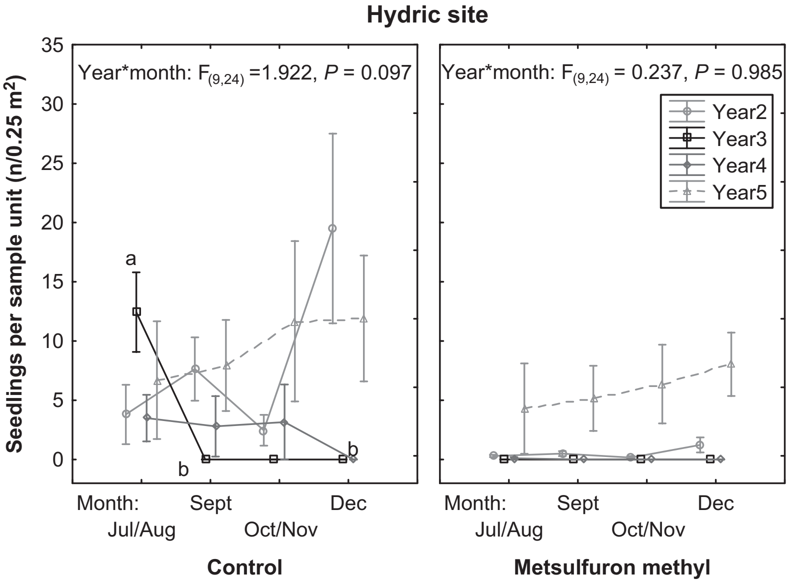

Fig. 7 Emergence and abundance of *Campuloclinium macrocephalum* seedlings during spring and early summer (Sep–Dec) in control and herbicide (metsulfuron-methyl) plots from the second to the fifth year at the hydric site only. Wildfires occurred in August of year three. Letters are used to indicate significant differences within year three only ($P < 0.05$) by the Tukey post hoc test for equal number of observations.

in contrast to leguminous species, for example (Mbalo & Witkowski, 1997).

It is becoming increasingly evident that the economic and logistical feasibility of eradicating or even containing weeds is much more difficult than imagined. Some of the reasons include the spatial distribution of infestations and accessibility, detectability (inconspicuousness especially prior to reproduction), fecundity/seedbank characteristics and control effectiveness (number of treatments required to eliminate local populations). The potential of weed eradication is greatest when the invasive species in question is in low abundance and relatively localised in its distribution in the host country (Panetta, 2009). Neither of these conditions is met concerning *C. macrocephalum* (Henderson, 2007). The economic feasibility of containing *C. macrocephalum* is also questionable, because of the large scale of the infestation and chemical control being only moderately successful (Panetta, 2009).

The effectiveness of application timing was ambiguous. Metsulfuron-methyl and picloram were more effective in autumn compared with summer applications in the first year only, but this was also a year in which uncontrolled factors that negatively affected *C. macrocephalum* were absent. The importance of timing may depend on the growth form of the weed, the type of herbicide intended for use and environmental constraints. Selective herbicides in general may be more effective on broad-leaved weeds in rangelands when applications are made in autumn (DiTomaso, 2000; Payne *et al.*, 2010), but some species are controlled more effectively in spring (Lym & Messersmith, 1990).

Foliar sprays of picloram are usually applied at rates of between 0.14 and 3.4 kg a.i. ha^{-1} for the control of perennial broad-leaved weeds in grass ecosystems (Humburg *et al.*, 1989). Picloram residues in soil samples taken from the upper 25 cm of the soil profile were not detected at application rates ranging from 0.28 to 0.56 kg a.i. ha^{-1} (Lym & Messersmith, 1987). [Correction added after publication on 20 January 2014: In the preceding sentence, ‘upper 15 cm’ was corrected to ‘upper 25 cm’.] The registered rate of 0.252 kg a.i. ha^{-1} picloram on *C. macrocephalum* presents a low risk of soil persistence in South African grasslands. The benefits of picloram persistence in controlling *C. macrocephalum* seed germination and seedling establishment may not be important at the proposed registered rate, which explains why picloram and metsulfuron-methyl were equal in terms of suppression of total growth over the treatment period.

Environmental factors play a critical role in herbicide performance (Kudsk, 2001). A deficit in soil moisture can cause a significant reduction in herbicide efficacy (Coupland, 1989; Morrison *et al.*, 1995; Roche *et al.*, 2002) if target plants are water-stressed prior to the time of application. Cool temperatures and low-light intensity are also important factors, but less so than water stress (Coupland, 1989). Herbicide-induced mortality of *C. macrocephalum* was poorest in the drought year (year three), the only time when mature plants in the untreated control also died. Disease severely disrupts assimilate transport in infected host plants (Livne & Daly, 1964), and it is also feasible that infected leaves may have a reduced capacity for herbicide adsorption and translocation. Vitelli and Madigan (1999) found metsulfuron-methyl efficacy on *Cryptostegia grandiflora* Roxb. ex R. Br. was reduced significantly with increasing levels of infection by the rust *Maravalia cryptostegiae*. In the case of *C. macrocephalum*, the effect of disease and drought on herbicide performance cannot be quantified because they did not form part of the experimental design, and as variables, their occurrence was random and uncontrolled. This does not detract from the fact that disease and soil moisture deficit are recurring constraints that could affect the chemical control of *C. macrocephalum*. On this basis, it is recommended that herbicide applications be constrained to the summer months because environmental factors that negatively affect plant growth, if present, will become increasingly more severe as the season progresses.

Future research on *C. macrocephalum* should focus on rust–herbicide interactions and the role of fire in the integrated control of the weed. Casual observations in the months after the wildfires suggested that fire affects seedlings and regrowth in different ways. Seedlings and possibly seeds on and close to the soil surface could be killed by heat generated by grass fires (Mbalo & Witkowski, 1997). Fire might be a trigger that causes rootstocks to produce regrowth spontaneously, a result which will benefit management strategies that utilise herbicides (Paynter & Flanagan, 2004). The use of fire in vegetation management and problem plant control is controversial, and some studies have found burning natural vegetation leads to an increase in invader biomass (Kettenring & Adams, 2011).

The extraordinary regenerative capacity of pompom from rootstocks and seeds in the soil may prove it a difficult species to control with herbicides alone. Rather than looking at control strategies for *C. macrocephalum* in terms of years, decision makers need to plan on strategies lasting decades or even longer (Panetta *et al.*, 2011). The rust currently does not appear to offer any control benefits. Interactions of the thrips and flower-feeding moth with the rust fungus will hopefully provide sufficient predation pressure to bring pompom under complete biological control and reduce the reliance on herbicides in the long term.

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6. IMPACTS OF CHEMICAL AND PHYSICAL CONTROL OF *Campuloclinium macrocephalum* (ASTERACEAE) ON GRASSLAND SPECIES COMPOSITION

INTRODUCTION

Selective broadleaf herbicides damage both alien and native broadleaf species and thus have the potential to affect forb composition in grasslands. The decline in forb competition would likely benefit graminoids in terms of increased cover and leaf production (Thilenius *et al.* 1975; Rice *et al.* 1997; Koger *et al.* 2002, Sheley & Denny 2006). Herbicide effects on native species have largely been overlooked in studies evaluating herbicide efficacy against alien weeds in natural ecosystems (Rice *et al.* 1997, Crone *et al.* 2009, Reid *et al.* 2009; Kettenring & Adams 2011); making it difficult to determine acceptable trade-offs in the loss of indigenous species from alien plant control activities. A review of alien plant control experiments showed that most studies that assessed the impacts on native species provided inconclusive or ambiguous verdicts (Kettenring & Adams 2011). Collective competition in the grassland forb functional group have been shown to produce a dampening effect on alien species dominance that can be counteracted by non-target herbicide damage (Rinella *et al.* 2009), and exacerbate the invasion process including novel invasions of exotic species (Sheley *et al.* 2006). The aim of chemical control in rangelands should be to reduce the density and spread of invasive species while at the same time implementing measures that mitigate non-target damage and promote restoration.

Manual weeding (physical control) has been practised ever since mankind learnt how to grow crops. It is a low-tech method of weed control and is always recommended in alien plant control situations where no herbicides are registered. The effectiveness of manual weeding, e.g. hoeing, is dependent on life history and re-sprouting traits of the invasive species in question. Annual weeds are effectively controlled by hoeing, but it has to be repeated often because the disturbance created by the scraping action facilitates flushes of weed seedlings from the soil seed bank. Perennial species with large root systems that regrow after cutting are poorly controlled by physical methods. Physical control is labour intensive and less cost-effective than chemical control (Goodall & Naudé 1998), but also less likely to be as damaging to nearby desirable species than herbicides.

Grassland forbs in general have a modest seed dispersal and short-lived seedbanks (Lunt 1995; Soons & Heil 2002; Soons *et al.* 2005) making them vulnerable to both alien plant invasions and clearing operations that utilise herbicides and manual hoeing/uprooting. The ecology of grassland forbs has not received the same levels of attention as their graminoid cohorts and thus it becomes challenging to make management decisions regarding activities that reduce alien species without harming the native non-graminoid elements. The effects of herbicides applied to alien plant infestations on grassland species composition is essentially unknown in South African.

Campuloclinium macrocephalum (Less.) DC. (Asteraceae) (pompom weed) is an alien perennial forb that is rapidly spreading in disturbed grasslands in South Africa (Goodall *et al.* 2011). Research into the chemical control of *C. macrocephalum* was carried out from 2005 to 2010 (Goodall & Witkowski 2014) at two grassland sites; one being a xeric grassland (25.80694° S; 28.16444° E) and the other a hydric grassland (25.8778° S, 28.2746° E) in Gauteng Province. Metsulfuron-methyl and picloram provided similar levels of control but did not achieve 80% kill rates one would have expected from registered herbicides. Uncontrolled factors such as the first appearance of *Puccinia eupatorii* Dietela, a host-specific rust on *C. macrocephalum*, wild fires and drought may have contributed to reduced herbicide efficacy. Taking into account that uncontrolled factors are natural constraints it was estimated that a minimum of five annual applications of herbicide in summer would be required to reduced weed density to <1 plant per plot (5 x 5 m), making chemical control prohibitively expensive on a large scale.

Grassland plant diversity appears to be more resilient to alien forb invasion, e.g. *C. macrocephalum* (Goodall *et al.* 2011) than alien shrub invasion, e.g. *Chromolaena odorata* (Goodall 2000). Highveld grasslands invaded by *C. macrocephalum* can still have many native species and growth forms, especially dry and rocky grasslands. In grasslands infested with *C. macrocephalum* it is not practical to spray herbicides selectively, i.e. spot-spraying, as the uneven application will likely result in many of the plants being missed. In addition, spot-spraying in dense infestations will increase the volume of herbicide mixture per treated hectare and the time taken (person-day costs) to treat the area compared to broadcast spraying.

The study carried out by Goodall & Witkowski (2014) employed broadcast applications of the registered, selective herbicides, metsulfuron-methyl and picloram to dense infestations of *C. macrocephalum.* Although vegetation assessments were conducted annually during the study period the effects of the treatments on other plant species was not reported at the time. Sequential broadcast applications of herbicide in grasslands provide a “worse-case” scenario for assessing herbicide damage on native species. The objective of this chapter is to determine the spatial and temporal effect of three years of herbicide application to monocotyledonous (monocots) and dicotyledonous (dicots) species in xeric and hydric grasslands. Also included is the effect of repeated hoeing (physical control) on alien and native species.

MATERIALS AND METHODS

The study commenced in 2005 and was repeated at two localities; an open grassland (xeric site) and a wetland (hydric site), near Pretoria, Gauteng Province, South Africa. Soils at the xeric site were shallow and well-drained in contrast to the mesic site which had deep, poorly drained clay soils. Average summer rainfall for the area is about 700 mm per annum (October to March). Rainfall varied over the course of the study with above average precipitation in 2006 and 2008 (900 and 1 000 mm) and a severe drought in 2007 (250 mm). The xeric site has a higher forb component than the hydric site. Both sites are disturbed communities with dense *C. macrocephalum* infestations. The hydric site has been modified and subjected to soil moisture stress for many years (Goodall & Witkowski 2014), which has caused a shift away from aquatic plant species to increasing invasions of native terrestrial grasses. The hydric site is tapped by numerous boreholes that supplement water supply to the city and also has old drainage channels from an earlier history (ca. pre-1940) when large sections of the wetland was planted to crops.

Experimental design and treatments

The experiment was a randomised block design with four treatments per block, namely two herbicides (picloram and metsulfuron methyl), manual weeding (hoeing) and an untreated control. Each site had three blocks, i.e. treatments were replicated three times at both hydric and xeric sites. Plots measured 5 x 5 m (25 m^2) with a 3 m buffer zone between plots within a block. Picloram as potassium salt (Access® 240 SL, 240 g a.i. L^{-1}, Dow AgroSciences) and metsulfuron methyl (Brush-Off®, 600 g a.i. kg^{-1}, DuPont) are registered for the control of *C. macrocephalum* in South Africa at rates of 252 g a.i. ha^{-1} and 45 g a.i. ha^{-1} respectively. These herbicides were applied at equivalent registered rates in plots. Herbicide treatments were applied as broadcast sprays when *C. macrocephalum* was fully developed (± 1 m) in February. Herbicides were applied once per annum over a course of three years. Manual weeding with a hand-held hoe was carried out twice per annum in the hoe treatments, once in the growing season (February) and again in early autumn (April). Deep hoeing was practised aimed as severing *C. macrocephalum* rootstocks about 5cm below the soil surface across the manual weeding plots. For further information on experimental design and application of herbicide treatments refer to Chapter 5. Soil samples were collected weekly up to 56 days after treatment and analysed for herbicide persistence on a Gas Chromatograph (GC) fitted with an ECD detector. Picloram and metsulfuron methyl were untraceable in all the samples (Goodall & Witkowski 2014).

Vegetation assessments

Campuloclinium macrocephalum was the only species that was counted in each plot over the course of the study. All species, including *C. macrocephalum,* were enumerated using the line intercept method (Canfield 1941) which produces paired measurements, number of intercepts (*Ni*) and intercept distance (*Ti*) per species. Assessments were carried out in late January each year prior to the application of treatments from 2005 (year one) to 2007 (year three), with a final survey in February 2008 (year four) a year after the last spray. Five permanent transects, each 5 m in length, were established 0.75 m apart in each plot. The first transect was positioned randomly in a band 50 to 75 cm in from the edge of the plot. A tape measure was used as the line transect. Plants that intercepted the tape were identified and measured. Total intercept distance (cm) per plot for each species was converted into percentage cover ($Ti\% = Ti/2500*100$). Intercept distances of graminoid species were from measurements taken at the ground level (basal cover) and measurements of forbs were from canopy intercept measurements because their basal area is generally very small.

The relation between *C. macrocephalum* counts and *Ni* was significant ($r=0.75$, $r^2=0.563$, $t=17.5$, $P<0.001$, $n=240$), hence *Ni* also serves as an indicator of relative abundance for this species. As only pompom was counted, *Ni* per species per plot (25 m) most equitably approximated individual species abundance and was used in conjunction with percentage cover for the analysis of compositional change over time. Uncontrolled factors such as a rust outbreak on *C. macrocephalum* (Goodall *et al.* 2012), rainfall variability and wildfires affected *C. macrocephalum* in different ways. For example, the rust *Puccinia eupatorii* Dietel (Pucciniaceae) on *C. macrocephalum* caused a reduction in aerial cover towards the end of summer but had very little effect on its density/abundance, while wildfires promoted regrowth. The role of these uncontrolled factors on the chemical control of *C. macrocephalum* was reported in Goodall & Witkowski (2014).

It is likely that treatments and uncontrolled factors (drought and fire) also affected abundance (*Ni*) and cover (*Ti*) of native species and growth forms differently. Both values are important in the interpretation of herbaceous layer composition. Equation 1 was used to provide a single measure of relative abundance and cover (*NT*) for each species. The *NT* value ascribes equal importance to both *Ni* and *Ti,* which may assist in reducing the effects that uncontrolled factors had on individual aspects of abundance and cover. It also brings conformity between species with basal cover (grasses) and canopy cover (forbs) measurements.

$$\text{Equation 1: } NT = \frac{\ln(Ni+1)\times\ln(Ti\%+1)}{0.3611}$$

The *NT* index has a maximum value of 100 when a species has an intercept for every cm of transect (*Ni*=2500) and 100% cover (*Ti%*=100); it does not discriminate between the number of intercepts and intercept distance, i.e. low relative abundance-high cover (e.g. 5-100) and high abundance-low cover (e.g. 100-5). The NT index was used in the analysis of monocots, dicots and growth form data. The *Ni* index was only used to illustrate changes in abundance over time of individual common species.

Species nomenclature follows Germishuizen and Meyer (2003). Species were arranged according to Pteridophyta (one fern species), monocots and dicots. Two growth forms were identified for the purposes of susceptibility to selective broadleaf herbicides, namely graminoids (grasses and sedges =monocots) and forbs (ferns, dicots and non-graminoid monocots, e.g. *Asparagaceae*, *Asphodelaceae, Commelinaceae, Hyacinthaceae* and *Hypoxidaceae*). Both growth forms were allocated additional characteristics such as origin (native or alien) and life form (annual or perennial) (Appendix A). A single alien graminoid species, *Paspalum dilatatum* Poir., occurred in low abundance at the beginning of the study at the xeric site only. Analysis of alien graminoids was thus not possible because of their rarity.

Statistical analysis

The objective was to determine if treatments were the primary cause of changes in growth forms and species composition over time using *NT, Ni* and species richness (*S*). Repeated Measures Analysis of Variance under the General Linear Model (GLM) was used for this purpose. Analyses were performed on Statistica version 12 (StatSoft Inc. 2013). Turnover in *S* was not analysed and only presented as means ± standard deviations for samples. This is because forb richness was large, especially at the xeric site, but individual species abundance was low with random distributions across the sites. Even the common forb species were not present in every plot; hence there were constraints of unequal representation across replicates and treatments.

RESULTS

Base line surveys prior to the application of treatments at both sites provided information on the most abundant species at the start of the study. At the xeric site *Cynodon dactylon* (L.) Pers., *Eragrostis curvula* (Schrad.) Nees and *Setaria sphacelata* (Schumach.) Moss var. *torta* (Stapf) Clayton were the most abundant native graminoids and *Indigofera oxytropis* Benth. ex Harv. and *Thesium utile* A.W.Hill were the most abundant native forbs. *Campuloclinium macrocephalum* was the only alien forb at the xeric site at the start. The most abundant species at the hydric site, aside from *C. macrocephalum*, were *Hyparrhenia hirta* (L.) Stapf, *Imperata cylindrica* (L.) Raeusch. and *Leersia hexandra* Sw. (graminoids); while *Diclis reptans* Benth. (native perennial) and *Verbena bonariensis* L. (alien annual) were the most commonly encountered forbs.

A total of 124 species were recorded at both study sites but only 73 species occurred in the treatment plots over the study period, namely 28 at the hydric site, 55 at the xeric site with 10 species occurring at both sites (Appendix A). Changes to the hydrology at the hydric site are the most important factors that have affected the survival of many wetland species at the hydric site.

Dicots

Treatment interactions were not significant between sites over the study period (*P* >0.1) and hence sites were pooled. This was because *C. macrocephalum* was dominant at both sites. Dicot *NT* did not change significantly in the untreated control (Fig.1A). Picloram and metsulfuron methyl caused a significant reduction in dicot *NT* ($P<0.001$). Hoeing had a superficial impact on dicot species as a functional group (NS).

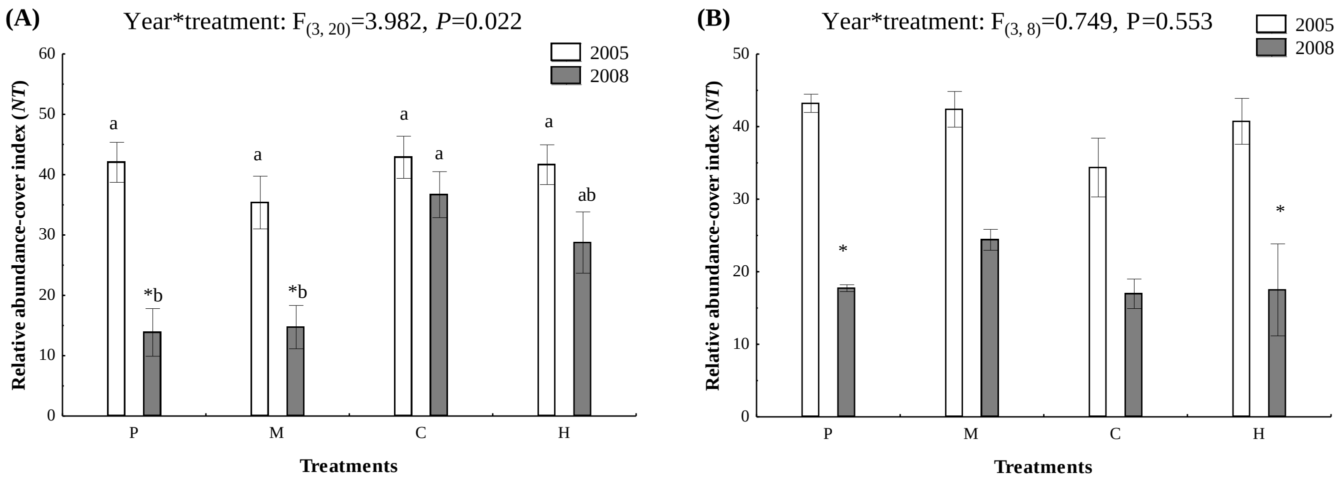

Fig. 1 The effect of treatments (n=4) applied annually (n=3) on mean *NT* values (± SE) of (**A**) dicot species, xeric and hydric sites pooled, and (**B**) monocots species at the hydric site. Treatments were picloram (P), metsulfuron methyl (M), untreated control (C) and hoeing (H). Significant differences between treatments within a time period (year) are indicated by letters (P<0.05), Tukey post hoc test for equal number of observations. Differences over time within a treatment are indicated by an asterisk (*).

Monocots

Sites were analysed separately because monocot density and cover was dissimilar ($F_{(1,16)}$=72.231, P<0.001). No significant differences in monocot *NT* occurred between and within treatments at the xeric site with all treatments recording increases by the end of the study (site means 2005=23±2, 2008=29±1). Monocot *NT* had declined in all treatments at the hydric site by the end of the study (Fig. 1B) due to man-made constraints on soil moisture as mentioned in the methods. Graminoid species made up the majority of the monocot composition; non-graminoid monocots made up 2.2% for both xeric and hydric sites respectively (Appendix A). The manipulative treatments, viz. herbicides and hoeing, had limited impact on monocot composition compared with the untreated control.

Growth forms (forbs)

Alien forbs

Alien forb *NT* was similar across sites at the start and end of the study with no significant differences between treatments within a time period (Fig. 2). Picloram was the only treatment that caused a significant reduction in the density of broadleaf weeds at the xeric site. *Campuloclinium macrocephalum* was the most dominant weed. Significant changes in *NT* from 2005 to 2008, after three consecutive years of herbicides applications, only occurred in the picloram treatment at the xeric site, but was still <80% kill rate threshold expected of registered herbicides (-76±9%). The annual weeds *Tagetes minuta* (L.) and *Bidens pilosa* (L.) appeared in the hoe treatment from 2007 and at the xeric site only. *Verbena bonariensis* (L.) was the second most encountered alien broadleaf species (annual) at the hydric site and persisted in all treatments. The number of perennial alien species remained constant (n=1) at both sites, that is *C. macrocephalum* persisted in all treatments. Annual weeds did not occur at the xeric site at the start and only the two species mentioned above were recorded at the site in 2008, and only in the hoe treatment.

Native forbs

Native forb *NT* declined in all treatments at the xeric site (Fig. 3A), although only significantly in the picloram treatment (*P*=0.03). Three annual forb species were recorded in 2005 at the xeric site in the picloram treatment only and were not observed in 2008. Species richness was also only affected significantly by picloram (Fig. 3B). Native forbs were very low in abundance and cover at the hydric site and *NT* did not differ significantly between and within treatments over the study period (means 2005=3±1, 2008=4±1).

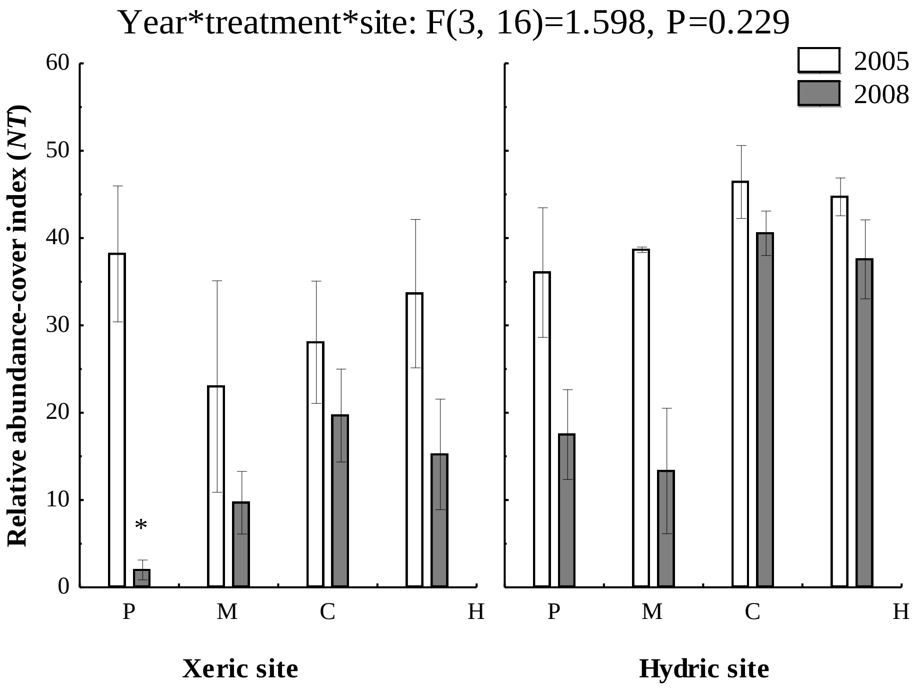

Fig. 2 The effect of treatments (n=4) applied annually (n=3) on mean *NT* values (± SE) of alien forbs species at two grassland sites (xeric and hydric). Treatments were picloram (P), metsulfuron methyl (M), untreated control (C) and hoeing (H). Significant differences between treatments within a time were not detected at either site (P<0.05), Tukey post hoc test for equal number of observations. Differences over time within a treatment are indicated by an asterisk (*).

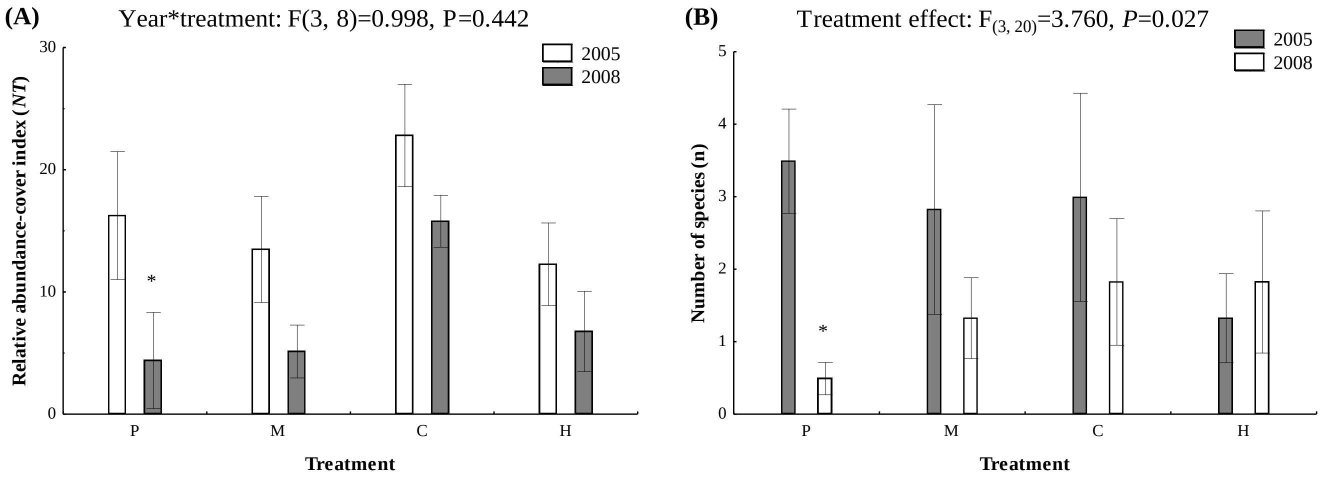

Fig. 3 The effect of treatments (n=4) applied annually (n=3) on mean values (± SE) of native forb *NT* (**A**) and (**B**) number of species at the xeric site, life forms (annual and perennial) combined. Treatments were picloram (P), metsulfuron methyl (M), untreated control (C) and hoeing (H). Significant differences between treatments within a time were not detected (P<0.05), Tukey post hoc test for equal number of observations. Differences over time within a treatment are indicated by an asterisk (*).

Changes in common broadleaf species

Alien forbs

Campuloclinium macrocephalum was suppressed by herbicides but not eliminated in plots after three annual applications (Fig. 4A xeric, Fig. 4B hydric). Hoeing had no effect on suppressing the weed. Populations in the untreated control remained relatively stable over the study period at the xeric site but declined at the hydric site. This phenomenon occurred with several species and is attributed to hydrological constraints as previously mentioned. Repeated soil disturbances from hoeing activities appeared to facilitate the establishment of the annual weeds *T. minuta* (Fig. 4C) and *B. pilosa* (Fig. 4D) at the xeric site. *Oenothera rosea* L'Hér. ex Aiton was eliminated by herbicide application at the hydric site and no new invasions of annual weeds occurred. *Verbena bonariensis* (annual) persisted in all treatments and herbicides did not control this common wetland invader over the short term (Fig. 4E).

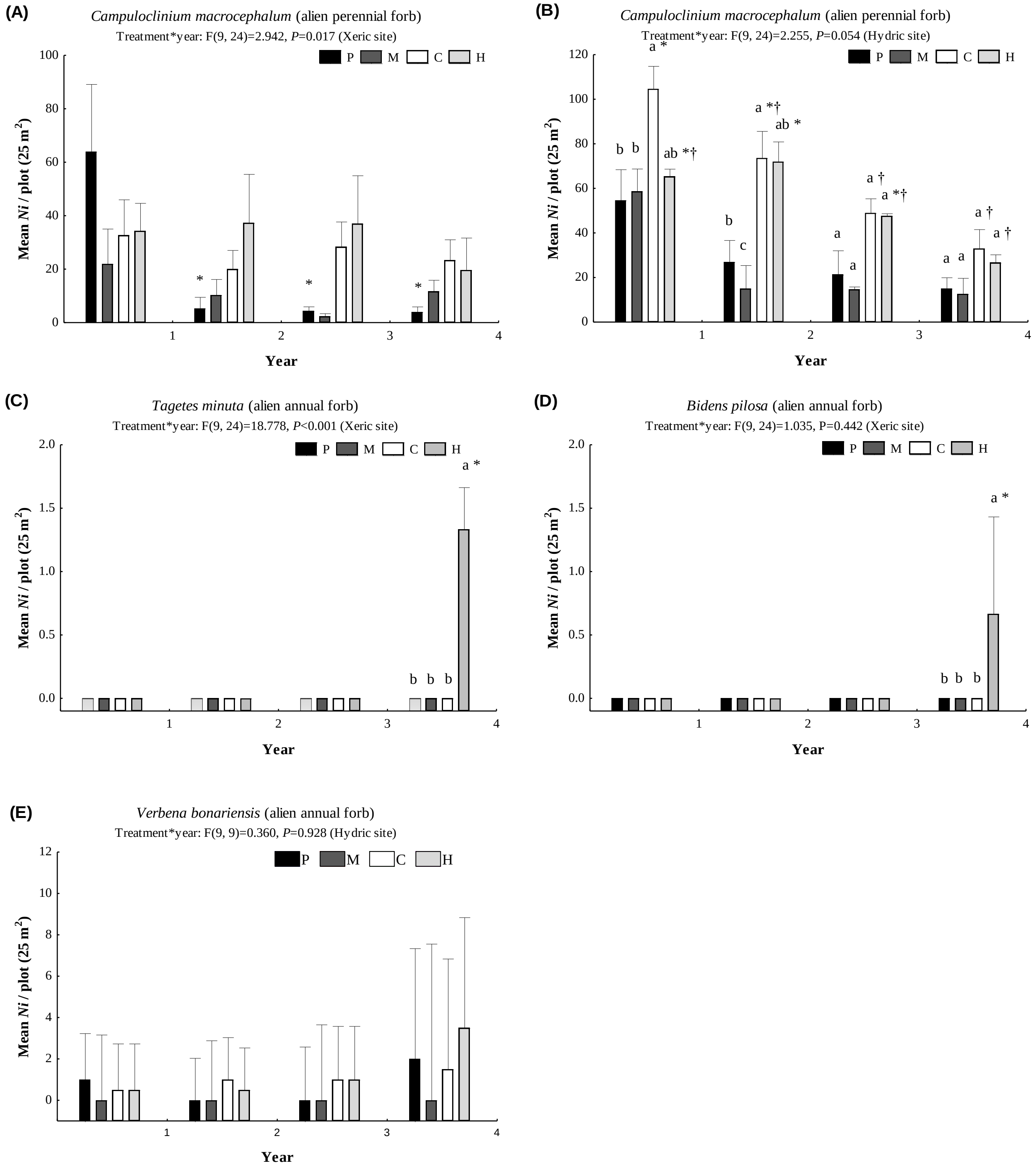

Fig. 4 The effect of treatments (n=4) applied annually (n=3) on the abundance (*Ni*) of specified alien forbs at both study sites. Treatments were picloram (P), metsulfuron methyl (M), untreated control (C) and hoeing (H). Significant differences between treatments within a time are indicated by letters (P<0.05), Tukey post hoc test for equal number of observations. Differences over time within a treatment are indicated by the symbols (*, †).

Native forbs

Native forbs displayed variable tolerance to herbicides and hoeing, but it is difficult to arrive at definitive conclusions on individual species (Fig. 5) because of large variations in abundance and distribution across treatments at both study sites. Most native forbs were unique to the xeric or hydric sites (Appendix A).

Crabbea hirsuta Harv., *Indigofera oxytropis* Benth. ex Harv. and *Ipomoea ommaneyi* Rendle were examples of species that were intolerant to either metsulfuron methyl or picloram, without being adversely affected by hoeing (Figs 5A-5C). The hemi-parasite *T. utile* remained stable in the untreated control but was eliminated in the herbicide and the hoe treatments (Fig. 5D). The hoe treatment specifically targeted *C. macrocephalum*, thus it is possible that *T. utile* could be using the weed as a host species and was killed indirectly when root haustoria were severed by the hoeing action. Populations of the wetland plant *Diclis reptans* (Fig. 5E), a small mat-forming forb, remained stable in the herbicide treatments, possibly because the taller grass canopy shielded it from direct herbicide contact. It declined dramatically in the untreated control possibly because of weed competition and soil moisture constraints.

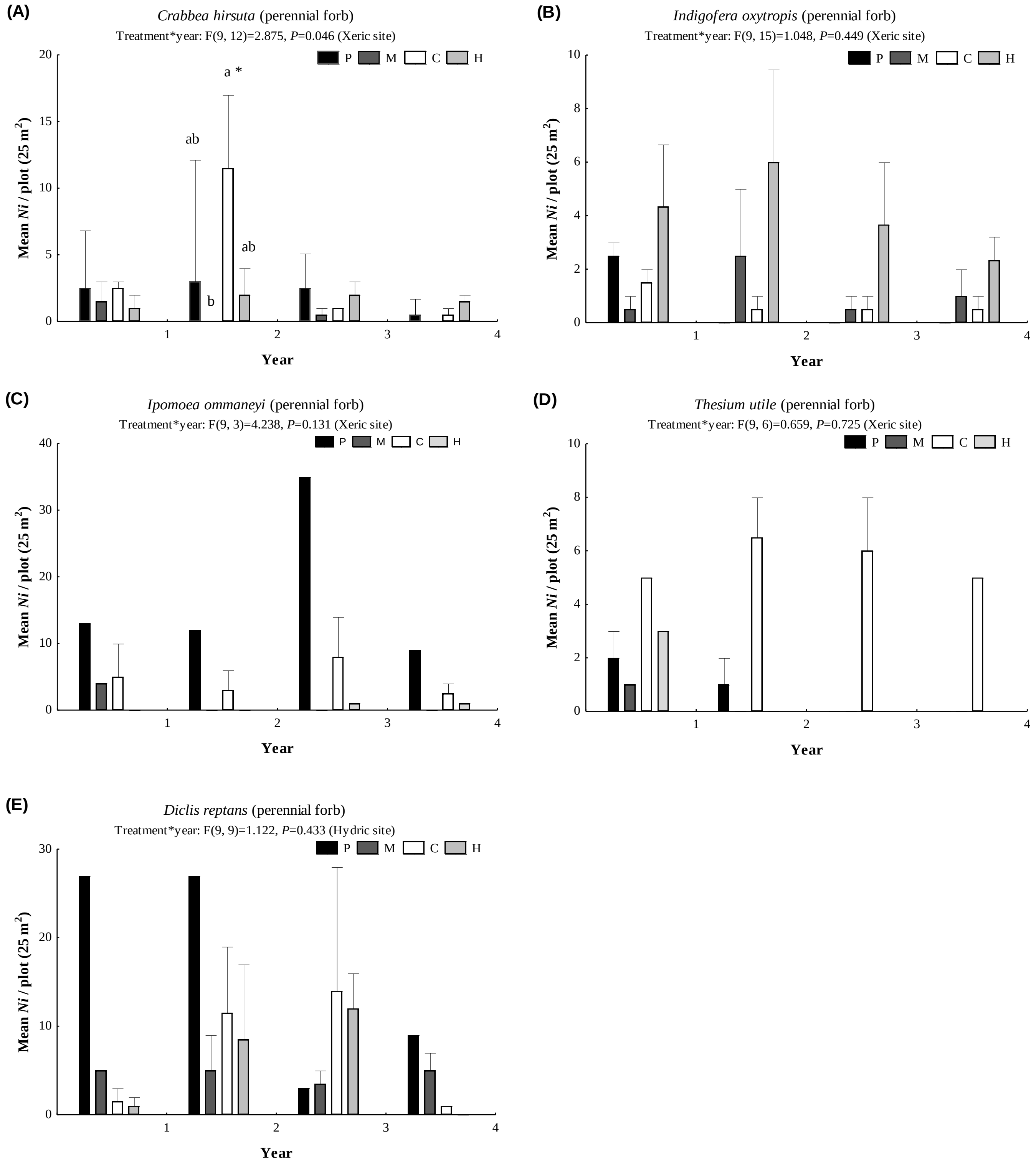

Fig. 5 The effect of treatments (n=4) applied annually (n=3) on the abundance (*Ni*) of specified native forbs at both study sites. Treatments were picloram (P), metsulfuron methyl (M), untreated control (C) and hoeing (H). Significant differences between treatments within a time are indicated by letters (P<0.05), Tukey post hoc test for equal number of observations. Differences over time within a treatment are indicated by the symbols (*, †).

Changes in common grass species

Cynodon dactylon, a stoloniforus pioneer, was unaffected by selective herbicides and remained stable in the untreated control. It was not present in the hoe treatment at the start but had increased over the course of the study (Fig. 6A) at the xeric site. *Diheteropogon amplectens* (Nees) Clayton, a tufted species with high grazing value (decreaser species), was not affected by picloram or hoe treatments in relation to the untreated control. Metsulfuron methyl appeared to have an impact on *D. amplectens* as it was eliminated at the end of the study (Fig. 6B).

Hyparrhenia hirta, a tufted species of low grazing value but an important thatching grass was present at both sites. At the xeric site *H. hirta* had increased significantly by the end of the study in all treatments and therefore unlikely to have been a release effect caused by manipulative treatments (Fig. 6C). The opposite occurred at the hydric site with declines in *H. hirta* abundance across all treatments over the study period (Fig. 6D). *Imperata cylindrica*, a common wetland grass, was essentially eliminated in all treatments (Fig. 6E). The other dominant wetland grass *L. hexandra* disappeared in all plots after the initial vegetation assessments.

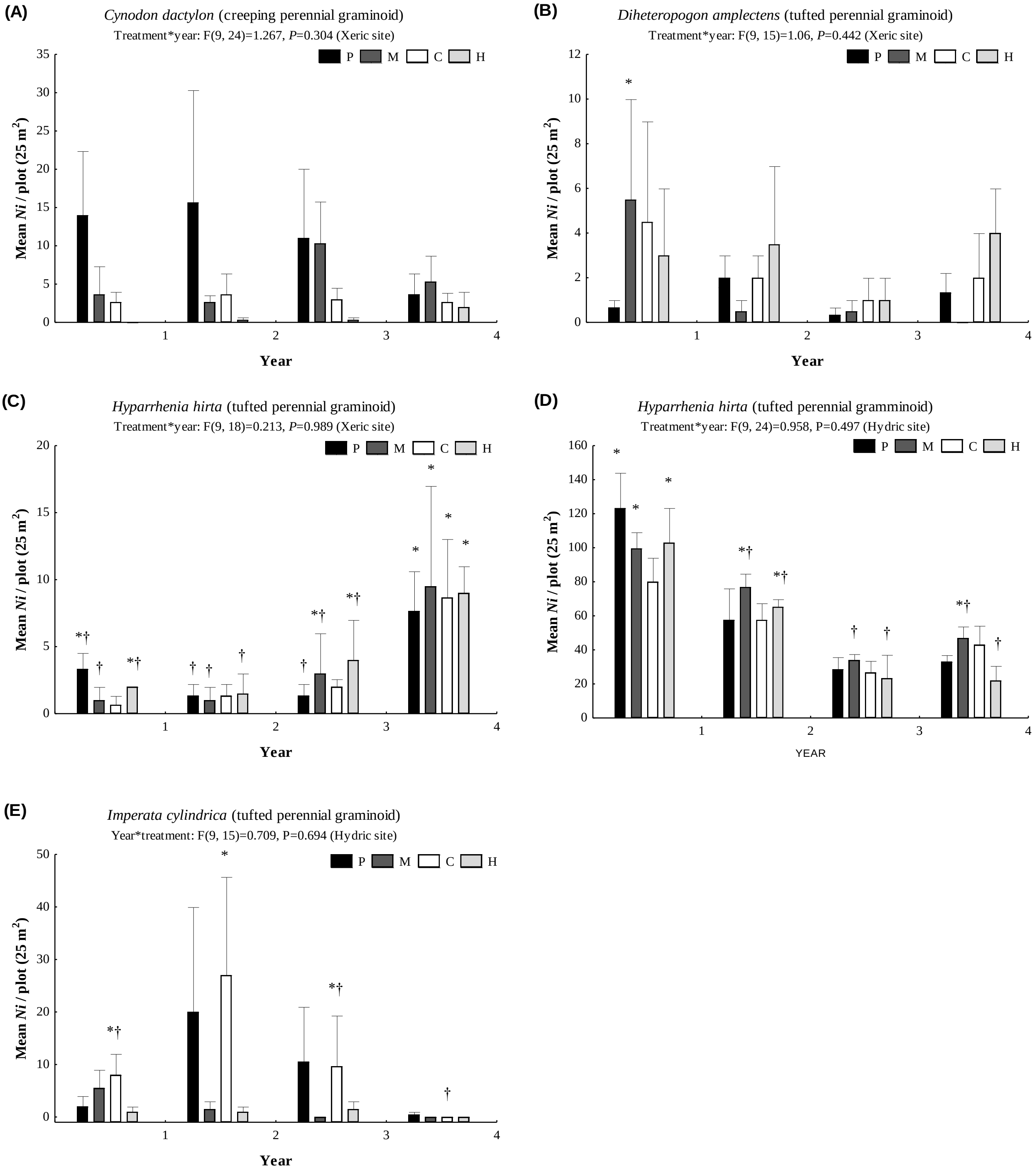

Fig. 6 The effect of treatments (n=4) applied annually (n=3) on the abundance (*Ni*) of specified native grasses at both study sites. Treatments were picloram (P), metsulfuron methyl (M), untreated control (C) and hoeing (H). Significant differences between treatments within a time were not detected (P<0.05), Tukey post hoc test for equal number of observations. Differences over time within a treatment are indicated by the symbols (*, †).

Turnover in native species richness

Species turnover accounts for the changes in native forb and graminoid richness over the study period after the base line data had been collected. Turnover variables included death (missing), ephemerality and persistence in addition to baseline and final assessment data. Forbs and graminoids are presented separately, life history attributes were pooled. The xeric site had more forbs and graminoid species per treatment than the hydric site (Table 1, Appendix A). Data are presented as means ± standard deviations for samples. No additional analyses were carried out because of small means and large variances in all growth forms across all treatments. Absolute variables such as percentage survival are not likely to be significantly different between treatments in each growth form category at each site. All that can be deduced are trends based on average scores.

Forbs

Forb turnover was very high across all treatments at both sites (Table 1). This may be a natural phenomenon as not much is known about the community ecology of grassland forbs other than taxonomic characteristics. Hoeing appeared to have the least impact on the survival of forbs present at the start, followed by the untreated control. Herbicides had the greatest impact on forb diversity at the xeric site with picloram accounting for the lowest survival rates at both sites.

Graminoids

Survival of graminoids species at the xeric site was high but highly variable at the hydric due to incursions of terrestrial grasses replacing aquatic grasses. Picloram and hoeing favored grass survival at the hydric site with low survival rates in the metsulfuron methyl and control treatments. The hydric site has shifted from aquatic vegetation to greater representation of terrestrial species and for this reason treatment effects on species turnover are probably less causative than man-made constraints imposed at this site.

Table 1 Turnover in native species richness (*S*) in two growth forms, forbs and graminoids (Gram.) across the four treatments (P, M, C, H) in dense *Campuloclinium macrocephalum* infestations at the xeric and hydric study sites respectively. The treatments were picloram (P), metsulfuron methyl (M), untreated control (C) and hoeing (H). Both P and M were broadcast sprays applied annually for three consecutive years, while hoeing of *C. macrocephalum* was carried out twice a year also for three consecutive years. Turnover variables are number of species: at the start and end of the study; ephemeral species (Eph.) which only occurred between start and end dates and new species that were found only at the end of the study; species that were only present at the start (Died) and species which persisted throughout the study (Pers.). Data are presented as means ± standard deviations for samples.

Treat.	GF	Start	End	Eph.	Died	Pers.	% survival
XERIC SITE							
P	Forbs	4.0±2.6	1.0±0.0	3.0±1.4	3.3±2.5	0.7±0.6	26.6±36.6
M	Forbs	5.7±4.0	2.7±1.5	3.7±1.3	4.7±3.1	1.0±1.0	31.6±50.3
C	Forbs	6.0±2.6	3.7±2.5	4.3±1.7	3.7±2.1	2.3±1.2	46.0±34.2
H	Forbs	2.7±0.6	3.7±2.1	4.3±1.3	1.0±0.0	1.7±0.6	65.3±28.5
P	Gram.	5.0±1.0	6.0±1.0	4.3±0.8	1.7±0.6	3.3±0.6	68.8±18.9
M	Gram.	4.3±2.5	5.0±1.7	2.7±1.5	1.7±2.1	2.7±0.6	86.3±59.8
C	Gram.	6.3±1.5	6.7±1.2	4.0±1.7	1.3±1.2	5.0±1.0	82.8±26.3
H	Gram.	4.3±2.1	6.3±2.1	4.7±1.6	1.3±1.5	3.0±1.0	86.4±56.0
HYDRIC SITE							
P	Forbs	1.7±2.1	1.0±1.0	0.7±0.5	1.0±0.7	0.7±0.6	-42.6±139.5
M	Forbs	1.0±1.0	1.3±1.2	1.7±0.8	0.3±0.6	0.7±0.6	27.3±36.0
C	Forbs	1.7±2.9	2.0±1.0	1.3±0.8	1.0±1.7	0.7±1.2	-7.6±83.7
H	Forbs	0.3±0.6	1.3±1.5	2.0±1.3	0.3±0.6	0.0±0.0	0.0±0.0
P	Gram.	1.3±0.6	1.7±1.2	1.0±0.8	0.3±0.6	1.0±0.0	90.6±43.0
M	Gram.	2.0±0.0	1.0±0.0	0.3±0.4	1.0±0.0	1.0±0.0	50.0±0.0
C	Gram.	2.7±1.2	1.0±0.0	0.3±0.4	1.7±2	1.0±0.0	45.0±21.6
H	Gram.	1.3±0.6	1.3±0.6	0.7±0.5	0.3±0.6	1.0±0.0	90.6±43.0

DISCUSSION

The registered herbicides picloram 240 g a.i. L^{-1} and metsulfuron methyl 600 g a.i. kg^{-1} were unsuccessful at eliminating *C. macrocephalum* on a small scale (25 m^2 plots) after three annual broadcast applications. Weed density increased after cessation of treatments through seedling recruitment and it was estimated that at least a 7-year follow-up plan would be required to bring the weed under complete control at the plot level (Goodall & Witkowski 2014). From a purely economic perspective this effectively makes chemical control unfeasible for most enterprises where invaded grasslands are still utilised for animal production.

The impacts of herbicides on individual forb species in communities invaded by *C. macrocephalum* was largely inconclusive because most of these species were underrepresented and an analysis of treatment effects versus random effects could not be ascertained. Nevertheless, there was evidence to suggest the herbicides had the greatest impact on collective forb abundance (Fig. 3A) and species richness (Fig. 3B) compared to the untreated control. The primary challenge in this study in dealing with forb diversity was their largely low levels of abundance and patchy distributions (low redundancy, high noise). This has been a common problem encountered by researchers in other parts of the world assessing the impacts of chemical control of alien species on native species in rangelands (Fuhlendorf *et al.* 2002).

The ecological impact of repeated long-term herbicide applications on susceptible non-target species, native forbs specifically, is likely to be profound. Crone *et al.* (2009) focusing on the common forb *Balsamorhiza sagittata* (Pursh) in Montana found that a single application of picloram as potassium salt (24.4%) applied at a dosage of 1.24 L/ha^{-1} at intervals >5 years would be beneficial for this species, but more frequent applications (<5 years) would be detrimental. This is clearly not a practical solution that will win favour amongst landusers that rely on rangelands for animal production or national programmes that have a mandate to control declared noxious weeds (e.g. USDA's APHIS Federal Noxious Weed Program, the Australian Weeds Strategy and South Africa's Working for Water Programme).

Repeated herbicide applications in grasslands have provided inconsistent efficacy when used exclusively without intergradation with other methods or practises and provide little assistance in ecosystem restoration (Sheley & Denny 2006). Grass production responds positively to the herbicidal suppression of alien broadleaf weeds (Thilenius *et al.* 1975; Rice *et al.* 1997; Rice & Toney 1998, Koger *et al.* 2002; Sheley & Denny 2006). Increased stocking rates or improved livestock production, however, are not a guaranteed outcome of improved grass production through herbicide release (Fuhlendorf *et al.* 2009; Rinella *et al.* 2009). Stocking rates are reliant on sward palatability, soil fertility and bioclimatic influence (Fynn & O'Connor 2001; Fensham *et al.* 2005). As a general rule, heavily invaded rangelands do not revert back to quality pasturage through chemical control alone (Papanastasis 2009), a misconception of why farmers apply herbicides in weedy grasslands.

In the study testing selective herbicides and hand-removal to control *Potentilla recta* L., grass cover and biomass increased and native forb density decreased with herbicide application, but species richness and diversity were not adversely affected (Sheley & Denny 2006). In our study we detected declines in general forb abundance and richness (Figs. 1 and 5) but did not detect a general graminoid release-effect from the chemical control of *C. macrocephalum* (Figs. 2 and 7). Hoeing twice a year was labour intensive, completely ineffective as a control measure against *C. macrocephalum* and facilitated the establishment of annual broadleaf weeds (*B. pilosa* and *T. minuta*) and native pioneer grasses (*C. dactylon*). Hoeing also did not favour the common perennial forb *T. utile* (Santalaceae). All species in the genus *Thesium* are root hemi-parasites of grass species primarily, but haustorial connections have also been found attached to members of the Asteraceae and several other dicot families (Suetsugu *et al.* 2008). In their study haustoria were examined in sod samples excavated to a depth of 20 cm, which is quite shallow and falls within the depth zone affected by hoeing *C. macrocephalum*, which may have severed haustorial connections to other host species in the plots that received hand-weeding. *Thesium utile* may also have formed a new-association with *C. macrocephalum* making any form of control unfavourable for the native species. *Thesium utile* remained unchanged in the control plots but was killed outright by herbicide treatments, probably through direct contact with the spray.

Selective herbicides clearly have a highly variable effect on a diverse array of native forb species across the globe. Differences in herbicide-tolerance thresholds are clearly evident from past studies and this was also observed in our study (Fig. 5). Life form may favour some species over others, e.g. therophytes (short term effect) versus perennial species; for example, the role of soil seed reserves in buffering the effects of herbicide-induced mortality of mature plants. Species turnover and survival remains unknown for most forb species under different environmental and management conditions. Grassy biomes throughout the world have diverse non-graminoid species compositions coupled with relatively high levels of endemism (Cowling *et al*. 1989; Ratter *et al.* 1997; Myers *et al*. 2000; Bond & Parr 2010) making the task of gleaning knowledge on autecology and community ecology a near impossible undertaking.

These conflicting and confounding findings are quite alarming because herbicides are clearly a very valuable tool, and most often the only method for the control of unwanted alien species worldwide. Classical weed biological control is obviously the most cost-effective and environmentally-friendly method of suppressing declared weeds on a large scale if it is able to reduce naturalised populations to levels where they have minimal impacts in habitats they invade. Weed biocontrol is extremely target-specific and testing for host-specificity takes several years before enough data guaranteeing each agent’s specific host requirement is sufficient before release permits are issued. In South Africa the time constraints to complete these biosecurity measures unfortunately is out of sync with the already extensive noxious weeds listed in the 2014 Alien and Invasive Species Regulations of the National Environmental Management: Biodiversity Act, 2004 (Act No. 10 of 2004). In addition to extensive promulgated weed lists there are issues relating to the management of new weed introductions, casual alien invaders where repeated introductions are a requirement for persistence over a < 10 year period (Henderson 2007), and sleeper weeds which display a lag phase of several years/decades before becoming invasive (Grice & Ainsworth 2003). *Campuloclinium macrocephalum* was a sleeper weed for about 30 years before it suddenly became highly invasive (Goodall *et al.* 2011, Henderson L *pers. comm.* 22 Dec 2015).

In light of these findings careful consideration needs to be given to the best management practises for the integrated management of *C. macrocephalum* in the grassland biome of South Africa. Herbaceous alien species generally invade disturbed grasslands (Keely 2003; Goodall *et al.* 2011) and the benefits of chemical control are unlikely to result in increased sward palatability. This is because palatable grasses may have been reduced through sustained grazing pressure, many of which have low seed production potential and poorly adapted long-distance dispersal mechanisms (O’Connor 1991), thereby facilitating the invasion of alien species and increased competition from less palatable grass species and native forbs. The main purpose for applying herbicides in natural grasslands should be to reduce invasive species and curb their spread rather than as a remedy to fix poor pasture management. Knowledge of selective herbicides and their impacts on native broadleaf plants in grasslands is thus very important for conserving and restoring grassland integrity when invasion of alien species and mismanagement are significant threats. Grass production from year to year has a strong association with rainfall, more so than with grazing (Fynn &

O’Connor 2001; Fensham *et al.* 2005) and release from competing broadleaf species through the application of selective herbicides (Fuhlendorf *et al.* 2009). Additional methods such as restoration/rehabilitation (Kettenring & Adams 2011) and sound rangeland management practises are essential to facilitate the process towards improving pasture condition.

There is a great need for a handbook on the integrated management of invasive alien plants in the grassland biome of South Africa (e.g. Brown & Brooks 2002; Cal-IPC 2015); however, many knowledge gaps exist that currently prevent the development of a holistic approach. Quantifying the effects of selective herbicide on native forb composition in rangelands is always going to be challenging. More research is required on the integrated management of *C. macrocephalum* to improve weed suppression and to reduce non-target impacts in the grassland biome of South Africa. Exciting opportunities are now possible for researching the holistic management of C. *macrocephalum* with the recent release of a new insect agent (McConnachie & McKay 2015) in addition to the existing rust *P. eupatorii* (McConnachie *et al.* 2011), the role of fire to reduce the weed seedbank and promote early regrowth (Goodall & Witkowski 2014), timing and target-specific or selective application techniques using selective broadleaf herbicides to minimise non-target effects (Boerboom & Wyse 1988) and habitat restoration by reintroducing key native species (Kettenring & Adams 2011).

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APPENDIX A: LIST OF SPECIES RECORDED IN PLOTS AT THE XERIC AND HYDRIC STUDY SITES ARRANGED ACCORDING TO NATURAL ASSOCIATION AND FAMILIES

Taxa	**Growth form**			**Life span**		**Site**	
	Non-graminoid monocots	Forbs, ferns	Graminoid	Annual/Biennial	Perennial	Hydric	Xeric
PTERIDOPHYTA							
Pteridaceae							
Cheilanthes involuta var obscura		✓			✓		✓
DICOTYLEDONS							
Acanthaceae							
Crabbea angustifolia Nees		✓			✓		✓
Crabbea hirsuta Harv.		✓			✓		✓
Apiaceae							
*Hydrocotyle americana** L.		✓			✓	✓	
Apocynaceae							
Asclepias L. sp.		✓		✓		✓	
Xysmalobium undulatum (L.) Aiton		✓			✓	✓	
Asteraceae							
*Bidens pilosa** L.		✓		✓			✓
*Campuloclinium macrocephalum** (Less.) DC.		✓			✓	✓	✓
Conyza podocephala DC.		✓			✓	✓	✓
Helichrysum nudifolium (L.) Less. var. *nudifolium*		✓			✓		✓
Helichrysum rugulosum Less.		✓			✓	✓	✓
Senecio erubescens Aiton var. *crepidifolius* DC.		✓			✓	✓	
Sonchus dregeanus DC.		✓			✓	✓	✓

Taxa	**Growth form**			**Life span**		**Site**	
	Non-graminoid monocots	Forbs, ferns	Graminoid	Annual/Biennial	Perennial	Hydric	Xeric
*Tagetes minuta** L.		✓		✓			✓
Vernonia oligocephala (DC.) Sch.Bip. ex Walp.		✓			✓		✓
Boraginaceae							
Cynoglossum hispidum Thunb.		✓		✓		✓	
Caryophyllaceae							
Pollichia campestris Aiton		✓			✓		✓
Chrysobalanaceae							
Parinari capensis Harv. subsp. *capensis*		✓			✓		✓
Convolvulaceae							
Ipomoea ommaneyi Rendle		✓			✓		✓
Crassulaceae							
Crassula capitella Thunb. subsp *nodulosa* (Schönland) Tölken		✓		✓			✓
Fabaceae							
Chamaecrista comosa E.Mey. Var. *capricornia*		✓			✓		✓
Eriosema cordatum E.Mey.		✓			✓		✓
Indigofera oxytropis Benth. ex Harv.		✓			✓		✓
Rhynchosia totta (Thunb.) DC. var. *totta*		✓			✓		✓
Lamiaceae							
Becium obovatum (E.Mey. ex Benth.) N.E.Br. var. *obovatum*		✓			✓		✓
Malvaceae							
Hibiscus microcarpus Garcke		✓		✓			✓
Hibiscus trionum L.		✓		✓		✓	
Onagraceae							

Taxa	**Growth form**			**Life span**		**Site**	
	Non-graminoid monocots	Forbs, ferns	Graminoid	Annual/Biennial	Perennial	Hydric	Xeric
*Oenothera rosea** L’Hér. ex Aiton		✓			✓	✓	
Polygonaceae							
Oxygonum dregeanum Meisn. subsp. *canescens* (Sond.) Germish.		✓		✓			✓
Rumex sagittatus Thunb.		✓			✓	✓	
Rosaceae							
Agrimonia procera Wallr.		✓			✓	✓	
Rubiaceae							
Galium capense Thunb. subsp. *garipense* (Sond.) Puff		✓			✓	✓	
Pygmaeothamnus zeyheri (Sond.) Robyns var. *zeyheri*		✓			✓		✓
Santalaceae							
Thesium ultile A.W.Hill		✓			✓		✓
Scrophulariaceae							
Diclis reptans Benth.		✓			✓	✓	
Hebenstretia comosa Hochst.		✓			✓		✓
Solanaceae							
Solanum panduriforme E.Mey.		✓			✓		✓
Withania somnifera (L.) Dunal		✓			✓		✓
Sterculiaceae							
Hermannia cordata (E.Mey. ex E.Phillips) De Winter		✓		✓			✓
Verbenaceae							
Lantana rugosa Thunb.		✓			✓	✓	✓
*Verbena bonariensis** L.		✓		✓		✓	

Taxa	**Growth form**			**Life span**		**Site**	
	Non-graminoid monocots	Forbs, ferns	Graminoid	Annual/Biennial	Perennial	Hydric	Xeric
MONOCOTYLEDONS							
Asparagaceae							
Asparagus laricinus Burch.		✓			✓	✓	
Asphodelaceae							
Aloe zebrina Baker		✓			✓		✓
Commelinaceae							
Commelina africana var. *africana* C.B.Clarke		✓			✓	✓	
Cyperaceae							
Cyperus esculentus L. var. *esculentus*			✓		✓	✓	✓
Hyacinthaceae							
Ledebouria ovatifolia (Baker) Jessop	✓				✓		✓
Ledebouria revoluta (L.f.) Jessop	✓				✓		✓
Hypoxidaceae							
Hypoxis rigidula Baker var. *rigidula*	✓				✓		✓
Poaceae							
Aristida congesta Roem. & Schult.			✓		✓	✓	✓
Brachiaria serrata (Thunb.) Stapf			✓		✓		✓
Cymbopogon nardus (L.) Rendle			✓		✓		✓
Cynodon dactylon (L.) Pers.			✓		✓		✓
Digitaria eriantha Steud.			✓		✓		✓
Diheteropogon amplectens (Nees) Clayton			✓		✓		✓
Elionurus muticus (Spreng.) Kuntze			✓		✓		✓
Eragrostis capensis (Schrad.) Nees			✓		✓		✓
Eragrostis curvula (Schrad.) Nees			✓		✓		✓
Eragrostis gummiflua Nees			✓		✓		✓
Heteropogon contortus (L.) Roem. & Schult.			✓		✓		✓

Taxa	**Growth form**			**Life span**		**Site**	
	Non-graminoid monocots	Forbs, ferns	Graminoid	Annual/Biennial	Perennial	Hydric	Xeric
Hyparrhenia hirta (L.) Stapf			✓		✓	✓	✓
Imperata cylindrica (L.) Raeusch.			✓		✓	✓	
Ischaemum fasciculatum Brongn.			✓		✓	✓	
Leersia hexandra Sw.			✓		✓	✓	
Melinis nerviglumis (Franch.) Zizka			✓		✓		✓
Melinis repens (Willd.) Zizka			✓	✓		✓	✓
Panicum natalense Hochst.			✓		✓		✓
*Paspalum dilatatum** Poir.			✓		✓		
Pogonarthria squarrosa (Roem. & Schult.) Pilg.			✓		✓		✓
Schizachyrium sanguineum (Retz.) Alston			✓		✓		✓
Setaria sphacelata (Schumach.) Moss var. *torta* (Stapf) Clayton			✓		✓	✓	✓
Themeda triandra Forssk.			✓		✓	✓	✓
Trachypogon spicatus (L.f.) Kuntze			✓		✓		✓
Urochloa brachyura (Hack.) Stapf			✓	✓			✓
* = alien							
Total	3	44	26	12	61	28	55

7. CONCLUSION

SYNTHESIS

Campuloclinium macrocephalum is a long-lived herbaceous perennial forb with a well-developed tuberous root system and a semi-woody root collar with an abundance of perennating buds that produce annual stems. The association with a diverse collection of natural enemies in its native range (McConnachie *et al.* 2011) and the ability to withstand the devastating effects of *Puccinia eupatorii* in South Africa (Goodall *et al.* 2012) suggests that the morphological characteristics of the plant may be evolutionary adaptions to herbivory, predation and disease. This is clearly not a pioneer species but one which has evolved a niche in grasslands maintained at disclimax states by a range of agencies of disturbance. Its success as a grassland invader include the ability to survive under intensive fire regimes, annual stems that grow rapidly after fire, produce copious numbers of wind-borne seeds within three months of emerging from the soil, long-distance seed dispersal and successful local recruitment in invaded areas.

In South Africa human activities are the greatest cause of disturbances that facilitate invasion and establishment (Goodall *et al.* 2011), e.g. mowing, fire and heavy grazing, leading to low grass basal cover and soil erosion. The weed also appears to be constrained by rainfall and semi-arid grasslands which receive <600 mm per annum may be considered low risk areas.

An absence of host-specific natural enemies prior to 2006 is believed to be the primary reason why *C. macrocephalum* became a weed in South Africa. The Absence of Predators Hypothesis (Huffaker *et al.* 1976) and Enemy Release Hypothesis (ERH) (Keane & Crawley 2002) are still upheld based on the vast difference in colony densities in its native range (Williams 1976; Cabrera 1978; Breedlove 1986) compared to its introduced range in South Africa (Henderson 2007). Some members of the closely related *Aster* genus are morphologically (Hilliard 1977; van Wyk 1997) very similar to *C. macrocephalum* (subfamily Asteroideae) in both above ground (densely hairy leaves and on short lived / annual stems) and underground structures (perennial rootstock, tuberous fusiform roots). The native grassland forb *Aster bakerianus* (Burtt Davy ex) is a widespread species throughout the grassland biome but is seldom found in great abundance locally, except in areas of open grassland where cover has been removed or reduced, e.g. frequent fires and/or grazing (Hilliard 1977), as is the case with *C. macrocephalum* (Goodall *et al.* 2011). *Aster* *bakerianus* is morphologically similar to *C. macrocephalum* and both species appear to share an equivalent niche in their respective native ranges, perhaps due to associations with specialist organisms that regulate their abundance. Several members of the *Aster* genus have become weeds in introduced regions; e.g. *A. subulatus* Michx. (from North America) in Australia (Downey *et al.* 2010), former *A. ericoides* L. and *A. novae-angliae* L. (North America) in Canada (Chmielewski & Semple 2003).

The Novel Weapons Hypothesis (NWH) postulates that some plants are allelopathic and their allelochemicals may be detrimental to other plant species growing in close proximity (Bais *et al.* 2003; Callaway & Ridenour 2004). In native countries the "neighbourhood" allelopathic effect may be dampened through evolutionary adaptation. On the other hand, if the species in question is invasive, in introduced regions there could be neighbourhood effects on overall community composition and structure with weed density expansion.

Manipulative laboratory experimentation and field assessments indicate that the NWH does not apply to *C. macrocephalum* (Goodall *et al.* 2010). Varying concentrations (1, 10 and 25% w/v) of root and shoot extracts of on seeds of *Lactuca sativa* L., *Eragrostis curvula* (Schrad.) Nees and *E. tef* (Zucc.) Trotter failed to yield allelopathic effects on seed germination under controlled conditions in growth chambers. Stunting of *E. curvula* radicles from leaf extracts was the most pronounced indication of allelopathy. When *C. macrocephalum* and *E. curvula* were grown together in pots there was no evidence of allelopathy or competitive exclusion in either species based on biomass production. Field assessments confirmed coexistence between the two species lending further weight to alternative casual reasons for invasion, the most obvious being ERH. Both species prevail under disturbed conditions although pompom has a much broader niche evident by infestations in a wide range of soil types and moisture gradients, whereas *E. curvula* was restricted to well drained soils. Coexistence would only be possible if these species were not allelopathic, have different resources requirements and/or harness resources from different regions/sources in the soil (Tilman 1985).

The gradient analysis of invaded grasslands (Goodall *et al.* 2011) established that *C. macrocephalum* was an effective weed in areas with poor veld condition and low basal. It was not possible identify the cause of declining biodiversity; i.e. poor veld management, pompom weed or soil disturbance/erosion. Selectively grazed and moribund (under-utilized / neglected) grasslands seem more susceptible to invasion than grasslands dominated by increaser species adapted to sustained non-selective defoliation (Dyksterhuis 1949). None of the 80 invaded study sites were in pristine condition and low abundance of decreaser species and low basal cover could be used as indicators of high susceptibility to invasion. Grassland mismanagement is of grandiose proportions in South Africa with <2% of the grassland biome in protected areas (Reyers & Tosh 2003), hence the potential for further spread of *C. macrocephalum* is many times greater than its current distribution (Trethowan *et al.* 2011).

The invasion of *C. macrocephalum* and terrestrial plants in seasonally inundated wetlands (Goodall *et al.* 2011, 2014, Chapter 6) may be due to activities that affect hydrology (van Loon 2009), e.g. boreholes and drainage canals, for harvesting groundwater or modifying waterlogged land for agricultural purposes (e.g. cropping, grazing). The weed does not invade inundated or seasonally inundated wetlands. Engineering practices that raise groundwater levels may be a simple and effective method to reverse the invasion of terrestrial species in semi-aquatic habitats and facilitate the natural succession of hygrophilous vegetation in invaded wetlands (Sorrel *et al.* 2007). Monitoring in restored wetlands is essential to detect and implement remedial actions to prevent the invasion of undesirable aquatic weed species in future (van Bodegom *et al.* 2009).

The *P. eupatorii* isolate from Argentina held in quarantine was, by way of sequencing, found to be the same species as the rust infecting *C. macrocephalum* populations throughout its distribution in South Africa (Retief *et al.* 2016). *Puccinia eupatorii* may yet prove to be a very valuable and destructive biocontrol agent in time. It is too early to speculate its future impact on pompom weed biocontrol at this time (McConnachie *et al.* 2011).

The control of *C. macrocephalum* using registered herbicides is unlikely to bring the weed under control irrespective of the scale of inference (Goodall & Witkowski 2014). On the one hand there is the expectation that registered herbicides will achieve an average mortality rate of 80% with adherence to the instructions of the label concerning mixing (concentration and carrier), application method (correct equipment, dose and rate of application per unit area, precision), target plant quality (healthy, not stressed) and suitable environmental conditions for herbicide absorption and translocation (temperature <30°C, adequate soil moisture for healthy growth, wind-free, plants not dusty, rain-free for several hours after application). Meeting these requirements is essential for effective control for plants that received herbicide. The remaining 20% relates to plants that were not affected because they were missed (operator error, fatigue) and uncontrolled factors such as gusts of wind (disperses spray pattern in foliar applications) and rapid changes in atmospheric conditions resulting in untimely rainfall (incomplete absorption due to herbicide runoff). What is not mentioned on any herbicide label is the fact that what is seen above ground is usually a very small faction of the problem that will require years of annual follow-up application to bring the problem species under adequate control in the target area (5% cover) (Goodall & Naudé 1998). These factors include the residual seedbank in the soil, seed rain and hardy perennial species which require more than one application to kill.

In the case of *C. macrocephalum* the size of the weed seedbank and seed rain in areas targeted for herbicide control on the highveld is massive and out of proportion with the affordability of committing to long-term follow-ups in each treatment area while at the same time trying to expand the area under herbicide management annually (Goodall & Witkowski 2014; Chapter 6). In addition, the widespread biocontrol agent *Puccinia eupatorii* affects plant quality and condition from as early as February each year (Goodall *et al.* 2012), which reduces the window of opportunity for applying herbicides; November to April prior to the appearance of the rust in 2006 and post-rust December to February at the latest (Goodall *et al.* 2014). On the highveld it is not uncommon to have below average rainfall in spring and early summer in these years this window may only remain open for one to two months before the rust affects plant health. The long-term chemical control of pompom weed in grasslands will also have profound negative impact on native species diversity and composition, with the costs and detriments far exceeding the benefits from the reductions in pompom densities (Chapter 6).

A recommendation is that controlled burning in August and herbicide application in early November, before many forbs have emerged, will neutralize weed seeds on the soil surface, flush regrowth, help reduce non-target damage and provide an opportunity for a second treatment before the plants become too damaged by the rust. It is now clear that research into the integrated control of pompom weed is a top priority in the grassland biome, especially since this biome has become a hot topic for urgent conservation internationally (Reyers & Tosh 2003; Bond & Parr 2010; Veldman *et al.* 2015; Bond 2016).

RECOMMENDATIONS

Research

The competiveness of *C. macrocephalum* as a causal agent for declining biodiversity and carrying capacity, and for determining key species for ecosystem restoration is a research priority. Competition/interference studies are complex involving contrasts under controlled conditions using manipulative experimentation with additive factorial designs with and without replacement (Zimdahl 2004, Didham *et al.* 2005; MacDougall & Turkington 2005;

Froud-Williams 2008) to separate above from below ground competition and explain the interactions of above ground structures on below-ground organs and *vice versa*. The studies become even more elaborate in multi-species designs, e.g. pompom weed × native grass × native perennial forb. Lessons learned then have to be applied under field conditions where the weed is not currently present and would involve long-term studies (repeated measures) before one is able to reject the hypothesis that *C. macrocephalum* introduced (transplanted seedlings/sown seed) at varying intensities is not the cause for declining biodiversity in invaded grasslands.

The plight of the grassland biome is receiving international attention by eminent scientists across the globe. Now is the right time for a new research initiative towards producing a comprehensive handbook on the integrated management of invasive alien plants in the grassland biome of South Africa, including restoration and best management practices. This research initiative is multi-disciplinary and therefore requires inter-institution collaboration from National Government (Department of Environmental Affairs and the affiliated "Working for/on" programmes, e.g. ecosystems, fire, land, water, wetlands), Provincial Governments in the biome, universities (Free State, Pretoria and Witwatersrand) and science organisations (Agricultural Research Council, Centre for Invasion Biology, South African National Biodiversity Institute (SANBI)). Many invasive alien plant species are problematic within the grassland biome (Henderson 2007) but *C. macrocephalum* is one which shows great potential for expansion. Although comprehensive literature regarding *C. macrocephalum* has recently been published (Goodall *et al.* 2010, 2011; Farco & Dematteis 2012, Goodall *et al.* 2012, McConnachie *et al.* 2011; Gitonga *et al.* 2014; Goodall & Witkowski 2014, McConnachie & McKay 2016; Retief *et al.* 2016) that would be invaluable to the handbook on integrated weed management and best practises, gaps in our understanding of its biology, ecology and control still exist that prevent the development of best management practices. These key areas for research make ideal projects for post-graduate students (Fig. 1).

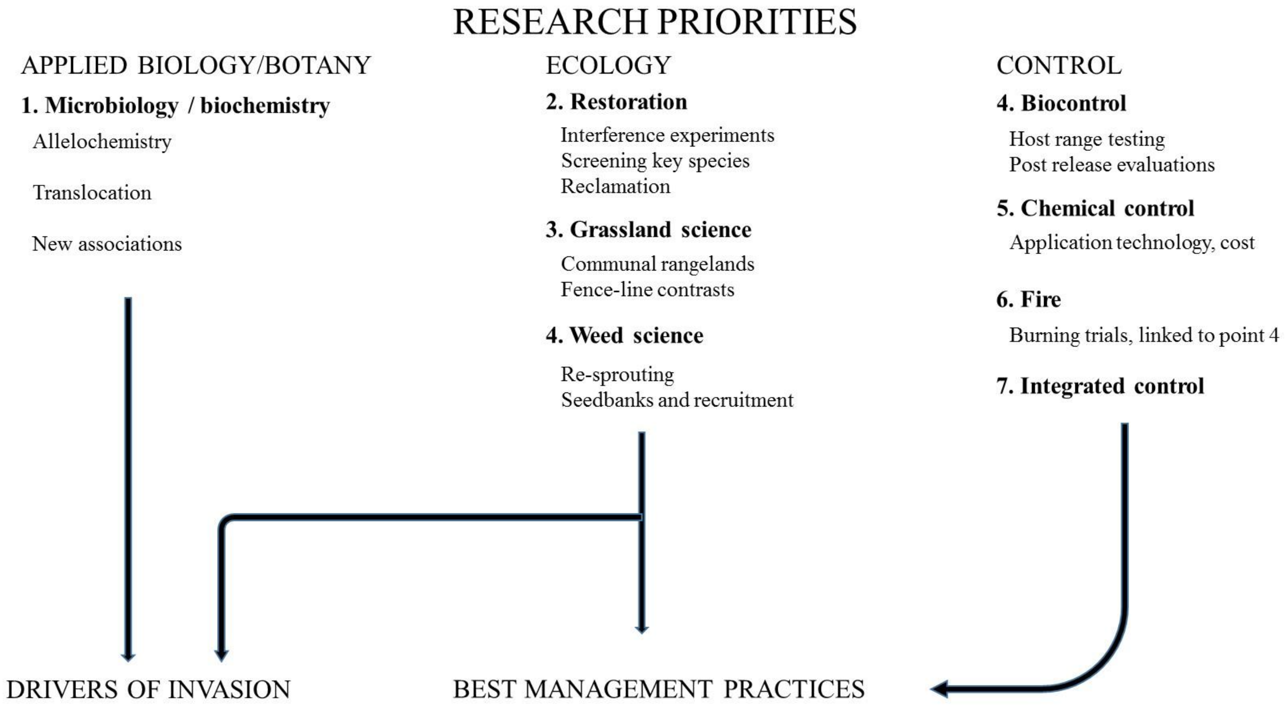

Fig. 1: Flow diagram illustrating core areas of research where gaps in knowledge concerning the biology, ecology and control of *Campuloclinium macrocephalum* exist. Numbers refer to the order of research priorities described below.

1. Applied microbiology and botany (→drivers of invasion)
    - Identify the chemicals produced by the glandular trichomes and their evolutionary function and purpose to lend support or reject the Novel Weapons hypothesis.
    - Study of *P. eupatorii* – herbicide interactions on *C. macrocephalum* from initial infection to advanced senescence, in particular herbicide absorption and the translocation of assimilates. The results of this study should provide empirical evidence of how herbicide transport and impact on meristematic tissue is affected under different infection rates and provide recommendations for chemical control.
    - Study of new associations in native parasitic and hemi-parasitic plants with special emphasis on associations between the Santalaceae and the invasive alien Asteraceae.
2. Restoration ((→best management practices)
    - Interference experiments are essential for determining above and below ground competition and provide a mechanistic model for invasions under a range of limiting resource conditions, invasion intensity and competitive vegetation. This information

is vital to provide links between declining biodiversity, weed invasion and veld management. The information will also be relevant to restoration initiatives.

- Screening key species (monocots & dicots) for restoring invaded grasslands and wetlands affected by disturbance and herbicides.
- Study on wetland reclamation using engineering practices to correct hydrological imbalances and raise groundwater levels in invaded sites and the impact on pompom weed populations and natural succession. This will entail long-term pre- and post-reclamation monitoring.

3. Grassland science ((→best management practices)

- Study on the impact of pompom weed in communal rangelands and comparative analysis with commercially grazed rangelands in the grassland biome. Results from this study should provide a novel case study of indigenous knowledge vs. profit-driven models in conservation management.
- A study of fence-line contrasts along road reserves in invaded and uninvaded grassland may yield additional understanding of the drivers of invasion.

4. Weed Science

- Re-sprouting will entail basic biology of the root crown and its survival, growth and responses to treatment (biocontrol, herbicide and fire) and treatment exclusion.
- Seed bank studies should include –
  - (a) seed trapping (long term) to determine seasonal volumes of seed drop from mature plants in the vicinity, air-borne seed entering the study area and their relative contributions to the seedbank
  - (b) seedbank sampling (long term) to determine size variations in response to seed rain, atrophy, predation, infertility, longevity, viability, dormancy, recruitment and integrated control
  - (c) seedling studies under differing moisture and grass biomass gradients to determine seedling survival, attrition and seedling to adult transition, i.e. time required for the seedling develop a rootstock with perennating buds and/or more than one natural stem (not as a result of damage)
  - (d) populations studies incorporating the previous elements and how these influence the success of control initiatives

5.Biocontrol

- Host range testing of biocontrol agents in certified quarantine laboratories (ongoing – Agricultural Research Council). Included in this aspect is application for release of agents proving to be host-specific and getting agents established in the field once permission has been granted.
- Long-term evaluations of agent impacts on the weed, including rate of spread, population density, soil seedbank size, recruitment and agent interactions (synergy or incompatibility).

6. Chemical control

- Refine herbicide application technology to minimise not target damage. Various options are available that include low volume application techniques, wiping and spot-spraying but the efficacy and costs of these practices needs to be established under variable pompom weed densities along with neighbour impacts.

7. Fire (see 4. Weed Science)

- Burning experiments should be carried to determine the effect of fire and heat on pompom weed regrowth, seedlings and seedbanks under high and low grass biomass conditions. Burning should be applied at three frequencies, namely fire exclusion (zero), annual and triennial burns. Base line data (one to two years) is essential prior to the application of treatments to establish before and after trends in weed density, recruitment and grass biomass in association with rainfall.

8. Integrated control ((→best management practices)

- Large, long-term manipulative experiments must be implemented in invaded grasslands and wetlands once the gaps in research (points 1 to 7 above) have been concluded. Integrated control should include biological control (*P. eupatorii* and at least one insect agent), chemical control (one registered herbicide), fire and restoration. Proper trial design (full factorial with replication), adequate duration (at least 10 years) and appropriate sampling methods are critical to separate individual effects from treatment interactions and provide robust solutions for best management practises in the grassland biome.

Management guidelines

The Australians arguably have the most experience concerning weed risk assessment, management, containment and extirpation (Groves & Panetta 2002; Panetta & Lawes 2005;

Downey & Sheppard 2006; Panetta 2007, 2009; Downey *et al.* 2010; Grice *et al.* 2010). Until the handbook of best management practices of invasive alien plants in the grassland biome of South Africa has been produced / published it is recommended we adopt the Australian principles to managing pompom weed in South Africa. These same principles were used to develop the National Parthenium Strategy of the Department of Environmental Affairs.

- Commitment: The collaboration of all affected stakeholders in the grassland biome is essential for a national initiative against pompom weed to be successful. Affected land use includes conservation land on mine properties, communal rangelands, farms, small holdings, protected areas, road and rail reserve and vacant lots. The affected parties will require funding and support from organisations mentioned in the first paragraph in the Research subsection under Recommendations.
- Management zones: The Australians have identified four types of zones, namely Prevention, Extirpation, Containment and Asset Protection zones.
    - Prevention zones are regions where there are no known populations of pompom weed and would include vulnerable grasslands in Free State, North West, Limpopo and Mpumalanga provinces and the Drakensberg Transfrontier Park in KwaZulu-Natal. Actions include vigilance / surveillance and reported population sightings would be extirpated with registered herbicide by SANBI rapid response teams.
    - Extirpation zones are areas where the populations of pompom weed are at low densities and infestations are widely distributed such as in the Free State and North West provinces. Actions include vigilance / surveillance and reported population sightings would be extirpated by SANBI rapid response teams.
    - Containment zones are regions in which pompom weed is frequent to common in Limpopo, Mpumalanga and KwaZulu-Natal provinces. Actions include awareness, management plans, biological and chemical control, and long-term monitoring. Multi-party by-in (municipalities, landowners, road and rail agencies, government and science organisations) is key to sustainability.
    - Asset Protection zones are where the weed is widespread and dense, e.g. Gauteng Province. The weed is beyond economic management currently and natural renewable resources, e.g. conservation areas and grasslands utilized for grazing (commercial and communal), are under integrated weed management.

Affected parties will require government support to prevent loss of the resource, bankruptcy, job losses and poverty. Actions include integrated control, restoration and long-term monitoring.

- Strategic goals: The initiation of a national initiative against pompom weed focusing on research and development of integrated control methods culminating in the publication of a handbook of best management practices for invasive alien plants in the grassland biome. Key performance indicators would include:
    - Securing adequate funding and commitment / by-in from stakeholders
    - Detection and extirpation of new infestations
    - Reduction in the density and extent in containment zones using integrated control methods.
    - Restoration of important grasslands and wetlands in Gauteng province.
    - Collaboration between different levels of government, science organisations including universities, the private sector, tribal authorities and neighbouring countries.
    - Formulate and disseminate best management practices and guidelines for all land users.
    - Publication of a handbook of best management practices for invasive alien plants in the grassland biome

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