NeoBiota 90: 97–121 (2024)
doi: 10.3897/neobiota.90.113069
https://neobiota.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
NeoBiota
Advancing research on alien species and biological invasions

Root hemiparasites suppress invasive alien clonal plants: evidence from a cultivation experiment

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Academic editor: R. Bustamante | Received 22 September 2023 | Accepted 29 November 2023 | Published 15 January 2024

Citation: Těšitelová T, Knotková K, Knotek A, Cempírková H, Těšitel J (2024) Root hemiparasites suppress invasive alien clonal plants: evidence from a cultivation experiment. NeoBiota 90: 97–121. https://doi.org/10.3897/neobiota.90.113069

Abstract

Alien invasive plants threaten biodiversity by rapid spread and competitive exclusion of native plant species. Especially, tall clonal invasives can rapidly attain strong dominance in vegetation. Root-hemiparasitic plants are known to suppress the growth of clonal plants by the uptake of resources from their belowground organs and reduce their abundance. However, root-hemiparasites' ability to interact with alien clonal plants has not yet been tested.

We explored the interactions between native root-hemiparasitic species, *Melampyrum arvense* and *Rhinanthus alectorolophus* and invasive aliens, *Solidago gigantea* and *Symphyotrichum lanceolatum*. We investigated the haustorial connections and conducted a pot experiment. We used seeds from wild hemiparasite populations and those cultivated in monostands of the invasive plants to identify a possible selection of lineages with increased compatibility with these alien hosts. The hemiparasitic species significantly suppressed the growth of the invasive plants. *Melampyrum* inflicted the most substantial growth reduction on *Solidago* (78%), followed by *Rhinanthus* (49%). Both hemiparasitic species reduced *Symphyotrichum* biomass by one-third. Additionally, *Melampyrum* reduced the shoot density of both host species. We also observed some transgenerational effects possibly facilitating the growth of hemiparasites sourced from subpopulations experienced with the host.

Native root hemiparasites can effectively decrease alien clonal plants' biomass production and shoot density. The outcomes of these interactions are species-specific and may be associated with the level of clonal integration of the hosts. The putative selection of lineages with higher performance when attached to the invasive novel hosts may increase hemiparasites' efficiency in future biocontrol applications.

Keywords

Asteraceae, biological invasion, biotic resistance, Orobanchaceae, physiological integration, pot experiment, restoration

Introduction

Alien plant invasions represent a component of global change with profound effects on diversity, ecosystem functioning and services. Invasive species broadly vary in their specific impacts on the habitats they invade due to different abilities to spread and achieve dominance or mechanisms of interaction with native biota (Blackburn et al. 2014). Of particular concern are the so-called transformer invaders (Richardson et al. 2000), which can invade indigenous natural communities over large areas, attain high dominance and change ecosystem functioning. Alien tall clonal herbs with below-ground rhizomes are frequent examples of transformer invaders in grasslands due to their increased competitiveness leading to the exclusion of native plants from infested vegetation throughout temperate regions (Divíšek et al. 2018; Wang et al. 2019; Wan et al. 2021; Lanta et al. 2022). Although conventional control management, represented by mowing or grazing, can reduce the density of the invasive clonal herbs to some extent (e.g. Nagy et al. (2020); Szymura et al. (2022)), they are usually not eliminated and may spread rapidly from the rhizomes if the management measures are ceased. More drastic restoration measures (e.g. use of herbicides, long-term shading) may eradicate the invaders, but can also adversely affect native species, making their use problematic in areas with conservation value (e.g. Weber and Jakobs (2005); Szymura et al. (2022)).

Native parasitic plants have recently been suggested as potential biocontrol agents for a wide range of invasive plants globally (Těšitel et al. 2020). Following the biotic resistance hypothesis (Maron and Vilà 2001), generalist native adversaries, such as parasitic plants, may impede the success of invaders due to the lack of defence or tolerance mechanisms of the host plants against parasitism (Cameron and Seel 2007). Clonal hosts could be especially harmed by parasitism, as the parasitic uptake of resources targets the cornerstone of their growth strategy, that is, the spatial spread of vegetative ramets and clonal integration (e.g. Song et al. (2013); Roiloa (2019)), i.e. the transfer of resources amongst interconnected ramets via rhizome network, which facilitates efficient resource acquisition and sharing (Kavanová and Gloser 2005; Gao et al. 2021). However, parasitic plants may turn this advantage into a liability. A parasite that attaches to one ramet may access nutrients within the network, leading to its vigorous growth and potentially marked biomass decline of the clonal host, including the non-infected ramets (Lepš and Těšitel 2015; Gao et al. 2021). This could explain a substantial decrease in the clonal hosts' abundance in the communities with parasitic plants observed in several studies (Decleer et al. 2013; Demey et al. 2015; Somodi et al. 2018). Moreover, field experiments have demonstrated the ability of root-hemiparasitic *Rhinanthus* species to significantly reduce harmful expansive clonal grass *Calamagrostis epigejos* from semi-natural grasslands (Těšitel et al. 2017, 2018), which was consequently introduced to ecological restoration practice (Lukavský 2020; SPPK D02 002 2021).

Amongst parasitic plants, species of root hemiparasites (or, more precisely, Euphytoid parasites in the new parasitic plant classification of Teixeira-Costa and Davis (2021)) appear to be particularly suitable candidates for suppressing clonal invasive species due to their low host specificity (e.g. Matthies (2017, 2021)), capacity to substantially suppress host growth (e.g. Press et al. (2005); Těšitel et al. (2015b); Matthies (2021)) and ability to form dense populations (van Hulst et al. 1987; Mudrák and Lepš 2010; Heer et al. 2018). Despite available evidence on the negative effects of root hemiparasites on expansive species (reviewed by Těšitel et al. (2020)), only one study has investigated the effect of a root-hemiparasitic species on an alien invader (Walder et al. 2019), which, however, did not show any adverse impact of the parasite on the host species. Two reasons may explain this lack of empirical research on interactions between root hemiparasites and alien invaders. First, hemiparasites and alien invaders may not share the same habitats. For instance, in Central Europe, an analysis of habitats of hemiparasitic species identified natural and semi-natural communities as their principal habitats (Těšitel et al. 2015a). These habitats are simultaneously characterised by low levels of alien invasions (Pyšek et al. 2012). Second, establishing a parasitic association with alien invaders may be difficult. Although hemiparasites are mostly host generalists, host quality (i.e. the extent of support of parasite growth) varies between species (e.g. Rowntree et al. (2014); Matthies (2017, 2021)). Native hemiparasitic species lack a common evolutionary history with non-indigenous plants. The lack of experience with an alien host may limit a hemiparasite's efficiency of resource withdrawal on the one hand, but also the host's resistance or tolerance to parasitism on the other, as predicted by the biotic resistance hypothesis. Compatibility with a host may also be affected by high intra- and interpopulation genotypic variability of the annual hemiparasites (Mutikainen et al. 2000; Rowntree et al. 2011; Unachukwu et al. 2017; Rowntree and Craig 2019; Moncalvillo and Matthies 2023). The recognised ability to rapidly evolve ecotypes adapted to various environmental conditions (Zopfi 1993; Pleines et al. 2013) may further facilitate the interaction with novel host species.

In this paper, we investigated the interactions between root-hemiparasitic *Rhinanthus alectorolophus* and *Melampyrum arvense* (Orobanchaceae) and the alien invasive clonal species *Solidago gigantea* and *Symphyotrichum lanceolatum* (Asteraceae). First, we examined the anatomy of haustoria to determine whether the hemiparasites can form functional parasitic connections with the novel hosts. Second, we set up a comprehensive pot experiment to study the effect of host identity on hemiparasite performance and the impact of hemiparasite infection on the two hosts. We expected to identify differences in vitality (measured by biomass production) of the two hemiparasite species (hypothesis 1), which should be reflected by a difference in host suppression (hypothesis 2). Specifically, we expected lower host suppression by *Rhinanthus*, given its general preference for grass or legume hosts (Matthies 2021), than in *Melampyrum*, which has been shown to flourish when attached to various forbs, including many Asteraceae

(Matthies 2017). Furthermore, we investigated the potential selection of hemiparasite lineages and their effect on host–hemiparasite interactions. To do so, we used seeds from hemiparasites that had grown for two years in monoculture stands of the two host species and compared their performance to plants from the original population from a species-rich grassland ('naïve' plants), i.e. all tested seed sources per hemiparasite species originated from a single hemiparasite population. We hypothesised that growth in a host monoculture might lead to a selection of lineages better adapted to the given host, reflected by improved hemiparasite growth and possibly a more deleterious effect on that host (hypothesis 3).

Materials and methods

Study species

Melampyrum arvense L. and *Rhinanthus alectorolophus* (Scop.) Pollich are annual xylem-feeding root-hemiparasitic species native to Europe. *Melampyrum* typically grows in dry grasslands and steppes, while *Rhinanthus alectorolophus* (Scop.) Pollich favours dry to mesic grasslands. *Solidago gigantea* Aiton and *Symphyotrichum lanceolatum* (Willd.) G. L. Nesom are perennial rhizomatous species from the Asteraceae family, originating from North America (Pyšek et al. 2012). They began spreading across Europe in the 19th century and have become serious invaders (Weber and Jakobs 2005; Jedlička and Prach 2006; Axmanová et al. 2021). *Solidago* and *Symphyotrichum* are considered typical wetland species, but they also occur in disturbed anthropogenic habitats, poorly-managed fields, pastures and meadows within their native range (Chmielewski and Semple 2001; Weber and Jakobs 2005). *Solidago* has a broader ecological niche in the invaded areas, also occupying drier and nutrient-poorer soils (Weber and Jakobs 2005). Both species have a perennial rhizome, which, in the spring, produces a cohort of shoots that start to flower in late summer (*Solidago*) or early autumn (*Symphyotrichum*) and yield numerous tiny wind-dispersed seeds. Jedlička and Prach (2006) noted the high viability of *Symphyotrichum lanceolatum* seeds, which, combined with the effective ability to penetrate established vegetation, triggers the high invasive potential of this species.

Haustorial connection

We initiated a pilot cultivation trial to examine the anatomy of haustorial connections between the hemiparasites and the two invasive hosts. The cultivation was set up in the experimental garden of the Department of Botany and Zoology at Masaryk University in Brno, Czech Republic. The hemiparasites' seeds were collected from species-rich vegetation in the summer of 2019 (see Suppl. material 1: appendix S1 for localisations). In the autumn of 2019, we transplanted rhizomes of host species into 15 × 15 × 20 cm pots (narrower at the bottom, corresponding to 3.6 litres), filled with a mixture of peat and garden soil (ratio 1:3). In October, we sowed 20 hemiparasite seeds on each pot. We established five replicates for each hemiparasite-host combination.

In June 2020, we rinsed the hosts' roots, harvested the haustoria and preserved them in 70% ethanol. Following the method of Soukup and Tylová (2014), we dehydrated the samples, transferred them to anhydrous butanol, infiltrated and then embedded them in paraffin. We prepared 12 μm sections using a sliding microtome (Reichert, Wien, Austria) and de-waxed and stained them with phloroglucinol-HCl (Wiesner solution) (Liljegren 2010) to colour the lignified cell walls.

Cultivation experiment

We established the main pot experiment in autumn 2021 to investigate and quantify the outcome of the novel interactions for the hemiparasites and the extent of host suppression. For each hemiparasitic species, we used three seed sources: (i) seeds from a wild population growing in a species-rich grassland and (ii) seeds from plants originally obtained from the same populations as in (i), but which had been growing since 2019 in monostands of the two invasive host species. The aim was to investigate the potential selection of genotypes more adapted to the specific hosts. More specifically, the monostands were mown in the autumn of 2019, after which we sowed the hemiparasites' seeds. In 2020, the monostands with hemiparasites were mown in July and October. We collected ripe hemiparasite seeds from all populations from June to July 2021. The seeds were stored at room temperature before use. As both host species produce a dense rhizome network in the topsoil layer, we collected soil blocks with rhizomes from monostands of each host species to establish host cultivation in September 2021. First, we removed the above-ground biomass and then cut approx. 12 × 12 cm rhizome blocks with a spade. The rhizomes were then inserted into the same pots and soil substrate described in the chapter 'Haustorial connection'. See Suppl. material 1: appendix S1 for GPS coordinates of the sites of hemiparasites' seed and host plants' origin.

The experimental design comprised: (i) an uninfected control treatment (host species without hemiparasite seed addition) and three types of 'infected' treatments (with hemiparasite seeds addition), i.e. treatments (ii) 'naïve' (seeds of hemiparasites originating from a wild population), (iii) 'home' (seeds from hemiparasites growing for two years in a monostand of a host species and then sown with the same host species in the pot) and (iv) 'cross' (seeds from hemiparasites growing for two years in a monostand of one host species and then sown into the pot with the other host species) (see the scheme of the origin of hemiparasites' seeds in Fig. 1). Both hemiparasites were sown with both invasive species, resulting in 14 treatments. Each treatment consisted of 10 replicates of the pots, totalling 140 pots. Each pot in the 'infected' treatments (treatments ii–iv) received 40 seeds of one of the hemiparasitic species. Seeds were spread on the surface and gently mixed with the topsoil layer. The pots were then placed in the experimental garden in Brno, following a completely randomised design and irrigated. During spring 2022, the pots were irrigated as necessary. In April 2022, seedlings of

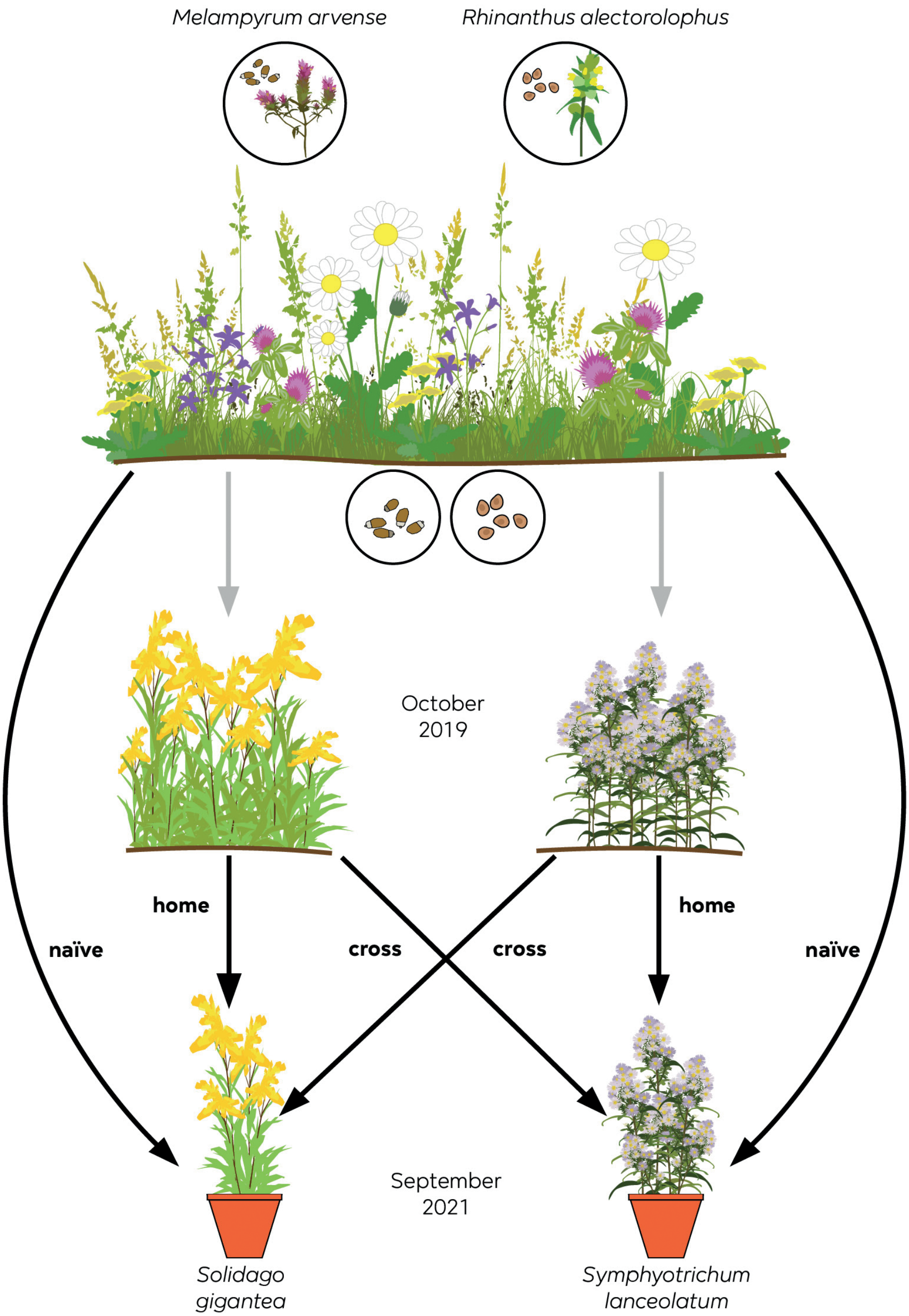

Figure 1. Scheme of origin of the hemiparasites' seeds used in the cultivation experiment. In October 2019, seeds of *Melampyrum arvense* and *Rhinanthus alectorolophus* from a single population per species, originating from a species-rich grassland, were sown in monostands of the host species *Solidago gigantea* and *Symphyotrichum lanceolatum*. By 2021, hemiparasite seeds collected from the host species' monostands and the original hemiparasite population were used in the cultivation experiment resulting in three types of hemiparasite seed sources: 'naïve', 'home' and 'cross'.

non-target species were removed from the pots. The pots were spaced 30 cm apart and their position within the experimental matrix was changed three times before harvest at the beginning of June 2022.

The experiment was harvested during hemiparasite flowering. We cut the above-ground biomass and counted the number of host shoots and hemiparasitic plants that survived in each pot. The hemiparasite and host biomass from each pot were dried separately at 80 °C and weighed. Schmid et al. (1995) revealed a strong dependency of sexual reproduction and clonal growth on plant size as well as a threshold size for sexual reproduction in *Symphyotrichum lanceolatum* and *Solidago canadensis*, a species closely related to *Solidago gigantea*. We thus expected the vegetative biomass to reflect host fitness and reproductive potential sufficiently.

Statistical analyses

Initially, we conducted an exploratory analysis of patterns in counts of hemiparasite individuals, host ramets and above-ground biomass production to identify pots that were not representative due to insufficient host or hemiparasite recruitment. Only pots with at least six host shoots and three hemiparasite individuals (in infected treatments) were subsequently included in the analysis (n = 132 out of 140 pots). Scatterplots of biomass vs. individual or shoot counts (Suppl. material 1: appendices S2, S3) demonstrated low correlations, indicating compensatory growth in pots retained for the analysis.

We used linear models to analyse the following parameters: hemiparasite above-ground biomass, the number of individuals, mean biomass per individual and host above-ground biomass, the number of shoots and mean biomass per shoot. All variables were log-transformed before analysis to improve the normality of residuals and homogeneity of variances. The analysis of each parameter, used as response variables, was conducted at two levels: (i) the species-level model included hemiparasite, host species and their interaction as predictors. Seed-source treatments were disregarded in this analysis; (ii) seed-source analysis consisted of a series of linear models, one for each host–hemiparasite combination, with seed-source treatment as a single predictor. In this analysis, we set treatment contrasts with the 'naïve' treatment as the baseline level, to which the two other treatments were compared. Only biomass data were tested in the seed-source level analysis.

We first built a saturated model for each analysis with all candidate predictors and interactions. Individual terms of the saturated models were tested by an F-test, the results of which are reported in ANOVA tables as in a classical two-way ANOVA with interactions. Non-significant ($P > 0.05$) terms were subsequently removed from the models in the backward predictor selection procedure. Non-significant main effects were retained if a predictor was involved in a significant interaction. The resulting minimal adequate models were then used to extract regression coefficients and their associated tests of significance. This approach was allowed by the nature of our data coming from a manipulative experiment with a balanced design, which implies the orthogonality of the predictors. We acknowledge that the orthogonality was not perfect because we removed a few pots with low establishment of hosts or parasites. Still, the collinearity between the tested effects (host and parasite predictors) was minimal as measured by the phi-coefficient ($\varphi = 0.026$; $\chi_1 = 0.0084$; $P = 0.927$), which justifies the validity of the interaction-term testing and supports backward selection as a suitable model-selection approach. All analyses were performed in R, version 4.2.2 (R Core Team 2022).

Results

Functional haustorial connection

Both hemiparasitic species formed fully developed haustoria on the roots and rhizomes of both host species. In all cases, the xylem bridge from hemiparasite haustoria reached the xylem vessels of the hosts. No signs of a defensive reaction by the hosts were observed (Fig. 2).

Host–Hemiparasite interaction on the species level

Hosts successfully resprouted from rhizomes in the transferred soil blocks; only four pots had to be omitted because of insufficient sprouting (Fig. 3, Suppl. material 1: appendix S4). The number of hemiparasite plants varied in the pots, but their establishment was generally successful, with only four pots omitted from the experiment due to poor hemiparasite establishment. On average, 10.9 *Melampyrum* plants were harvested in pots with both host species (max. 20 individuals). In contrast, significantly higher average numbers of *Rhinanthus* plants, 16.2 and 13.2, were harvested in pots with *Solidago* and *Symphyotrichum* (max. 23 individuals), respectively (Table 1, Fig. 4, Suppl. material 2 for the primary data). Hemiparasite biomass production differed between the two species and was also significantly affected by the host identity (Table 1). Specifically, *Melampyrum* grew larger than *Rhinanthus* ($t_{110} = 11.25$, $P < 10^{-6}$) and *Solidago* supported a more vigorous hemiparasite growth than *Symphyotrichum* ($t_{110} = 10.12$, $P < 10^{-6}$). These effects were additive, i.e. the difference in the host quality had a similar impact on both hemiparasitic species (Fig. 4). Similar trends and significant interactions were also found concerning the average biomass of hemiparasite individuals (Table 1). *Melampyrum* individuals were consistently larger than *Rhinanthus* and both hemiparasitic species produced larger specimens on *Solidago* than on *Symphyotrichum*. However, this trend was less pronounced in *Rhinanthus*, i.e. *Rhinanthus* individuals growing with *Symphyotrichum* were larger than expected by additive effects ($t_{109} = 2.57$, $P = 0.012$; Fig. 4).

Regarding host suppression, we identified strong interactive effects of host and hemiparasite species identities on the host biomass (Table 1). The hemiparasitic spe-

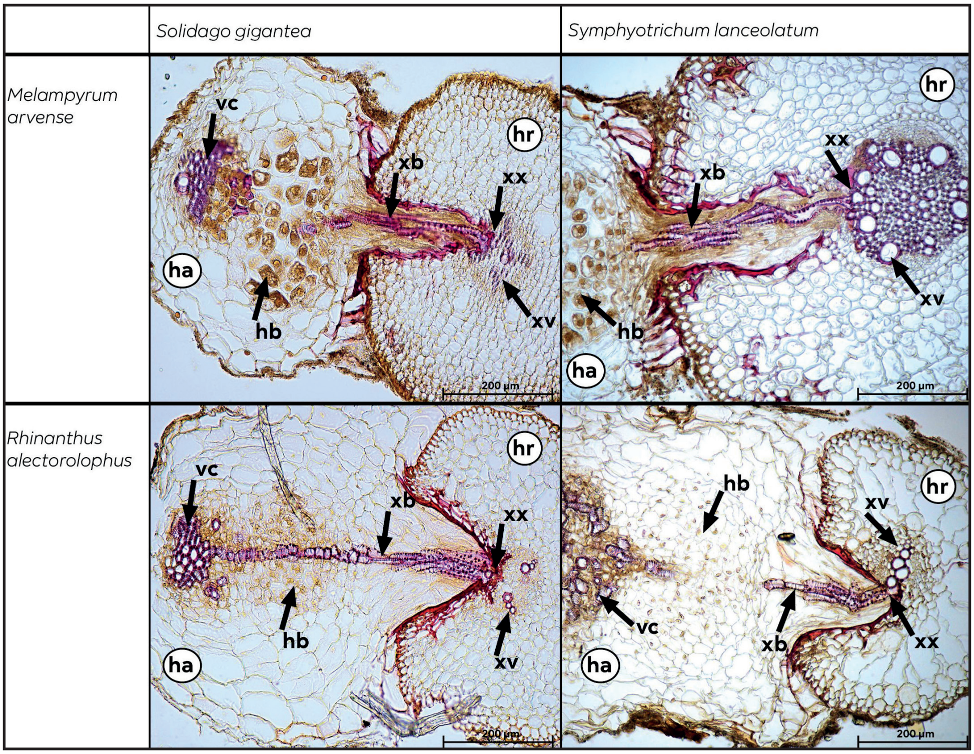

Figure 2. Cross sections of haustorial connections between two root-hemiparasitic species and their hosts. In the hemiparasite haustoria (ha), there is a hyaline body (hb), the vascular core of the haustorium (vc) and a xylem bridge (xb) leading to host xylem vessels (xv) in the host root (hr); xx – xylem–xylem contact.

cies significantly reduced host biomass relative to uninfected controls (*Melampyrum*: $t_{126} = -10.1$, $P < 10^{-6}$, *Rhinanthus*: $t_{126} = -4.53$, $P < 10^{-4}$), but the suppression was significantly more pronounced in *Solidago* infected by *Melampyrum* ($t_{126} = 4.50$, $P < 10^{-5}$; Fig. 5). Overall, *Solidago* biomass was reduced by 77.6% and 49.1% on average when infected by *Melampyrum* and *Rhinanthus*, respectively. *Symphyotrichum* biomass was reduced by 31.6% and 35.2% on average by *Melampyrum* and *Rhinanthus*, respectively. Host biomass was reduced by decreasing the number of host shoots or reducing the average biomass of host shoots. While *Melampyrum* acted in both ways, *Rhinanthus* mainly decreased the average host shoot biomass (Fig. 5). In detail, *Melampyrum* reduced the number of host shoots per pot ($t_{128} = -4.05$, $P < 10^{-4}$) by 33% in *Solidago* and 21% in *Symphyotrichum*. The effect of *Rhinanthus* on the host shoot number was not significant ($t_{128} = -0.76$, $P = 0.45$). Both *Melampyrum* ($t_{126} = -7.07$, $P < 10^{-6}$) and *Rhinanthus* ($t_{126} = -4.00$, $P < 0.001$) reduced the average biomass of host shoots. While *Rhinanthus* reduced the average shoot biomass of both hosts to a similar extent, *Melampyrum* was significantly less deleterious to *Symphyotrichum* than to *Solidago* ($t_{126} = 3.98$, $P < 0.001$).

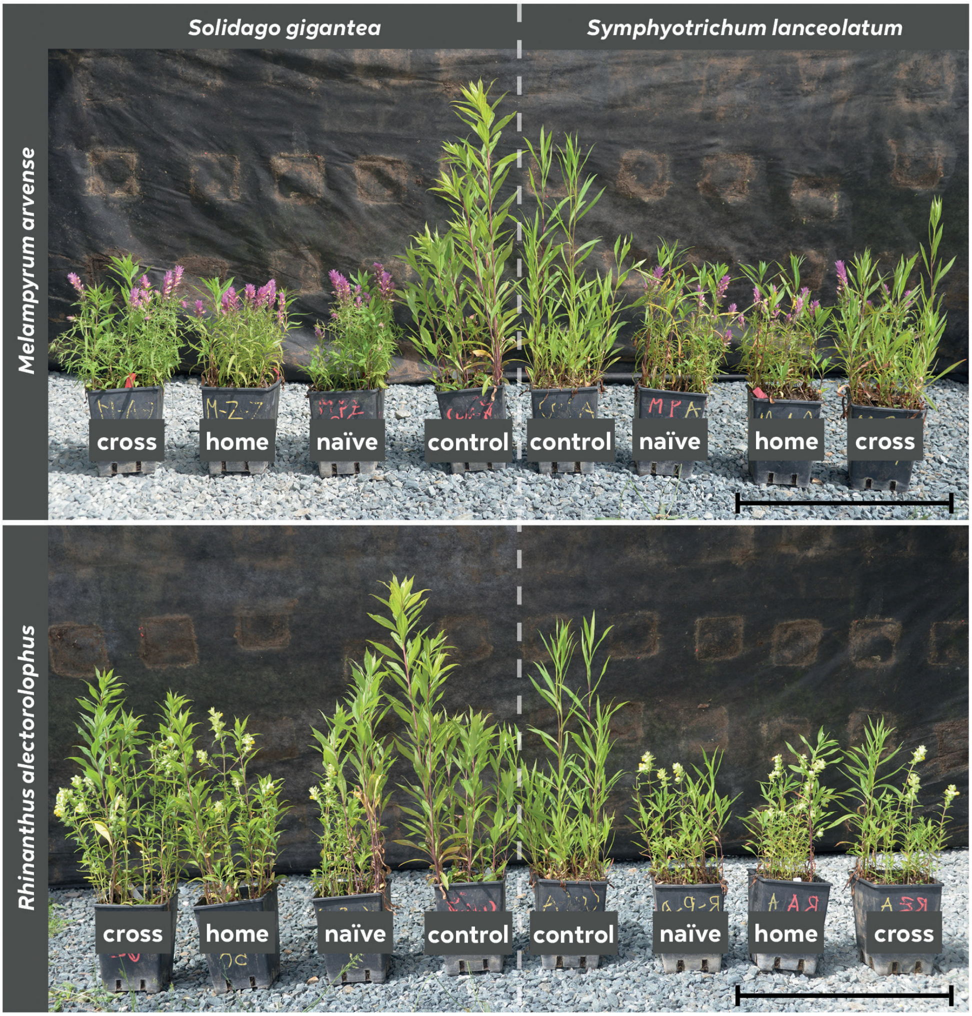

Figure 3. Representative pots for each hemiparasite seed-source treatment ('cross', 'home', 'naïve') and the uninfected control. *Solidago gigantea* (left) and *Symphyotrichum lanceolatum* (right) are infected by *Melampyrum arvense* (top) or *Rhinanthus alectorolophus* (bottom). The bottom photo is flipped vertically for clarity of the experiment presentation. Photographic documentation of all experimental pots is provided in Suppl. material 1: appendix S4. Scale bars: 50 cm.

Effect of the hemiparasite seed origin on the interaction

We identified the significant effects of the hemiparasite seed-source treatments on some interactions. Total hemiparasite biomass was affected in the case of *Melampyrum* growing on *Solidago* ($R^2 = 0.29$, $F_{2,24} = 4.98$, $P = 0.016$) and *Rhinanthus* growing on *Symphyotrichum* ($R^2 = 0.32$, $F_{2,25} = 5.81$, $P = 0.008$). Specifically, *Melampyrum* plants

Table 1. Analysis of variance tables summarising the effects of hemiparasite and host species identity on the growth of hemiparasites and hosts.

Response	Effect	df	Sum Sq.	*F*	*P*
Hemiparasite biomass	Hemiparasite	1	13.72	121.95	**$< 10^{-6}$**
	Host	1	11.65	103.48	**$< 10^{-6}$**
	Hemiparasite × Host	1	0.24	2.10	0.15
	Residuals	109	12.28		
Hemiparasite count per pot	Hemiparasite	1	3.89	19.64	**$< 10^{-4}$**
	Host	1	0.35	1.75	0.19
	Hemiparasite × Host	1	0.29	1.46	0.23
	Residuals	109	21.60		
Hemiparasite average biomass	Hemiparasite	1	32.23	203.10	**$< 10^{-6}$**
	Host	1	7.97	50.22	**$< 10^{-6}$**
	Hemiparasite × Host	1	1.05	6.61	**0.011**
	Residuals	109	17.30		
Host biomass	Hemiparasite[*]	2	16.11	46.12	**$< 10^{-6}$**
	Host	1	0.02	0.13	0.72
	Hemiparasite × Host	2	7.69	22.00	**$< 10^{-6}$**
	Residuals	126	22.00		
Host shoot count per pot	Hemiparasite[*]	2	2.53	13.09	**$< 10^{-5}$**
	Host	1	5.07	52.42	**$< 10^{-6}$**
	Hemiparasite × Host	2	0.31	1.59	0.21
	Residuals	126	12.18		
Host shoot average biomass	Hemiparasite[*]	2	7.28	18.35	**$< 10^{-6}$**
	Host	1	5.77	29.10	**$< 10^{-6}$**
	Hemiparasite × Host	2	5.04	12.71	**$< 10^{-5}$**
	Residuals	126	24.99		

[*] The hemiparasite effect on host biomass also comprises non-infected control as an extra level.

in the ‘cross’ treatment (seeds from plants previously grown with the alternative invasive host) produced significantly less biomass ($t_{24} = -2.80$, $P = 0.010$) compared to the ‘naïve’ treatment (seeds from species-rich vegetation), while the biomass of *Melampyrum* on *Solidago* from the ‘home’ (seeds from plants previously grown with the same host species) and ‘naïve’ treatment did not significantly differ (Fig. 6). Conversely, the biomass of *Rhinanthus* on *Symphyotrichum* was significantly higher in the ‘home’ treatment compared to the ‘naïve’ treatment ($t_{25} = 3.09$, $P = 0.005$) and the hemiparasite biomass in the ‘cross’ and ‘naïve’ treatment did not differ (Fig. 6).

Host biomass was significantly affected only in the case of *Solidago* infected by *Rhinanthus* ($R^2 = 0.27$, $F_{2,27} = 5.09$, $P = 0.013$) (Fig. 7). Here, *Rhinanthus* of ‘home’ and ‘cross’ treatments suppressed *Solidago* biomass more than ‘naïve’ *Rhinanthus* plants ($t_{27} = -2.73$, $P = 0.011$ and $t_{27} = -2.80$, $P = 0.009$ for ‘home’ and ‘cross’ treatments, respectively).

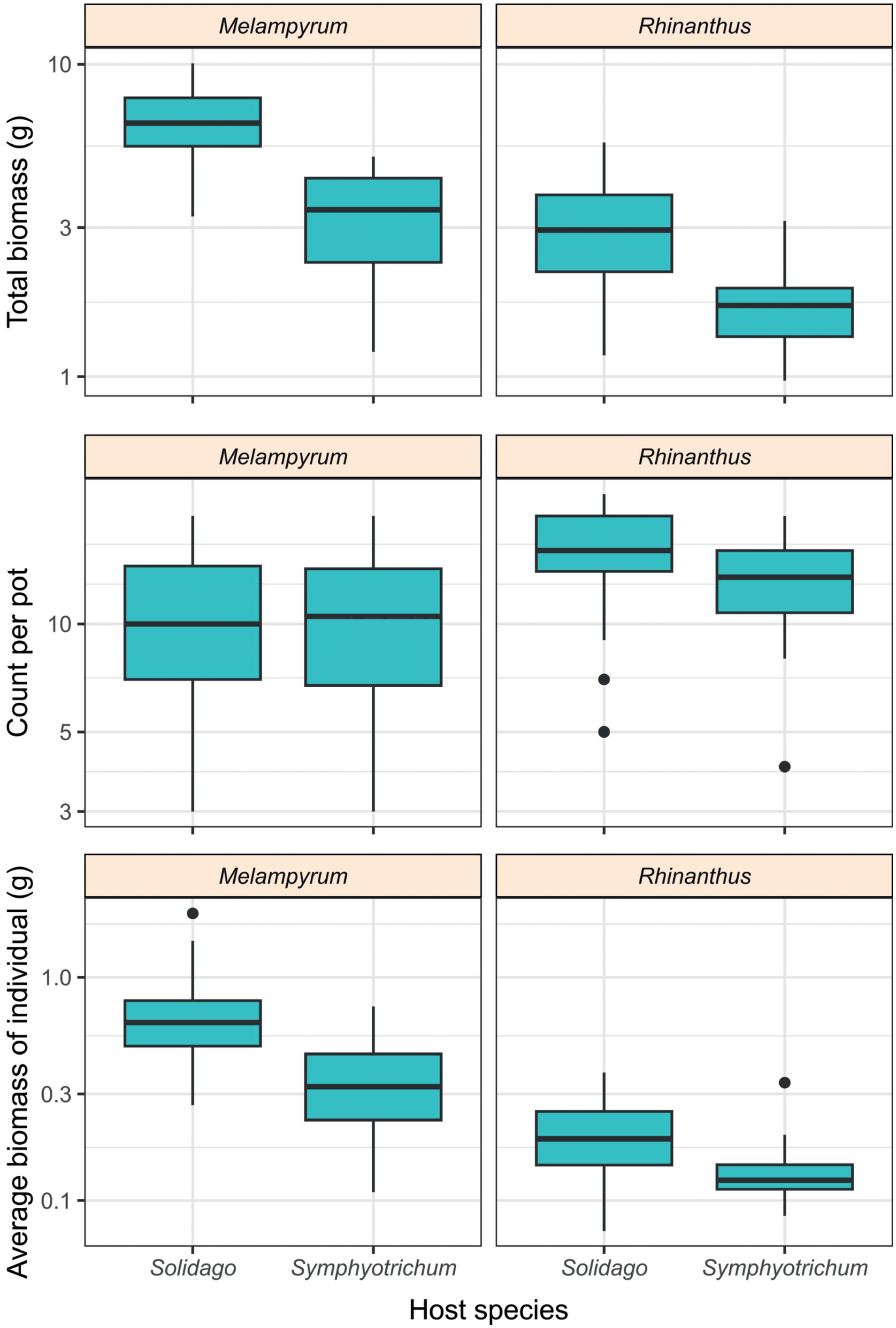

Figure 4. Effects of host species on the total biomass, number of individuals per pot and average biomass of the individuals of the two hemiparasitic species. Boxplots represent median, quartiles and ranges. See Table 1 for the ANOVA tables summarising significance tests. Note the logarithmic scale of the y-axes.

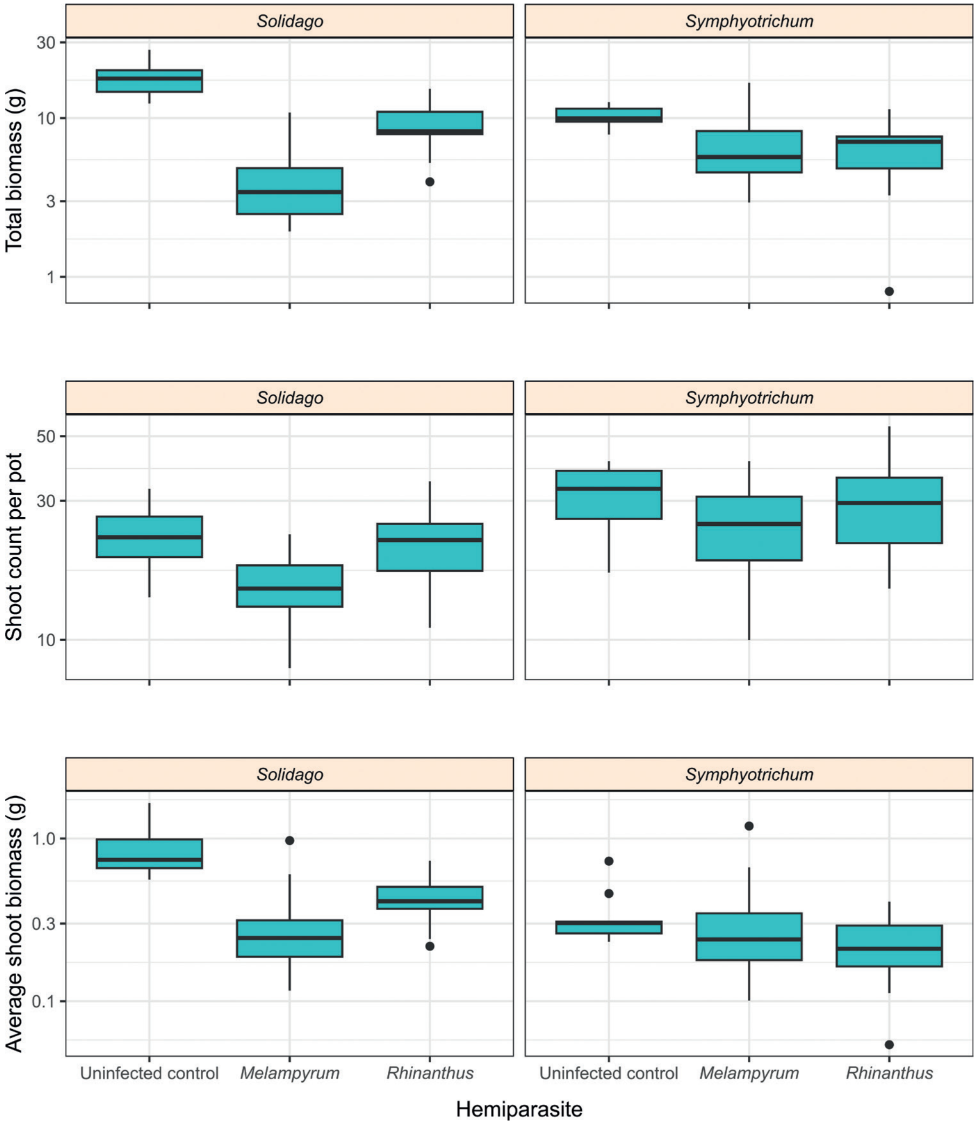

Figure 5. Effect of hemiparasite infection on total biomass, number of shoots per pot and average shoot biomass of the two host species. Boxplots represent median, quartiles and non-outlier ranges, with outliers displayed as points outside the non-outlier ranges. Note the logarithmic scale of the y-axes. See Table 1 for the ANOVA tables summarising significance tests.

Discussion

The outcome of the novel host–hemiparasite interactions

Both root-hemiparasitic species established a functional parasitic association with the two novel host species, as evidenced by functional haustorial connection, vital growth

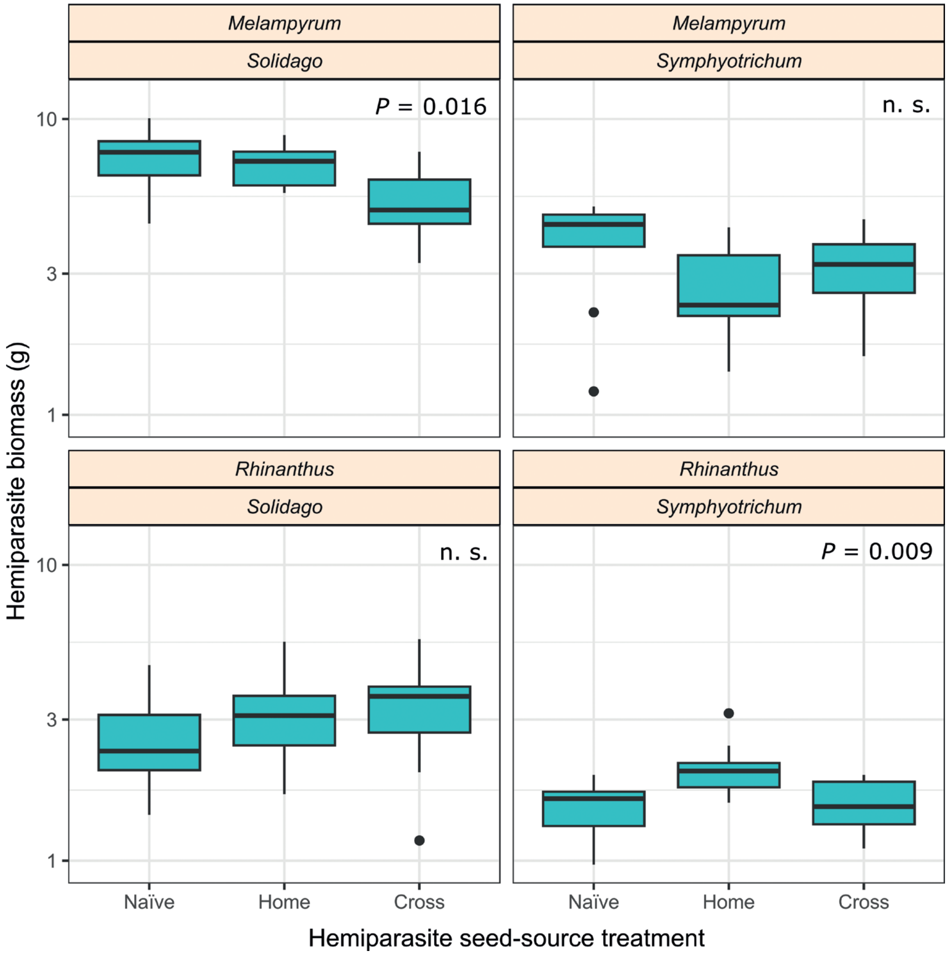

Figure 6. Effect of seed-source treatments on hemiparasite biomass production categorised by the individual host–hemiparasite combinations. Boxplots represent median, quartiles and non-outlier ranges, with outliers displayed as points outside the non-outlier ranges. *P*-values indicate significant effects of seed-source treatments. Note the logarithmic scale of the y-axes.

and flowering of both parasites (Figs 2, 3). In line with our first hypothesis, the hemiparasite species differed in compatibility with the two invasive hosts from Asteraceae, with *Melampyrum* proving a more efficient parasite than *Rhinanthus*. This outcome is not surprising, as *Melampyrum* has previously been shown to thrive when attached to a series of forbs. Asteraceae species, such as *Achillea millefolium*, *Matricaria chamomilla* and *Taraxacum officinale*, were even amongst the top five hosts out of 27 potential hosts tested (Matthies 2017). The average biomass of *Melampyrum* individuals of ca. 500 mg on *Solidago* and 300 mg on *Symphyotrichum* classifies these species amongst the best or moderately good hosts, respectively (in comparison to Matthies (2017)). *Rhinanthus* spp. have been repeatedly reported to grow better when attached to grasses or legumes

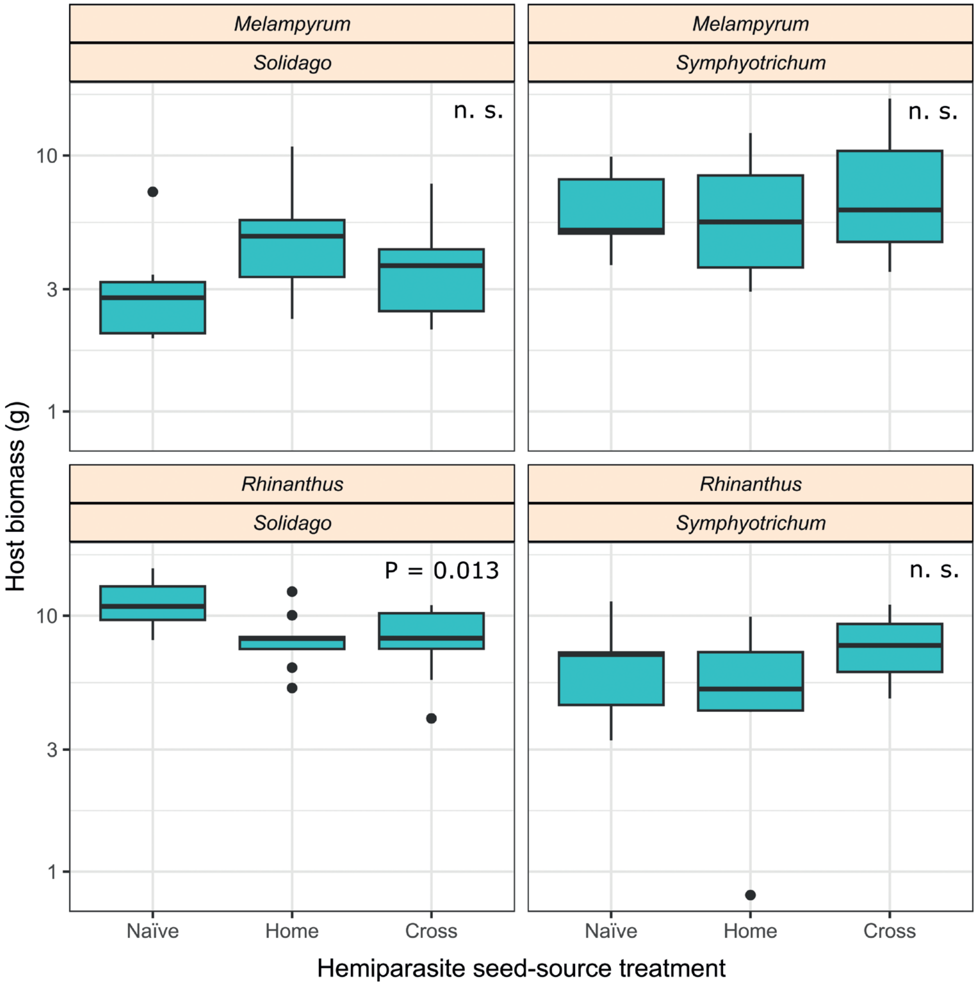

Figure 7. Effect of seed-source treatments on host biomass production in infected pots categorised by the individual host–hemiparasite combinations. Boxplots represent median, quartiles and non-outlier ranges, with outliers displayed as points outside the non-outlier ranges. *P*-values indicate significant effects of seed-source treatments. Note the logarithmic scale of the y-axes.

than forbs (Cameron and Seel 2007; Rowntree et al. 2014; Matthies 2021). The biomass production of *Rhinanthus alectorolophus*, converted to values per 1 m^2, amounted to 136 g DW and 75.6 g DW when attached to *Solidago* and *Symphyotrichum*, respectively. These values are lower than those reported for the best grass hosts in a recent field cultivation experiment (Hejduk et al. 2020). Still, *Solidago* can be considered a similarly good host for *Rhinanthus alectorolophus* as *Lotus corniculatus*, the best host amongst legumes. *Symphyotrichum* is a host of lower quality, but still comparable to some grasses (*Festuca rubra*) or legumes (*Trifolium hybridum*) (Hejduk et al. 2020). Compared to pot cultivations, the two invasive hosts can also be considered of at least moderate quality for *Rhinanthus alectorolophus* with an average biomass of individuals of about 220 mg and 150 mg on *Solidago* and *Symphyotrichum*, respectively. The biomass values per individual may be up to five times higher with the best host species in greenhouse pot cultivations (Těšitel et al. 2015b; Matthies 2021). However, the hemiparasitic plants in these cultivation experiments could benefit from optimal greenhouse conditions, including sufficient soil nutrients and reduced intraspecific competition due to the presence of only a single hemiparasite individual in each pot (Matthies 2021).

Both hemiparasitic species significantly suppressed the growth of both host species, which is the first experimental demonstration of an adverse effect of root hemiparasites on invasive species. We expected that the growth of the hemiparasites would correlate with the level of host suppression (hypothesis 2), which was only partially supported. Both hemiparasitic species reduced *Symphyotrichum* above-ground biomass by a third despite a significant difference in hemiparasite biomass (Figs 4, 5). Conversely, *Solidago* growth was reduced by 80% and 50% when parasitised by *Melampyrum* and *Rhinanthus*, respectively, corresponding to the difference in biomass production of the two hemiparasitic species and also to maximal levels of host biomass suppression reported from previous pot experiments (70% and 65% by *Melampyrum arvense* and *Rhinanthus alectorolophus*, respectively; Těšitel et al. (2015b); Matthies (2017); Sandner and Matthies (2018)). The difference in the host suppression could be related to their clonal growth characteristics, specifically the persistence of ramet connection. The clonal connections of *Solidago* ramets may persist for several years, while connections amongst *Symphyotrichum* ramets decay after one year (Schmid et al. 1995; Klimešová and Klimeš 2006). Schmid and Bazzaz (1987) suggested stronger physiological integration in *Solidago* due to the larger effects of experimental rhizome severance on *Solidago gigantea* growth than *Symphyotrichum*. The persistent clonal spread was identified as a significant positive predictor of hemiparasite-induced growth reduction (Demey et al. 2015); thus, the putatively stronger integration of *Solidago* ramets could be one of the reasons for the more extensive damage inflicted by the parasites. Physiological integration may be a trait contributing to a species' susceptibility to plant parasitism.

Examining the interactions between clonal hosts and hemiparasites presents a challenging task. Pot experiments are necessary to isolate the interaction between the host and the generalist hemiparasites from the natural community context, ensuring no other plant serves as a host. Typically, hosts are grown from seeds in these experiments, with hemiparasites later germinating in the pots or being transplanted as pregerminated seedlings. Consequently, hemiparasites attach to young host individuals that have not yet developed clonal growth. Furthermore, arbitrary numbers of host and hemiparasite individuals (sometimes as low as one host with one hemiparasite) are used in most of the pot experiments (e.g. Cameron and Seel (2007); Rowntree et al. (2014); Těšitel et al. (2015b); Matthies (2017); Sandner and Matthies (2018); but see, for example, Matthies (1995) and Hejduk et al. (2020) for hemiparasite density-manipulation experiments). These issues limit the potential of such experiments to elucidate the clonal host–hemiparasite interaction because, in natural communities, hemiparasite seedlings mostly attach to mature individuals of perennial plants with a fully-developed root system and clonal-growth organs. Thanks to transplanting whole soil blocks from the host population, our experiment maintains the host plant properties (developmental stage, ramet density) as close to natural conditions as possible. In addition, the hemiparasite seedlings were permitted to develop under natural climatic conditions, host phenological development and at densities close to realistic values (van Hulst et al. 1987; Mudrák and Lepš 2010). Hence, our experiment paves the way to more realistic pot experiments studying clonal host–hemiparasite interactions, which are of particular significance in European grassland ecosystem (Demey et al. 2015; Lepš and Těšitel 2015; Těšitel et al. 2017).

Conservation perspective

Pronounced biomass suppression of *Solidago* and *Symphyotrichum* is noteworthy from the restoration perspective. Both species are invasive, often achieving dominance and significantly impacting above-ground diversity (Hejda et al. 2021; Cubino et al. 2022; Fenesi et al. 2023). *Solidago* spp. also affected below-ground soil properties and the activity and biomass of soil bacteria and fungi (Zhang et al. 2009a, b; Scharfy et al. 2010; Pergl et al. 2023). Both species are listed in the second most serious category in the Black List of invasive species (BL2) with a massive environmental impact (Pergl et al. 2016). Hence, reducing their populations is crucial, particularly at sites of high conservation values. Mowing twice, a standard management technique for vegetation infested with *Solidago gigantea*, reduces the species' dominance. Cover reduction by 75% of the initial cover was reported over the long term, but the species is still persistent in the vegetation (Nagy et al. 2020; Szymura et al. 2022). A more pronounced suppression of *Solidago* may be achieved through cattle and sheep grazing or flooding (> 95% suppression; Nagy et al. (2020)). Despite the rapid spread of *Symphyotrichum lanceolatum* in wetland habitats of high natural value (Lanta et al. 2022), no information on managing this invasion is available. Biological control by introducing specialised insects or fungi from the species' native range has not been established yet in the invaded ranges, though several non-native insect enemies may be available in the case of *Solidago* (Fontes et al. 1994; Sheppard et al. 2006). Another biocontrol option available in subtropical regions may represent the widely-spread fungus *Sclerotium rolfsii*, causing the southern blight disease. Wilting of *Solidago canadensis*, induced by this fungus, has been reported from China (Tang et al. 2010) and the fungus application combined with soil disturbance led to 90% decrease in *Solidago canadensis* stem density (Zhang et al. 2019).

Using native hemiparasitic plants in combination with standard mowing management may offer another tool for the biocontrol of the two study species without any potential risks of introducing alien organisms to the ecosystems. The effects of hemiparasites on the invasive hosts observed in our experiment are comparable to the level of the suppression of *Calamagrostis epigejos* by *Rhinanthus alectorolophus* reported in previous research (Těšitel et al. 2017). The reduction and even elimination of this expansive grass by *Rhinanthus* have been established as a standard tool of biodiversity restoration in nature conservation in the Czech Republic (SPPK D02 002 2021). In contrast, *Melampyrum arvense* has not been used in ecological restoration so far, possibly because it is now considered a vulnerable species confined mainly to steppes and protected areas in Central Europe (Těšitel et al. 2015a). However, this species used to be a noxious weed in winter cereal fields (e.g. Rau (1970); Çetinsoy (1980); Matthies (1995)). It can increase its biomass by 1/3 in nutrient-rich soil and prefers hosts from nutrient-rich environments (Matthies 2017). Such ecological characteristics align with the ecology of *Solidago* and *Symphyotrichum*, sometimes called 'old-field perennials' (Schmid and Bazzaz 1987; Schmid et al. 1995), which efficiently colonise bare ground, fallows and disturbed urban areas and thrive in humid, nutrient-rich soils. Our experimental results demonstrate the ability of both hemiparasites to suppress the invasive species, but implementing this finding in ecological restoration requires further testing in the field conditions over longer periods.

Genotype adaptation

We identified transgenerational effects in hemiparasitic interactions thanks to using hemiparasite seeds of the same population origin, but cultivated for two generations (= years) with the target host. The effects were not universal across all host–hemiparasite combinations; however, where present, they generally supported our hypothesis 3. Specifically, when the hemiparasites were exposed to the target host species during two previous generations, the offspring plants produced relatively more biomass (Fig. 6) or were more detrimental to the host (Fig. 7) in some host–hemiparasite combinations. The effects were more pronounced on the hemiparasite side of the association, a pattern identified in a previous study on genotype effects in root-hemiparasitic interactions (Rowntree et al. 2014). Two mechanisms may be at play here: classical genetics and the selection of alleles that provide better compatibility with a host species or epigenetic (maternal) effects acting in the same way (Anastasiadi et al. 2021). We are not able to distinguish between these two with the current data. Even in model organism studies, the state-of-the-art methodology struggles to provide absolute separation of selection and epigenetics (Schmid et al. 2018). However, any adaptive process facilitating the association with novel hosts is crucial for the biotic resistance role of the parasites.

The existence of transgenerational effects in host–hemiparasite compatibility suggests that the breeding of genotypes more compatible with the target invasive hosts or exposing the mother plants to the novel host species may potentially increase the success of biocontrol applications, at least in the case of *Rhinanthus*. The feasibility of such an approach is also supported by the observations of rapid adaptive evolution in *Rhinanthus alectorolophus* in response to environmental conditions and host species (Zopfi 1993; Pleines et al. 2013; Moncalvillo and Matthies 2023). The genetic diversity of hemiparasites was also demonstrated to be a significant predictor of their establishment success and fitness when cultivated with multiple host species (Rowntree and Craig 2019). Therefore, while breeding hemiparasites in monospecific host stands may be efficient for specific purposes, it is equally important to preserve the genetic diversity of the populations of hemiparasitic species in nature and in seed production for ecological restoration; for instance, by cultivating hemiparasites with various host species from different plant functional groups so that the pool of genotypes efficient in various host–hemiparasites combinations is not depleted.

Acknowledgements

The authors thank Pavel Dřevojan, Zuzana Plesková, Zdenka Preislerová, Terezie Chamrátová, Maroš Šlachtič for help with the experiment setting and harvesting and David Watson and Ramiro Bustamante for their insightful comments on the manuscript. This work was supported by the Czech Science Foundation (project 21-22488S).

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Supplementary material 1

Supplementary information

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Data type: docx

Explanation note: **appendix S1:** Location of source localities of hemiparasite seeds and invasive host plants. **appendix S2:** Overview of the number of hemiparasite specimens and their biomass in the experimental treatments. **appendix S3:** Overview of host shoot counts and host biomass in the experimental treatments. **appendix S4:** Photographic documentation of all pots representing the experimental treatments.

Link: https://doi.org/10.3897/neobiota.90.113069.suppl1

Supplementary material 2

Primary data table

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Data type: xlsx

Link: https://doi.org/10.3897/neobiota.90.113069.suppl2