Plant competition as an ecosystem-based management tool for suppressing *Parthenium hysterophorus* in rangelands

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Declarations of interest

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Introduction

Parthenium hysterophorus L. (Asteraceae) native to Mexico, Central and South America, is problematic invasive plant species in tropical and subtropical regions of Asia, Africa and Oceania.[1] Similar to other invasive species, increased movement of people and goods, as well as transport across continents appears to have facilitated the spread of *P. hysterophorus*.[2] Apart from causing biodiversity and agricultural loss, *P. hysterophorus* also reduces rangeland and grazing land quality, fodder quality, as well as quantity and productivity in invaded ecosystems.[1,2] It alters vegetation structure towards monospecific stands (*Parthenium*–dominated communities) as it releases allelochemicals, which inhibit growth of neighbouring co-existing plants in the recipient ecosystems.[3,4] Its Parthenin, which is toxic, makes *P. hysterophorus* non-palatable and responsible for the death of livestock and wildlife when consumed in large quantities (i.e. 10 to 50% of the diet).[5] Some people also develop skin allergies and respiratory illness if repeatedly exposed to *P. hysterophorus*, especially flowers or pollen, which contain Parthenin.[5] If *P. hysterophorus* is left uncontrolled, its invasion has the potential to impede the livelihood of local people, increase management costs associated with habitat restoration and herbicide application,[6] and adversely affect biodiversity and ecosystem function.[7]

While many studies have investigated the harmful effects of *P. hysterophorus* on ecosystems, and its distribution in sub-Saharan Africa,[4,7] little experimental work has been done to investigate sustainable and environmentally–friendly management approaches to control it. Even the control methods (i.e. bio–herbicides, synthetic herbicides, physical management, biological control and metabolites from fungal species) available in other countries have limited effects when used alone.[5,8] There are also drawbacks, for instance, physical management, which involves hand–weeding or uprooting of *P. hysterophorus* is tedious, time consuming, and can affect human health.[7] Manual removal techniques may further affect native flora and fauna communities through soil disturbance, which could disrupt soil dwelling organisms and roots or mycorrhizal systems of non–target species.[9] Other management methods such as fire, grazing, shading or mowing might have unexpected consequences to the wider plant and animal community.[9] Earlier studies reported that the most feasible way to reduce plant invasions in pastures or natural habitats is to maintain diverse assemblages of native or non–invasive forage plants.[6,8,10,11] Moreover, increased plant species diversity and/or density in grasslands have suppressed invasive plants in some studies.[6,10,12,13] For instance, the growth of the invasive weed, *Ipomoea cairica* L., was suppressed when planted with competitive native plants (*Pueraria lobata* [Willd.] Ohwi or *Paederia scandens* [Lour.] Merr.).[14] Hence, ecosystems or rangelands invaded by alien plants might be assisted in their recovery following invasive removal through the addition of competitive diverse forage species.[6,8,9]

Employing 'biocontrol plants' (i.e. plants introduced purposefully in ecosystems to enhance crop or habitat productivity, to manage pests, weeds or invasive plants) may also increase the efficiency of biological control agents and other management methods when they are integrated into the ecosystem.[8,15] Biocontrol plants outcompete invasives for water, growth space, light, and impede their germination and growth. Hence, competitive forage species might be a potential management tool in Tanzania to control *P. hysterophorus*, particularly in natural habitats where herbicide application is not recommended.[6,8,16] Though some forage plant species in Tanzania might be suitable biocontrol plants, they have not been studied for their suppressive ability against *P. hysterophorus*. Our primary objective was to test different plant species for their suppressive effect on *P. hysterophorus* growth. We hypothesized that higher numbers of competitive plant species negatively affect (i) growth parameters (i.e. stem height, diameter, root length, and biomass) and (ii) total leaf chlorophyll content of the invasive *P. hysterophorus* seedlings. We conducted field plot experiments to investigate the suppressive ability of legumes (*Desmodium intortum* L. [Fabaceae], *Lablab purpureus* L. [Fabaceae], and *Medicago sativa* L. [Fabaceae])) against *P. hysterophorus*.

Materials and methods

Characteristics of test plant species

Test plant species were selected based on their characteristics of being non–invasive, drought–resistant, a conservation cover crop species, and important fodder species for livestock due to their high protein content, high biomass production, and adaptability [17]. Additional competitive advantages of the test species over *P. hysterophorus* are their ability to fix atmospheric nitrogen.[17–19] The test plant species are unlikely to become invasive because they have been grown for >50 years in east Africa to feed cattle and have not become problematic.[17–19] *Lablab purpureus* (native to Africa) grows up to 6 m high in a diverse range of environmental conditions in bushland, grassland, and forest as it is highly adaptable.[18,20,21] This species endures temperatures of 18–35°C, and annual rainfall of 650–3000 mm.[20] During the dry season, it remains green, making it an attractive fodder to livestock when other forages are scarce and dry.[20] *Desmodium intortum* (native to South America) is an annual species, growing up to 7.5 m in height, in areas with annual rainfall of 900 – 3000 mm, and temperatures between 25 and 30°C.[22,23] This species tolerates flooding, waterlogged habitats and shade.[22,24] *Medicago sativa*, which is native to Asia, can live for several years at its ideal temperature range of 15 – 25°C and rainfall of 200 – 2500 mm.[25–27] This species can reach up to 1 m high with numerous branches.[25]

Competition experiments and seedling growth parameters

In our experiments, we grew *P. hysterophorus* for 50 days with different biocontrol plant species compositions, and we assessed *P. hysterophorus* fitness using seedling height, stem diameter, root length, dry biomass, and total leaf chlorophyll content in Tanzania. We obtained *P. hysterophorus* seeds from the Agricultural Division at the Tropical Pesticide Research Institute (TPRI) in Tanzania. *Desmodium intortum* and *M. sativa* seeds were purchased from Kibo Seed Company Ltd. in Arusha, Tanzania. *Lablab purpureus* seeds were collected from the Department of Sustainable Agriculture, Biodiversity and Ecosystem Management at Nelson Mandela African Institution of Science and Technology (NM–AIST) in Arusha.

We investigated the suppressive effects of test plants *D. intortum*, *M. sativa*, and *L. purpureus* on *P. hysterophorus* seedling growth vigor in 1 m^2 field plots at NM-AIST Tengeru campus (3º 24.149′ S and 36º 47.790′ E, 1197 m a.s.l). The mean annual temperature in Tengeru is 19.5°C and average annual rainfall is 1078 mm.[1] The area is characterised by black clay loam soil.[1]

Twenty–five seeds of *P. hysterophorus* and test plant species each were sown in 5 plots at varying combinations of mixtures with a monoculture as a control. Plant seedlings were grown at a density of 6 *P. hysterophorus/*10 test plants per plot (Table 1). The total of 11 planting combinations was replicated five times to make 55 planting plot trials (Table 1). Each plot was irrigated daily in the morning with 4 l of water.

The 50–day–old seedlings of *P. hysterophorus* were harvested from each plot without destroying roots to assess the test plants' suppressive effects on invasive growth. The harvesting time was within the critical competition period of *P. hysterophorus* with various plants or crops.[28,29] Growth metrics were stem height, stem diameter, root length, and above– and below– ground dry biomass (ADB and BDB, respectively). Harvested seedlings were washed in water to remove dirt prior to separating into ADB and BDB components. Each component was placed in separate paper bags, dried in an 70ºC oven for 72 h. Root length, and stem height (from soil level to the tip of the tallest plant part) was measured using a meter ruler. Stem diameter (above the first two seedling leaves) and biomass were measured using digital callipers and an analytical balance, respectively. Additionally, an index of competition (i.e., relative interaction intensity [RII], (Equation 1)) was determined to assess performance of *P. hysterophorus* grown with suppressive species at different densities.[30] If RII < 0 competition prevails, RII > 0 facilitation prevails, and if RII = 0 the interaction is neutral or non–existent.[30] RII ranges from 1 to -1.[30]

$$RII = (B_w - B_o) \div (B_w + B_o) \tag{1}$$

where, B_0 and B_w are *P. hysterophorus* biomass grown in monoculture and in mixture, respectively. We used the mean biomass over all replications for each planting combination.

Measurement of leaf chlorophyll content

Four young, fresh leaves from 10 *P. hysterophorus* individuals per plot were selected for analysis of total leaf chlorophyll content (i.e., total Chl).[31] The total Chl was measured as an index of *P. hysterophorus* seedling health in response to suppressive effects of the competitive plant(s). We used 70 mg of *P. hysterophorus* leaves immersed in 6 ml of dimethyl sulfoxide (DMSO) in a test–tube, and incubated at 65°C for 12 h.[32,33] Afterwards, the extract was diluted to a total volume of 10 ml with DMSO. We transferred 3 ml of *P. hysterophorus* leaf chlorophyll extract onto a microplate to determine absorbance or optical density (OD) of the sample. The OD of the blank liquid (DMSO) and samples was determined under Synergy HTX Multi–Mode Microplate Reader (BioTek, U.S) at 663 nm and 645 nm. Prior to calculating total Chl, the OD of the blank was deducted from the OD readings of every sample. We calculated total Chl using:[32]

$$Total\ Chl = 0.0202A_{663} + 0.00802A_{645} \tag{2}$$

Where, A_{663} and A_{645} are absorbance readings at 663 nm and 645 nm, respectively.

Percentage change (PC) of *P. hysterophorus* growth parameter was calculated using:

$$PC = (G_m - G_c) \div G_c \quad (3)$$

Where, G_m and G_c are *P. hysterophorus* growth parameters in mixture and control, respectively. A negative change was expressed as a decrease growth parameter value while a positive change was expressed as an increase in growth parameter value in planting mixture.

Statistical analysis

Stem height, stem diameter, root length, ADB, BDB, and total leaf Chl of *P. hysterophorus* were compared across suppressive species planting combinations using one–way ANOVA.[1,31] We verified normality and homogeneity of variance using a Shapiro–Wilk test and Levene’s test, respectively.[31,34] The post–hoc Tukey–Kramer test (Tukey's honest significance test)[34] was used to separate the means at $p \leq 0.05$. We used the statistical software OriginPro 9.0 for data analysis[34] at a significance level of $\alpha = 0.05$.

Results

Effect of suppressive plants on P. hysterophorus growth

Overall, *P. hysterophorus* growth was more reduced when its seedlings were grown with *L. purpureus* in all combinations compared to other test plant species. *P. hysterophorus* seedlings had lower stem height, root length, shoot diameter, and biomass in mixtures than when grown in a monoculture. The stem height ($F_{(7, 32)} = 3.26$, $p = 0.01$), stem diameter ($F_{(7, 32)} = 1.67$, $p = 0.0151$), and root length ($F_{(7, 32)} = 11.77$, $p < 0.0001$) of *P. hysterophorus* seedlings grown with suppressive plants differed significantly between the number of suppressive plant species present (Fig. 1). *Parthenium hysterophorus* seedling stem height was >40% shorter when grown with *L. purpureus* in all combinations, than when grown with *M. sativa* and/or *D. intortum,* or in a monoculture (Fig. 1). The root length of *P. hysterophorus* seedlings when grown with two or three suppressive species was >54%

shorter than when grown with one species or in a monoculture (Fig. 1). Also, it was 45% shorter when *P. hysterophorus* was grown with one suppressive species, in particular *L. purpureus*, compared to when grown alone or with either *M. sativa* or *D. intortum*. *P. hysterophorus* stem diameter was >38% smaller when grown with two or three suppressive plant species than when grown in a monoculture or with one suppressive species (Fig 1).

Mean ADB and BDB of *P. hysterophorus* seedlings differed significantly between planting diversity and density (ADB: $F_{(7, 32)} = 9.42$, $p < 0.0001$, and BDB: $F_{(7, 32)} = 3.85$, $p = 0.0038$, Fig. 2). *Parthenium hysterophorus* ADB was >66% lower when grown with two (only in mixture of *L. purpureus* and *D. intortum*) or three suppressive species together than when grown in a monoculture or with one suppressive species. Also, when *P. hysterophorus* was grown with two or three suppressive species, BDB was >50% lower than when grown either in a monoculture, with *M. sativa* or *D. intortum* (Fig. 2). The competition intensity of *P. hysterophorus* declined (lower values of RII) with increasing density of suppressive plant species (Table 2).

Effect of suppressive plants on P. hysterophorus leaf chlorophyll

Total Chl of *P. hysterophorus* differed significantly across suppressive plant species ($F_{(4, 20)} = 48.36$, $p < 0.0001$, Fig. 3). Suppressive species affected total Chl of *P. hysterophorus* negatively when grown with one (except *M. sativa*), two or three suppressive species. The total Chl of *P. hysterophorus* was reduced by 69% when grown with three suppressive plant species than when grown in a monoculture or in other combinations. Also, when *P. hysterophorus* was grown with two or one suppressive species (either *D. intortum* or *L. purpureus*), its total Chl was reduced by >40% than when grown in a monoculture or with *M. sativa* only (Fig. 3).

Discussion

We demonstrated that growth of *P. hysterophorus* seedlings was negatively impacted by suppressive forage species, particularly when the latter were combined. We found that *L. purpureus* was the primary species suppressing *P. hysterophorus* growth because all performance parameters were low across planting mixtures with *L. purpureus*. In contrast, in mixtures without this species, little or no significant suppressive effect was observed. For instance, in planting mixtures PMDL, PML, PDL, and PL, *P. hysterophorus* seedlings displayed lower biomass and reduced stem height. We showed that when the number of suppressive species in the plot was increased from one to two or three, *P. hysterophorus* stem height, biomass and total Chl decreased. This decrease followed a gradient of effectiveness (i.e., the gradient of most effective to least effective suppressive plant species: *L. purpureus* > *D. intortum* > *M. sativa*) with little evidence that *M. sativa* alone could exert a suppressive effect. However, we advocate that the more and less suppressive species can be used together as rehabilitative species to complement each other in suppressing *P. hysterophorus* and improving livestock or wildlife forage availability as well as increasing ecosystem resilience against *P. hysterophorus* invasions.

The competition intensity index (RII) showed that suppressive plants at higher density negatively affected the total biomass of *P. hysterophorus*. Our findings are supported by previous research, which reported that resistance imposed by a single plant species to invasive species is weak compared to when several species are present.[13,35,36] Our results suggest that high plant density in rangelands may reduce ecosystem invasibility,[10] and highlights the importance of keeping rangelands from becoming impoverished or dominated by only a few grazing–tolerant species.[37] Moreover, it also indicates competitive plant species seeded together with *P. hysterophorus* in communities of high species diversity may suppress invasion, which is in accordance with studies that found higher native or introduced plant diversity suppressed invasive plants in pastures.[8,10,37,38] For instance, Khan et al[38] reported that *Setaria incrassata, Cenchrus ciliaris, Panicum maximum,* and *Eulalia aurea* at higher abundance suppressed *P. hysterophorus* growth. Moreover, maintaining diverse forage plant communities may affect the amount of invasive species' seeds (or seed bank) in the soil.[6] As *P. hysterophorus* seeds have a long dormancy, management methods such as high suppressive species diversity might help decrease the accumulation of invasive species seeds and avoid future potential invasions. Since ecosystem invasibility is influenced by habitat resource availability,[6,39] increasing abundance and diversity of effective suppressive species may reduce ecosystem vulnerability to *P. hysterophorus* invasions as there is complete resource utilization.[10,37] Therefore, management of *P. hysterophorus* using competitive suppressive plant species might promote long–term ecosystem resilience against invasions, and ensure ecosystem health and stability.

In our study, we observed a large area of ground cover of *L. purpureus* shading the rosette or juvenile plants of *P. hysterophorus,* which likely reduced their growth. This observation is similar to the findings of Tamado et al[28] and Khan et al.[38] Thus, we suggest management approaches to mitigate *P. hysterophorus* invasion using competitive forage species should target rosettes at an early growth stage. While *D. intortum* has been recommended for conservation of ground cover and pasture,[22] we showed it can also be used to control *P. hysterophorus*. It is particularly effective when mixed with the most effective suppressive plant or grass species, such as *L. purpureus*, *Digitaria eriantha, Urochloa mutica,* and *Pennisetum clandestinum,* as it likely grows better in mixed stands.[18] In addition to its extensive ground cover, *L. purpureus* also exhibits high stem height, root length, and biomass. We highlight that biological control through competitive plants is an approach with potential for managing *P. hysterophorus* in rangelands. It may benefit small land holders on a large scale by offering high yield without direct costs to growers while protecting the environment from *P. hysterophorus*.[8,38] The use of non-native plant species is not advised for protected areas to control alien invasions, we used them because they are a financial benefit to pastoralists and agro–pastoralists.[18–20,22]

As *P. hysterophorus* causes allergic reactions in humans and animals in cases of skin contact during manual weeding or livestock handling,[40] our management approach would not require touching or uprooting it. Thus, our method could potentially reduce health risks to people, livestock, and wildlife. It is also a low–cost and sustainable management method for controlling this invasive species. Our selected suppressive species are readily available, drought tolerant, and can fix atmospheric nitrogen, which could possibly enhance their competitive fitness in mixtures over *P. hysterophorus*.[19,20,41] Their non–target impacts on the environment are less severe compared to other biological control agents such as insects or microorganisms.[15] However, we acknowledge that *P. hysterophorus* cannot be controlled by a single method *per se*, and thus, existing methods may be complemented with new management techniques.[5,8]

Conclusions

We found that *P. hysterophorus* seedlings can be suppressed by forage species alone or in mixtures, primarily *L. purpureus*. To effectively control *P. hysterophorus*, seeding suppressive plant species must be included at an early invasion stage. For instance, before the emergence of rosettes and immediately following pulling of mature *P. hysterophorus* seedlings to create appropriate conditions (e.g. enough space, nutrients, water, and light) for quick establishment (e.g. increase in abundance and biomass) of suppressive species. We recommend that local communities be empowered with knowledge about suppressive potential of plant diversity and how to facilitate the planting of suppressive fodder species on their land. A coordinated national strategy and policy to mitigate invasive species is needed to ensure effective *P. hysterophorus* management in Tanzania. Furthermore, since our study is limited to germination and early growth stage of *P. hysterophorus*, future studies should test suppressive ability of forage species in already established invasive stands. While bio–herbicides of alien plants could be considered for suppressing invasive species, ecologists should not plant them in protected areas to suppress *P. hysterophorus* as they may become deleterious or invasive in the ecosystem due to climate change and/or lack of their natural enemies. Accordingly, if ecologists or invasion biologists need to control the invasive species using non–native plants, they must first assess and quantify their impacts at various levels of ecological complexity.

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Table 1. Experimental design for planting plots with *P. hysterophorus* (P) and suppressive plant species (S). *P. hysterophorus* (P) was grown in combination with *Medicago sativa* (M), *Desmodium intortum* (D) and *Lablab purpureus* (L). S_0 = no suppressive plant species added, S_1 = one species added, S_2 = two species added, S_3 = three species added. We used five replications per treatment.

***Parthenium hysterophorus* (P) and suppressive plant species (S)**				**Suppressive plant species grown alone**
PS_0	PS_1	PS_2	PS_3	
6P	6P/10M	6P/10M/10D	6P/10M/10D/10L	10M
	6P/10D	6P/10M/10L		10L
	6P/10L	6P/10L/10D		10D

Table 2. Relative interaction intensity (RII) for *P. hysterophorus* within each suppressive plant community combination. *P. hysterophorus* (P) was grown in combination with *Medicago sativa* (M), *Desmodium intortum* (D) and *Lablab purpureus* (L).

Planting combination	PL	PD	PB	PLD	PLB	PBD	PLDB
RII	-0.143	-0.218	-0.356	-0.269	-0.320	-0.462	-0.473

Figure 1. Mean (±SD) stem height (cm), stem diameter (mm), and root length (cm) of *P. hysterophorus* seedlings when grown in a monoculture (light grey box), and with one (dark grey boxes), two (dashed boxes) or three (white box) suppressive plant species. Boxplots show the mean (square within boxes), 25% and 75% quartile ranges, and whiskers show standard deviations. Boxes with dissimilar letters are significantly different by Tukey–Kramer test at $p \leq 0.05$. P = *P. hysterophorus*, M = *M. sativa*, D = *D. intortum*, and L = *L. purpureus*.

Figure 2. Mean (±SD) above- and below- ground dry biomass (g) of *P. hysterophorus* seedlings when grown in a monoculture (light grey box), and with one (dark grey boxes), two (dashed boxes) or three (white box) suppressive plant species. Boxplots show the mean (square within boxes), 25% and 75% quartile ranges, and whiskers show standard deviations. Boxes with dissimilar letters are significantly different by Tukey–Kramer test at $p \leq 0.05$. P = *P. hysterophorus*, M = *M. sativa*, D = *D. intortum*, and L = *L. purpureus*.

Figure 3. Mean (±SD) index values of total leaf chlorophyll content of *P. hysterophorus* seedlings when grown in a monoculture (light grey box), and with one (dark grey boxes), two (dashed boxes) or three (white box) suppressive plant species. Boxplots show the mean (square within boxes), 25% and 75% quartile ranges, and whiskers show standard deviations. Boxes with dissimilar letters are significantly different by Tukey–Kramer test at $p \leq 0.05$. *P* = *P. hysterophorus*, M = *M. sativa*, D = *D. intortum*, and L = *L. purpureus.*

On the Ground Summary

- The exotic invasive plant *P. hysterophorus* is invading rangelands in Africa while causing negative effects on the biodiversity, environment, economy, and human and animal health.
- There is a lack of effective eco–friendly control methods.
- We conducted experiments to investigate the suppressive effects of forage legume plant species *Desmodium intortum* (Fabaceae), *Lablab purpureus* (Fabaceae), and *Medicago sativa* (Fabaceae) in suppressing *P. hysterophorus* growth vigour
- *Parthenium hysterophorus* growth was suppressed when grown with fodder plant species at high density. But, the effect seemed to be mediated by the presence of *L. purpureus*
- Our work highlights the importance of keeping competitive native plants in rangelands.
- Moreover, this control method could be part of an integrated control toolkit deployed in a community–based approach in other countries.