

Suppressing competitive dominants and community restoration with native parasitic plants using the hemiparasitic *Rhinanthus alectorolophus* and the dominant grass *Calamagrostis epigejos*

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Summary

1. Dominance of native or alien competitive plants causes competitive exclusion of subordinate species and represents a major mechanism reducing biodiversity following land-use changes. The successful competitive strategies may, however, be interfered with by parasitic plants, which withdraw resources from other plants' vasculature. Parasitism may strongly reduce the growth of the dominants, which may facilitate regeneration of other species and consequently trigger restoration of natural communities of high diversity.

2. Here, we aim to provide robust empirical evidence demonstrating this restoration potential of parasitic plants. We present a case study testing suppressive effects of hemiparasitic *Rhinanthus alectorolophus* on competitive grass *Calamagrostis epigejos*. In recent decades, *C. epigejos* has invaded many high-nature-value semi-natural grasslands of Central Europe, which is one of the prominent factors causing their biodiversity decline.

3. We conducted three manipulative field experiments testing the effect of sowing of *R. alectorolophus* in different vegetation types infested by *C. epigejos*. *Rhinanthus* sowing was compared to different mowing treatments recommended as the 'best practice' management at respective sites.

4. *Rhinanthus alectorolophus* established itself in most *C. epigejos*-dominated plots where sown. *Calamagrostis epigejos* was virtually exterminated in 2 years in two of the experiments (dry meadow and industrial area). In the wet-meadow experiment, the suppressive effect was variable as a result of uneven establishment success of *Rhinanthus*. In this experiment increased mowing intensity had an additional suppressive effect on *C. epigejos*. *Rhinanthus* also increased regeneration potential of other species by a temporary reduction of vegetation density. Restoration of target vegetation composition was, however, dependent on community context.

5. *Synthesis and applications*. We demonstrated that hemiparasitic *Rhinanthus alectorolophus* is an accessible and efficient tool for targeted biological control of *Calamagrostis epigejos*, with a great potential to restore infested grassland vegetation. The strong effect of *Rhinanthus* is caused by interference with the underground storage and clonal growth strategy of *Calamagrostis epigejos*, which are both traits that underlie its competitive ability. The potential of native parasitic plants should be considered in restoration management of sites infested by competitive dominants, either alien or native.

Key-words: clonal plant, competition, diversity decline, dominance, ecological restoration, ecosystem engineering, land use change, parasitic plant, rhizome, semi-natural grassland

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Introduction

Dominance of competitive plant species is associated with low community diversity (Wisheu & Keddy 1992). An increase of dominance is a major mechanism causing biodiversity decline following land-use change (Lepš 2014). Many competitive dominants are alien invasive species (Gioria & Osborne 2014) but native dominants may have comparable effects on plant diversity (Somodi, Virágh & Podani 2008; Lepš 2014). Regulation of competitive dominants and restoration of infested communities is a serious issue in nature conservation. Optimal restoration measures should suppress competitive dominants and simultaneously support spontaneous recovery species-rich communities such as high-nature-value (HNV) grasslands. From this perspective, drastic mechanical (e.g. topsoil removal) or chemical (herbicide application) measures do not represent a desirable solution.

Parasitic plants, which take up resources directly from their host's vascular bundles may be used as biocontrol agents suppressing the competitive dominants. This parasitic mechanism interferes with the resource translocation mechanism of vascular plants. As a result, parasitic plants can display high growth rates while strongly reducing growth of their hosts (Shen *et al.* 2005, 2010; Prider, Watling & Facelli 2009; Těšitel *et al.* 2015). Many parasitic plants preferentially attack hosts of high nutrient status (Kelly 1992) or grow vigorously when attached to faster-growing (Hautier *et al.* 2010) or clonal hosts (Demey *et al.* 2015; Lepš & Těšitel 2015). Parasitic plants may thus inflict disproportional harm to host plant species displaying fast growth, efficient nutrient use or clonality, that is, traits connected to competitive ability and local dominance (Herben, Nováková & Klimešová 2014). Empirical support for such suppressive potential has recently emerged, e.g. in the case of *Pedicularis palustris* L. (Orobanchaceae). This root hemiparasite suppressed dominant sedge *Carex acuta* L., which consequently facilitated transformation of species-poor tall sedge fens into species-rich transition mires (Decler, Bonte & van Diggele 2013). Similarly, stem-parasitic *Cuscuta campestris* Yuncker (Convolvulaceae) has been demonstrated to suppress invasive *Mikania micrantha* H.B.K. in South China (Yu *et al.* 2008). Extensive research is being conducted on stem-parasitic *Cassytha pubescens* R.Br (Lauraceae) as a native biocontrol of invasive leguminous shrubs in Australia (Prider, Watling & Facelli 2009; Shen *et al.* 2010; Cirocco *et al.* 2015). While these studies indicate a general ability of plant parasites to decrease vitality of some competitive dominants (including alien invasives), their use as a targeted measure in ecological restoration remains to be evaluated.

Here, we examine the potential of plant parasitism, namely root-hemiparasitic species *Rhinanthus alectorolophus* (Scop.) Pollich (Orobanchaceae), as a targeted restoration measure for biocontrol of the range-expanding grass *Calamagrostis epigejos* (L.) Roth. *Calamagrostis*

epigejos is a perennial rhizomatous species widespread across the whole of temperate Eurasia. It uses the guerrilla strategy of clonal growth (Rebele & Lehmann 2002) to spread rapidly in previously unoccupied communities diversity of which is consequently reduced by competitive exclusion of subordinate species (Somodi, Virágh & Podani 2008; Rebele 2014). The competitive success of the *C. epigejos* lies in its ability to store and translocate resources below ground (Rebele & Lehmann 2001; Kavanová & Gloser 2005; Gloser, Košvancová & Gloser 2007) and high nutrient use efficiency (Yuan *et al.* 2005). Despite the fact that it is relatively slow-growing (Holub *et al.* 2012), the canopy height of *C. epigejos* still reaches up to 100 cm and the grass accumulates a large amount of standing biomass over the growth season (Rebele & Lehmann 2001). *Calamagrostis epigejos* efficiently translocates nutrients from senescing shoots to its roots at the end of the growth season. As a result, its litter is nutrient-poor, decomposes slowly and accumulates in a thick layer, which reduces growth and establishment of other species (Mudrák *et al.* 2013).

Unlike most other competitive meadow grasses (e.g. *Arrhenatherum elatius* (L.) J. Presl et C. Presl, *Lolium perenne* L., *Dactylis glomerata* L.), *C. epigejos* does not require high soil nutrient availability to spread and attain dominance in the community. Instead, it benefits from low nutrient availability in HNV grasslands and their low-intensity conservation management (Háková, Klauisová & Sádlo 2004; Kleijn *et al.* 2009). Moreover, many European HNV grasslands of low productivity (and high diversity) have been abandoned (Lepš 2014), which further facilitates *C. epigejos* to attain dominance. *Calamagrostis epigejos* is difficult to suppress by re-establishment of low-intensity land use (e.g. single-cut mowing) since this usually does not result in its substantial decrease in the short term (Lehmann & Rebele 2002; Házi *et al.* 2011). More intense restoration measures (e.g. intense mowing) may be harmful for valuable species still remaining in the community (Somodi, Virágh & Podani 2008). In summary, the expansion of *C. epigejos* represents a major threat to the biodiversity of Central European semi-natural HNV grasslands, some of which are remarkable due to the globally unparalleled species-richness at the small spatial scale (Chytrý *et al.* 2015). Conventional land-use practices (mowing, grazing) applied in low intensities (otherwise favourable for maintenance of species-rich grasslands) are inefficient in terms of suppressing *C. epigejos* as the species is well adapted to non-frequent above-ground disturbance by mobilising its below-ground resources. The below-ground resource storage could, however, be the 'Achilles heel' of the successful *C. epigejos* strategy if susceptible to infection by a root parasitic plant. A recent pilot experiment demonstrated the ability of root-hemiparasitic *R. alectorolophus* to establish in *C. epigejos*-dominated vegetation (Mudrák *et al.* 2014) and form functional haustorial connections to its roots (Fig. S1, Supporting Information).

Here, we follow up this pilot study by three manipulative field experiments to demonstrate the potential of *R. alectorolophus* to restore grasslands overgrown by *C. epigejos*. Specifically, we test three hypotheses: (1) *Rhinanthus* has the capacity to suppress *C. epigejos* rapidly when sown into its stand and established, (2) *Rhinanthus* opens gaps in the sward (community features known to be crucial for seed or bud bank regeneration; Fibich *et al.* 2013), and (3) *Rhinanthus* can increase community diversity and drive community composition towards semi-natural HNV grasslands. Sowing of *Rhinanthus* was the main and identical treatment in all experiments. We compare its effects to mowing treatments corresponding to recommended 'best practice' management of respective vegetation types at individual experimental sites (Háková, Klaudivová & Sádlo 2004).

Materials and methods

GENERAL EXPERIMENT LAYOUT AND SEED SOURCE

Three experiments were established in different habitats in 2012 to test the effect of sowing of *R. alectorolophus* on grassland vegetation dominated by *C. epigejos*. Baseline data was collected prior to any experimental treatments. Further monitoring was done annually for the following 3 years (2 years in the case of experiment 3, see below). Monitoring was conducted in late June/early July to match the phenology of *C. epigejos* which peaks at this time. At that time, *Rhinanthus* plants were senescent, mostly in the fruiting phase with some leaves having already fallen off. The recorded *Rhinanthus* cover/biomass values thus may be lower than its peak standing crop. All plots were free of any hemiparasitic species at the start of the experiments. Seed origin of *R. alectorolophus* and the sowing approach was identical in all experiments. The seeds were collected in a wild population located close to Huslenky, Vsetínské vrchy Mts., Czech Republic (49°18'58"N, 18°05'39"E, 600 m a.s.l.) and sown in the corresponding experimental plots in October 2012; no additional sowing was conducted in experiments 2 and 3. In addition, experiment 1 also comprised two mowing treatments. Experiments 2 and 3 comprised sowing of legumes as an additional treatment, which was motivated by local field observations indicating a possible supportive effect on *Rhinanthus* establishment. However, no significant effect of legumes on any of the monitored parameters was detected. Therefore, the plots where these were sown were removed from the data and the results are not reported.

Experiment 1

Experiment 1 was established on an abandoned meadow close to Švihov, Železné hory Mts., Czech Republic (49°50'08"N, 15°51'44"E, 440 m a.s.l.). The meadow consisted of a mosaic of intermittently wet meadows (*Molinion*) and oligotrophic submontane grasslands (*Violin caninae*; Chytrý 2007–2013) that had been unmanaged for at least a decade. Most of the site was overgrown by *C. epigejos* with an admixture of other grasses (*Alopecurus pratensis* L., *Deschampsia cespitosa* (L.) P. Beauv.) but species typical of local species-rich meadows, such as *Carex hartmanii* Cajander, *Betonica officinalis* L., *Sanguisorba officinalis*

L., *Solidago virgaurea* L., and *Viola canina* L. were still scarcely present in the community.

The experimental layout consisted of six blocks each composed of four 3 m × 3 m plots. Whole blocks were mown in summer (after the vegetation composition data sampling and biomass collection) which is a standard management practice of this vegetation type. Two experimental treatments were combined in a full factorial design: (i) sowing of *R. alectorolophus* (500 seeds m⁻²) and (ii) an additional mowing in October (including litter removal and gentle moss layer disturbance by raking, a treatment known to potentially improve *Rhinanthus* establishment; Mudrák *et al.* 2014). This resulted in four treatment combinations within each block: (i) summer mowing only (control; corresponds to recommended conservation management), (ii) summer and autumn mowing (corresponds to conventional restoration management of *C. epigejos*-heavily infested sites), (iii) summer mowing + *Rhinanthus*, (iv) summer and autumn mowing + *Rhinanthus*. Sowing (using fresh seeds) and all treatments were repeated every year. Vegetation composition of the central 2 m × 2 m square of each of the plots was monitored every year in late June/early July by a visual estimate of cover (%). In addition, above-ground biomass of *C. epigejos* was harvested from a 1 m × 1 m permanent square located within the central square to determine its dry-weight.

Experiment 2

Experiment 2 was established on a dry meadow in the northern part of the White Carpathian Mts. (49°6'41"N, 18°03'00"E, 410 m a.s.l.). The meadow had been abandoned for approximately 40 years and regular summer mowing had restarted 8 years prior to the experimental layout. The plant community was dominated by *C. epigejos* and *Brachypodium pinnatum* (L.) P. Beauv., but many dicotyledonous forbs (*Centaurea jacea* L., *Knautia arvensis* (L.) J. M. Coult., *Pulmonaria mollis* Hornem., *Trifolium medium* L.) persisted on the site. The site was fenced to prevent browsing of roe deer.

The experimental layout included five blocks each composed of two 2 m × 2 m plots. *Rhinanthus alectorolophus* was sown (500 seeds m⁻²) onto one of the plots while the other was a control without any sowing. Whole blocks were mown in summer (after the vegetation composition data sampling and biomass collection), which follows the 'best practice' conservation management of this vegetation type. Vegetation composition of the central 1 m × 1 m square was monitored annually at the end of June using the calibrated weight-estimate method (Tadmor *et al.* 1975). Species biomass estimate was based on its estimated proportion in the community multiplied by the community biomass, which was harvested from the same square and its dry weight was determined.

Experiment 3

Experiment 3 was established on an abandoned site located on the campus of the University of South Bohemia and the Biology Centre of the Czech Academy of Science in České Budějovice (48°58'33"N, 14°26'47"E, 390 m a.s.l.). The site was unmanaged for c. 20 years and was largely overgrown by *C. epigejos*. The site was fenced which prevented access of larger wild mammals such as roe deer or wild boars.

The experimental layout included four blocks each composed of two 3 m × 3 m plots. *Rhinanthus alectorolophus* was sown

(500 seeds m^{-2}) onto one of the plots while the other plot was a control without any sowing. Whole blocks were mown twice a year – in summer (July) and autumn (October). Following the autumn mowing, the litter was removed and gentle raking was applied as in experiment 1. This mowing treatment corresponds to the conventional restoration management suitable for sites. Vegetation composition of the central 2 m \times 2 m square of each of the plots was monitored every year in late June/early July by a visual estimate of cover (%). Biomass of *C. epigejos* was not sampled in this experiment; therefore the percent cover was used as a dominance measure. The experimental site was destroyed in the autumn of 2014 due to establishment of an arboretum. Therefore, the monitoring could not extend to 2015.

DATA ANALYSIS

We analysed the following variables in each experiment: a measure of dominance of *C. epigejos* (biomass dry weight or its estimate or cover), which was used to test hypothesis 1, herb layer cover relevant to hypothesis 2, and cover of *Rhinanthus* as an indicator of *Rhinanthus* establishment. To test hypothesis 3, we computed the Shannon diversity index (H) from the vegetation composition data using natural logarithms of species abundances. In addition, we computed dissimilarity of vegetation composition of each sample to the corresponding potential target vegetation. Vegetation composition of reference grassland types was obtained from the Czech National Phytosociological Database (Chytrý & Rafajová 2003) from which we selected plots of intermittently wet meadows and submontane oligotrophic grasslands (*Molinion*, *Violion caninae*), semi-dry suboceanic grasslands (*Bromion*), and mesic meadows (*Arrhenatherion*; Chytrý 2007–2013) as targets for experiments 1, 2 and 3, respectively. The dissimilarities were subsequently computed as Bray–Curtis dissimilarity between composition of individual vegetation samples and the most similar plot in the corresponding reference set. The dissimilarities were based on the square-root of cover (experiments 1, 3) or square-root of biomass estimate values (experiment 2).

Mixed-effect linear models were used for all univariate statistical analyses. Biomass and cover data were log-transformed prior to analysis. A saturated model containing a year of sampling (re-coded as a year since the start of the experiment), all treatments and all possible interactions as fixed effects and plot identity nested within a block as random effects was first fitted for each response variable. Minimum adequate models were subsequently selected by sequential removal of non-significant fixed-effect terms or their interactions. We retained non-significant terms, of which interactions were significant. Significance tests of individual regression coefficients of the final models are reported in the text. All analyses were conducted in R, version 3.2.2 (R Core Team 2015) using R package *nlme*, version 3.1 (Pinheiro *et al.* 2015). We also conducted multivariate constrained ordination analyses (Šmilauer & Lepš 2014) of the community composition presented in Appendix S1.

Results

EXPERIMENT 1

In the wet meadow overgrown by *C. epigejos*, *R. alectorolophus* established in all sown plots in 2013 but its cover was variable (Fig. S2.1 in Appendix S2). *Rhinanthus*

decreased slightly in 2014 followed by a steep decline in 2015. Flowering *Rhinanthus* plants were regularly damaged by grazing roe deer which nibbled the inflorescences, but this damage never occurred before flowering and mostly affected a minor proportion of the plants. Deer damage was only apparent on *Rhinanthus*; other plants were left intact.

Biomass of *C. epigejos* was significantly reduced by both sowing of *Rhinanthus* and mowing twice per season (Fig. 1; Table 1). These negative effects (*Rhinanthus* \times year interaction $t_{69} = -4.33$, $P < 0.001$; mowing twice \times year interaction $t_{69} = -2.17$, $P = 0.033$) were additive (Table 1); therefore the largest reduction of *C. epigejos* biomass was observed in the plots where both treatments were applied. Reduction of *C. epigejos* was variable among the blocks, which was correlated with the abundance of *Rhinanthus*. More than 90% decline of *C. epigejos* was generally observed only in plots where *Rhinanthus* cover reached 20% at least once during the experimental period (Fig. 2). The experimental block where *Rhinanthus* failed to reduce *C. epigejos* was co-dominated by *A. pratensis*, a fast-growing tall grass, which *Rhinanthus* apparently did not parasitise and which probably prevented its better establishment.

In addition to the significant suppression of *C. epigejos*, *Rhinanthus* significantly reduced herb layer cover 2 years after its sowing (Fig. 1; *Rhinanthus* \times 2014 vs. 2012 interaction $t_{66} = -4.70$, $P < 0.001$) and increased Shannon index (Fig. 1; *Rhinanthus* \times year interaction $t_{70} = 2.88$; $P = 0.005$). Both sowing of *Rhinanthus* and mowing twice a year significantly decreased dissimilarity (i.e. increased similarity) to target vegetation (*Rhinanthus* \times year interaction $t_{69} = -2.64$, $P = 0.010$; mowing twice \times year interaction $t_{69} = -2.20$, $P = 0.031$). Significant directional effect of *Rhinanthus* on community composition was identified also by a community ordination analysis (Appendix S1).

EXPERIMENT 2

In the dry meadow, *R. alectorolophus* established itself in all sown plots in 2013 (Fig. S2.2 in Appendix S2). The establishment success was rather even among the blocks. *Rhinanthus* abundance did not substantially change in the following year but a marked decline was observed in 2015 (Fig. S2.2 in Appendix S2).

Calamagrostis epigejos was strongly suppressed by the sowing of *Rhinanthus* (Fig. 3, Table 1; *Rhinanthus* \times year interaction $t_{28} = -4.68$, $P < 0.001$). The suppression was considerable already in the first year after sowing but even more pronounced in the successive years, when the abundance of *C. epigejos* declined close to zero in all plots with *Rhinanthus* (Fig. 3).

Rhinanthus significantly decreased herb layer cover in the year following its sowing (Fig. 3; *Rhinanthus* \times 2013 vs. 2012 interaction $t_{24} = -2.58$, $P = 0.016$) and in the successive year (*Rhinanthus* \times 2014 vs. 2012 interaction $t_{24} = -2.56$, $P = 0.017$). The herb layer cover increased

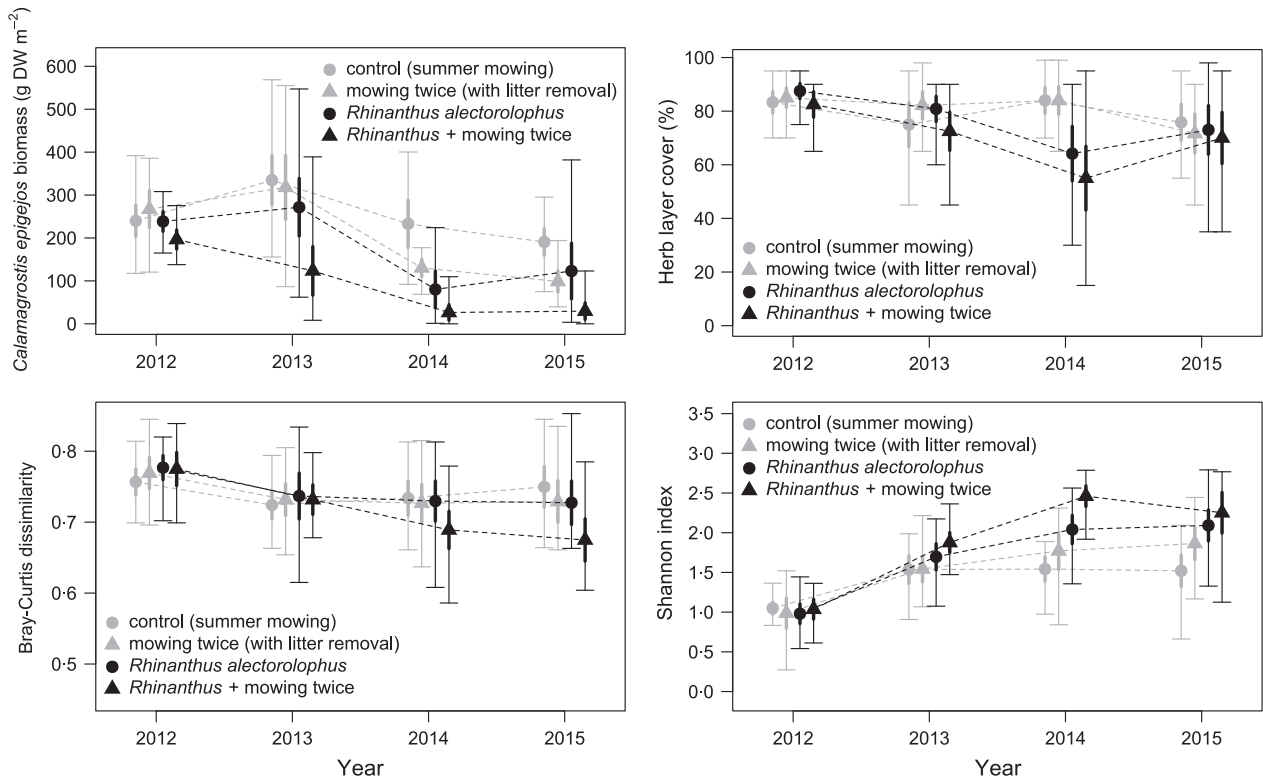


Fig. 1. Effects of the experimental treatments on *Calamagrostis epigejos* above-ground biomass, herb layer cover, dissimilarity of vegetation composition to target vegetation and Shannon index in the experiment 1. Means, one standard error intervals, and data ranges are displayed by points, bold lines, and whiskers, respectively.

Table 1. Summaries of minimal adequate models describing the effects of individual predictors on response variables in experiments 1–3

Exp.	Response	Model structure (fixed effects) [†]
1	<i>Calamagrostis epigejos</i> biomass	Mowing twice + <i>Rhinanthus</i> + Year + Mowing twice × Year* + <i>Rhinanthus</i> × Year***
1	Herb layer cover [‡]	<i>Rhinanthus</i> + Year + <i>Rhinanthus</i> × Year***
1	Distance to target vegetation	Mowing twice + <i>Rhinanthus</i> + Year + Mowing twice × Year* + <i>Rhinanthus</i> × Year*
1	Shannon index	Mowing twice* + <i>Rhinanthus</i> + Year*** + <i>Rhinanthus</i> × Year**
2	<i>Calamagrostis epigejos</i> biomass estimate	<i>Rhinanthus</i> + Year + <i>Rhinanthus</i> × Year***
2	Herb layer cover [‡]	<i>Rhinanthus</i> + Year + <i>Rhinanthus</i> × Year*
3	<i>Calamagrostis epigejos</i> cover	<i>Rhinanthus</i> + Year** + <i>Rhinanthus</i> × Year***

[†]Only models containing at least one significant fixed-effect predictor are listed.

[‡]Year was used as a categorical predictor in these models.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; non-significant regression coefficients are displayed in grey.

again in the last year of the experiment (Fig. 3). No significant effects of *Rhinanthus* on the Shannon index, dissimilarity to target vegetation or a directional change of community composition (Appendix S1) were observed.

EXPERIMENT 3

At the abandoned site, *R. alectorolophus* established itself in all sown plots in 2013 and retained its dominance also in the following year (Fig. S2.3 in Appendix S2). As a result of seed dispersal, few *Rhinanthus* plants were established at some of the control plots in 2014 (Fig. S2.3 in Appendix S2).

Calamagrostis epigejos declined in plots of all treatments throughout the experiment (Fig. 4; year main effect

$t_{14} = -3.89$, $P = 0.002$). However, the decline was significantly steeper in plots with *Rhinanthus* (Fig. 4; *Rhinanthus* × year interaction $t_{14} = -5.12$, $P < 0.001$). *Calamagrostis epigejos* cover approached zero in the second year after sowing of *Rhinanthus*. There was no significant effect of *Rhinanthus* on herb layer cover, the Shannon index, dissimilarity to target vegetation or a directional change of community composition (Appendix S1).

Discussion

All three experiments support hypothesis 1 by demonstrating the potential of *R. alectorolophus* to substantially and rapidly (in 1 or 2 years) suppress *C. epigejos*. The

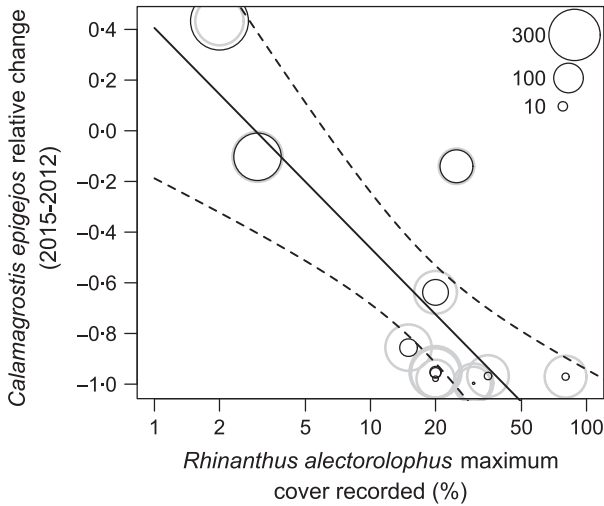


Fig. 2. Effect of maximum cover of *Rhinanthus alectorolophus* on the relative change of *Calamagrostis epigejos* above-ground biomass between the start and the end of experiment 1. Each experimental plot with *Rhinanthus* is represented by two concentric circles. Grey circles and black circles indicate *C. epigejos* above-ground biomass in 2012 and 2015, respectively. Size of the circles is proportional to the dry mass in g m^{-2} as indicated by the legend. Note the log-scale of the x-axis. Regression line with confidence intervals is displayed ($r^2 = 0.65$, $F_{1,10} = 18.43$, $P = 0.0016$).

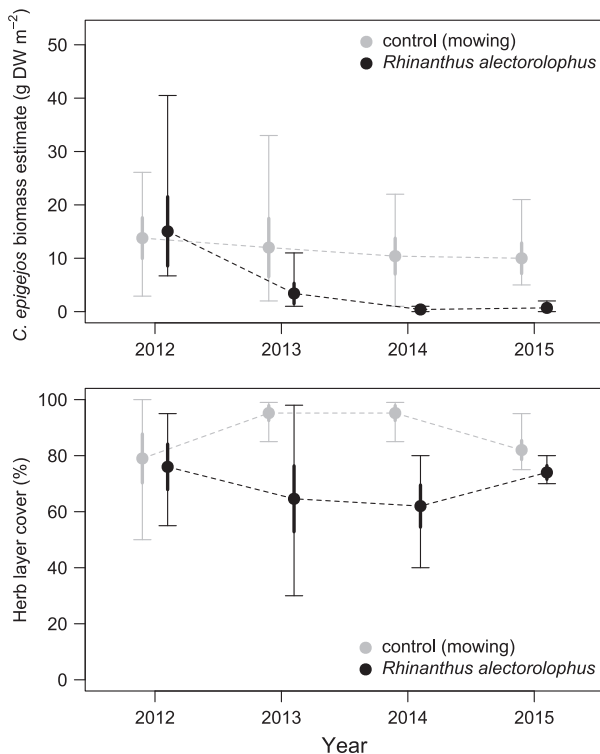


Fig. 3. Effect of *Rhinanthus alectorolophus* on *Calamagrostis epigejos* above-ground biomass estimate and herb layer cover in comparison with single mowing control in experiment 2. Means, one standard error intervals, and data ranges are displayed by points, bold lines, and whiskers, respectively.

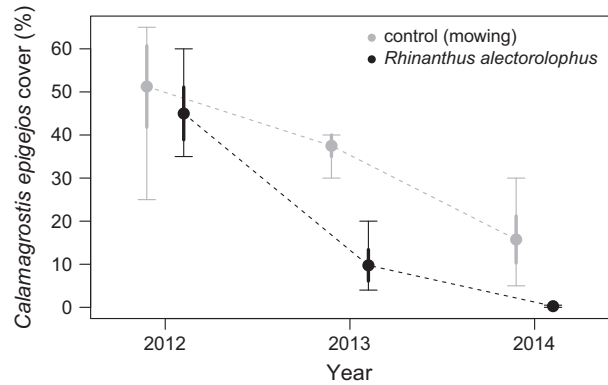


Fig. 4. Effect of *Rhinanthus alectorolophus* on *Calamagrostis epigejos* cover in comparison with mowing-twice control treatment in experiment 3. Means, one standard error intervals, and data ranges are displayed by points, bold lines, and whiskers, respectively.

level of suppression is tightly linked with the establishment success of *Rhinanthus*. Successful application requires a minimum threshold abundance of the hemiparasite (20% cover in the case of experiment 1). *Rhinanthus* establishment in favourable environmental conditions can be impeded by competition for light during its early development (before or shortly after the attachment to the host; Těšitel *et al.* 2011). Therefore, co-dominance of fast-growing dominants with early phenology may be a limiting factor for *Rhinanthus* as indicated by one block of experiment 1 co-dominated by fast-growing *A. pratensis*. This limitation due to competition for light from fast-growing dominants can be expected to be strongest at sites that are simultaneously moist and nutrient-rich where the relative advantages provided by hemiparasitism decrease as does the suppressive effect on the host (Těšitel *et al.* 2015).

The rapid decline of *C. epigejos* following the establishment of *Rhinanthus* was comparable to the effect of a selective herbicide. The initial rapid increase of the hemiparasite causing the rapid decline of *C. epigejos* followed by the decline of *Rhinanthus* in successive years indicates a relatively strong specificity of the hemiparasitic interaction. The specificity is furthermore supported by the moderate effect of *Rhinanthus* on the community composition in experiment 1 and the lack of directional effects in experiments 2 and 3. Such strong and specific interaction may seem surprising given the widely shared view on *Rhinanthus* species as generalists with preferences for grasses and legumes (Cameron & Seel 2007). However, this view has recently been challenged by Rowntree *et al.* (2014) who demonstrated host species identity was more important than functional group membership for the outcome of the hemiparasitic interaction. From this perspective, *C. epigejos* may be a host with traits that make it exceptionally susceptible to hemiparasite infection. Its extensive resource storage located in the roots (Kavanová & Gloser 2005) can be directly accessed by the hemiparasites.

Root-parasitism thus interferes with the key trait underlying *C. epigejos* growth and competitive strategy, which inflicts intense stress on the grass. Its relatively slow growth and late phenology decrease the above-ground competitive effects on hemiparasite seedlings (provided the slowly decomposing litter layer is removed or not too thick). Moreover, *Rhinanthus* growth and the massive harm inflicted to *C. epigejos* were likely further increased by the host clonality as suggested by recently formulated clonal integration hypothesis (Demey *et al.* 2015; Lepš & Těšitel 2015). Experiments 1 and 2, however, indicate that the hemiparasite population may collapse after depleting the clonal host resources. This is crucial for the practical use of the hemiparasites as biological control and restoration agents because it diminishes the possibility of their long-lasting dominance. However, it also means that the suppressive effect on *C. epigejos* is rather short-term and restoring its dominance must be prevented by standard methods of conservation management (e.g. mowing once a year). On a larger spatial scale than just a few square metres, *Rhinanthus* can be expected to establish patch dynamics within a site, which may result in a continuous and spatially heterogeneous suppressive effect on *C. epigejos* population.

The community effects of *R. alectorolophus* corresponding to hypotheses 2 (sward opening) and 3 (a directional community composition change and an increase of diversity) were only observed in two or one of the experiments, respectively. A temporary decrease of herb layer cover induced by *Rhinanthus* was observed in experiments 1 and 2, that is, the semi-natural HNV grassland sites. Such sward opening indicates potential for community composition change towards the target vegetation type. However, realisation of such potential differed between experiments 1 and 2. In addition, experiment 1 has demonstrated that the effect of the hemiparasites may be synergic with increased mowing intensity. This variability of the community effects was probably caused by differential community contexts. The vegetation in experiment 1 was largely overgrown by *C. epigejos*. Most other species had disappeared from the experimental plots but were still scarcely present at the site, in the seed bank or in dormant underground stages. Their re-appearance or re-establishment after opening the canopy increased species-richness up to 42 species 4 m^{-2} which approaches the maximal recorded species-richness (51 species 4 m^{-2}) of wet meadows in the region (Horník *et al.* 2012). In experiment 2, *C. epigejos* was less dominant than at the start of the other two experiments due to re-establishment of management 8 years before. Therefore, plant diversity had been preserved because the competitive exclusion of subordinate species occurs only after increasing dominance of *C. epigejos* (Somodi, Virágh & Podani 2008). Experiment 3 might have been too short-lasting to observe sward opening, although substantial gaps not captured by the monitoring must have occurred in late summer following the death of the *Rhinanthus* annuals.

The context dependency seems typical of *Rhinanthus* biodiversity effects as indicated by previously observed positive (Pywell *et al.* 2004; Westbury *et al.* 2006) or neutral (Westbury & Dunnett 2007, 2008; Mudrák & Lepš 2010) effects of *Rhinanthus minor* on community diversity despite almost universal reductions of standing crop biomass and relative proportion of grasses.

Root hemiparasites are recognised as a functional group with profound community and ecosystem effects (Press & Phoenix 2005; Watson 2009; Demey *et al.* 2015). Species of the genus *Rhinanthus* have been demonstrated to decrease community productivity (Ameloot, Verheyen & Hermy 2005), alter competitive relations in the communities (e.g. Davies *et al.* 1997; Westbury & Dunnett 2007; Mudrák & Lepš 2010; Hellström, Bullock & Pywell 2011; Demey *et al.* 2015) and affect nutrient cycling (Fisher *et al.* 2013; Demey *et al.* 2014). These effects may facilitate temperate grassland diversification. Specifically, native *R. minor* has been demonstrated to suppress grasses and increase subordinate forbs, an effect extensively used in grassland restoration in the United Kingdom (e.g. Pywell *et al.* 2004; Westbury *et al.* 2006; Hellström, Bullock & Pywell 2011). However, this application of the hemiparasites concerns the re-creation of semi-natural grasslands on ex-arable land or restoration of grasslands degraded by high land-use intensity (fertiliser application mainly), that is, ecosystems in which biodiversity has been negatively affected by human activity. The underlying community effect of the hemiparasites is largely non-specific as it reduces standing crop and the grass:forb ratio (i.e. relation between broadly defined functional groups). By contrast, complete eradication of the competitive dominant and pronounced decrease of herb layer cover inflicted by *R. alectorolophus* in our study, represent extreme forms of these ecological effects of root hemiparasites.

Several studies testing the impacts of other (hemi)parasitic plants on particular competitive dominants revealed similarly drastic and at least partially specific effects (Yu *et al.* 2008; Prider, Watling & Facelli 2009; Shen *et al.* 2010; Decler, Bonte & van Diggelen 2013; Cirocco *et al.* 2015). Such suppressive effects suggest that native (hemi)parasitic plants may be considered as potential biological controls of competitive dominants possibly including invasive species. However, any such application must be underlain by detailed knowledge of both host and parasite biology and experimental field assays to ensure its efficiency and minimise risks of possible adverse side-effects.

Given the profound effects of many parasitic plants on ecosystems (Press & Phoenix 2005; Watson 2009) extensive damage may be incurred by an alien parasitic plant; therefore, only native parasitic plants may be considered. Local propagule sources should be used preferably to conserve local genetic resources. However, the lack of a clear phylogeographic pattern in *Rhinanthus* species in Europe (Vrancken, Brochmann & Wesselingh 2009, 2012) suggest a lower importance of this rule in this particular case. A

more pragmatic approach to seed origin may be considered; e.g., the recently suggested genetic diversity approach, which involves sowing a mixture of seeds originating from multiple sources (Kettenring *et al.* 2014).

Conclusions and applications

We demonstrated the potential of *R. alectorolophus* to act as a largely specific native biological control of competitive dominant *C. epigejos*. Depending on the community context, *Rhinanthus* may also facilitate restoration of biodiversity of *C. epigejos*-infested grasslands. Our study is one of the pioneering works to demonstrate native parasitic plants as promising tools to control problematic, mainly competitive, plant populations across the globe. The parasites' interference with the growth strategy of the targeted competitive species underlies the strong suppressive effect. Such targeted use of (hemi)parasitic plants complements their current generic use to facilitate diversification and increase forb abundance in HNV semi-natural grassland restoration.

Authors' contributions

J.T., J.M. and J.H. conceived the ideas and designed the methodology. J.T., J.M., J.H., T.T. and V.A. collected the data. J.T. and L.T. analysed the data. J.T. led the writing of the manuscript. All authors contributed to the drafts and gave final approval for publication.

Acknowledgements

We thank Czech Science Foundation (project 14-26779P) for financial support, Pavla Mládková, Ondřej Nezval, Miroslav Dvorský, David Opálka, Eva Holá, Julie Jandová, Šárka Jiráská, Adéla Krejčí, Jirí Sladký, Lukáš Svobodník and Petra Ebermannová for field assistance, and Richard Kidd for English revision.

Data accessibility

Vegetation composition and *Calamagrostis epigejos* biomass data are available at Dryad Digital Repository <https://doi.org/10.5061/dryad.4r390> (Těšitel *et al.* 2017).

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Received 24 October 2016; accepted 8 February 2017

Handling Editor: Lara Souza

Supporting Information

Details of electronic Supporting Information are provided below.

Fig. S1. Anatomical section of haustorium of *Rhinanthus alectorolophus* on the root of *Calamagrostis epigejos*.

Appendix S1. Community ordination analysis.

Appendix S2. Relative dominance of *Rhinanthus alectorolophus*.