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The effect of multiple host species on a keystone parasitic plant and its aphid herbivores.

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Summary

1) The exploitation of shared resources by diverse organisms underpins the structure of ecological communities. Hemi-parasitic plants and the insect herbivores feeding on them both rely, directly and indirectly, on the resources supplied by the parasite's host plant. Therefore, the identity and

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number of host plant species providing these resources is likely to be critical for parasite and herbivore performance.

2) We tested the effect of single and multiple host species on the biomass of the generalist parasitic plant *Rhinanthus minor* and the abundance of its aphid (*Aphis gossypii*) herbivores.
3) Parasite biomass was proportional to the number of haustorial connections to host roots and was determined by host species identity rather than host functional group. Host species identity was also an important influence on aphid population size, and parasites attached to *Lotus corniculatus* experienced a considerable reduction in aphid herbivory.
4) The effects on the parasite of attaching to multiple hosts depended on the combination of species present. However, host mixtures generally benefitted aphids by diluting the negative effects of particular host species.
5) Our findings suggest that the specificity of host attachment alters the impact of this keystone parasitic plant on its own herbivores and, potentially, on the wider plant and herbivore community.

Key words *Rhinanthus minor*, *Aphis gossypii*, *Lotus corniculatus,* direct and indirect effects, mixed hosts, herbivory

Introduction

Parasitic plants are present, often in high abundance, in many ecosystems where they affect not only their hosts, but indirectly impact on many other organisms. They have been shown to have major effects on the structure and function of ecological communities (Press & Phoenix 2005), including changing plant community diversity

and structure (Joshi, Matthies & Schmid 2000; Ameloot, Verheyen & Hermy 2005), influencing nutrient cycling (Quested, Press & Callaghan 2003; Fisher *et al.* 2013), altering soil microbial communities (Bardgett *et al.* 2006) and affecting the performance of invertebrate herbivores (Marvier 1996; Ewald, John & Hartley 2011).

Rhinanthus minor is a generalist hemi-parasitic plant (Gibson & Watkinson 1989). It is a widespread component of grasslands throughout Europe and North America (Westbury 2004) and exerts much of its influence by dramatically reducing host plant biomass (Cameron *et al.* 2005). Like many other parasitic plants, *R. minor* establishes cellular continuity with the xylem stream of its hosts via specialised organs (haustoria), thereby extracting nutrients (Kuijt 1969; Riopel & Timko 1995). The severity of the impact of *R. minor* on its hosts, and its consequent community level effects, depend, at least in part, on host species identity and the ability of the host to tolerate or resist infection (Gibson & Watkinson 1991; Cameron, Coats & Seel 2006; Rowntree, Cameron & Preziosi 2011).

Just as *R. minor* has differential effects on host species, hosts can be more, or less, beneficial to the parasite. Variation in the performance of *R. minor* appears to result largely from how well the host plant can defend its xylem stream from the hemi-parasite (Cameron, Coats & Seel 2006; Cameron & Seel 2007), and to variation in the types and amounts of solutes that the hemi-parasite can remove from its host (Seel, Cooper & Press 1993; Press 1995), although host growth rate is also a determining factor (Hautier *et al.* 2010). In general, legumes and grasses are thought to be "good" hosts for *R. minor* while non-leguminous forbs are regarded as "poor" hosts (Seel, Cooper & Press 1993; Seel & Press 1993; Cameron, Coats & Seel 2006),

but thus far, only a limited range of potential host species has been tested in terms of impact on parasite performance.

Host and parasitic plants do not interact with each other in isolation. Host plants can indirectly influence parasite herbivores (e.g. Marvier 1996; Adler 2002), whilst parasitic plants indirectly affect host plant herbivores (Ewald, John & Hartley 2011). The performance of invertebrate herbivores is dependent on the nutritional quality of the plant on which they are feeding (Douglas 1993), as well as the plant's physical (Hanley *et al.* 2007) and chemical (Bennett & Wallsgrove 1994) defences. As the biomass and nutritional quality of hemi-parasites depends on the host species they are attached to (Seel, Cooper & Press 1993; Seel & Press 1993), it follows that the parasite's host plant may also determine the success of parasite-feeding herbivores (Adler 2002).

As a parasite with the ability to infect and utilise numerous host species simultaneously, it is unlikely that *R. minor* will be attached to a single host species in the field (Gibson & Watkinson 1989). Parasites attached to multiple host species can gain different nutritional components from each host (Govier, Nelson & Pate 1967) and may also receive protection against multiple environmental stressors (Pate *et al.* 1990), including herbivores (Marvier 1998). Therefore, a mixed host or "generalist" strategy has the potential to benefit the parasite, particularly in the presence of herbivores. However, the extent of such benefits has not been tested and to our knowledge this is the first experimental study where the effects of different multiple host combinations on the performance of the parasite and its herbivores have been identified. Investigating the effects of these complex multi-trophic interactions on the performance of the organisms concerned will enable us to understand more fully the

key role that parasitic plants can play in structuring terrestrial communities and regulating their dynamics.

We used a series of glasshouse experiments to understand the consequences of a generalist host strategy by a keystone parasitic plant, *R. minor*, for itself and other associated organisms. We used a range of host species alone, and in combination, to test the effects of host identity on the performance of the parasite and an aphid herbivore feeding upon it. First, we investigated the effect of individual host species on the parasitic plant and predicted that host functional group would be more important than host species identity in determining the performance of the parasitic plant. Next we investigated the effect of single host species on the performance of aphid herbivores feeding on the parasite and predicted that host identity would indirectly influence the success of the parasite's aphid herbivores. Finally, using the results from the first two experiments to inform the choice of host species used, we investigated the effects of the parasite attaching to multiple host species on its aphid herbivores. We predicted that aphid performance would depend on the combination of host species used, with the cumulative effect on aphids reflecting the balance of "good" and "bad" hosts within the mixture.

Materials and methods

Experiment 1: effect of host species on R. minor

We collected and dried at room temperature *Rhinanthus minor* L. seed from Castle Hill National Nature Reserve in East Sussex (UK OS grid ref: TQ 370 070; WGS84 Lat-long: 50° 50' 46.7126", -0° 3' 19.2953"). We purchased host plant seeds from Emorsgate Seeds, King's Lynn, Norfolk. Host plant species were selected on the basis of their presence at Castle Hill and fall into three functional groups: legumes (*Lotus*

corniculatus L.*, Ononis repens* L. and *Trifolium pratense* L.); non-leguminous forbs (*Achillea millefolium* L.*, Plantago lanceolata* L. and *Sanguisorba minor* Scop.) and grasses (*Briza media* L.*, Dactylis glomerata* L. and *Holcus lanatus* L.).

We surface-sterilised *R. minor* seeds using 5% household bleach for 60 seconds, rinsing four times with sterile water. Seeds were placed onto 9 cm petri dishes containing damp sterile filter paper and capillary matting, which were sealed with parafilm and placed at 4°C for 84 days. Host plants were germinated on damp vermiculite 28 days after sterilisation of the *R. minor* seed. After a further 14 days, we transplanted host plant seedlings into 9 cm pots (one per pot) containing six parts sand and one part John Innes No. 2 compost.

Forty-two days after transplanting host plants, we added five germinating *R. minor* seeds into each pot. Hemi-parasite seedlings were thinned to one per pot when the majority showed signs of attachment (see Klaren & Janssen 1978 for details) approximately 21 days after transplanting. This gave us one host plant and one parasitic plant in each replicate pot. All plant material was grown in glasshouses at the University of Sussex (15-25°C) with supplementary lighting (16:8 light:dark). Experimental pots were supplied with tap water *ad libitum* and treatments arranged randomly within blocks on benches. We harvested above-ground plant material when the first parasitic plants began to show signs of senescence (at 110 days). All plant material was dried at 60°C for two days and weighed. Roots were washed and the number of haustoria counted using a binocular dissecting microscope. Fifteen replicates were set up for each species (N = 135), but not all *R. minor* plants survived until the end of the experiment. See Table S1 in supporting information for final numbers of replicates.

Experiment 2: effect of host species on R. minor *aphid herbivores*

Host and parasitic plant sources and preparation were as in Experiment 1. We collected the generalist melon aphid *Aphis gossypii* from plants of *R. minor* previously grown at the University of Sussex. We reared cultures of aphids on non-experimental *R. minor* plants grown in trays containing two of the potential hosts (*T. pratense* and *H. lanatus*), providing the aphids with prior indirect exposure to these

hosts. Seventy days after the germinating *R. minor* seeds were introduced to the pots, we added 20 adult aphids onto each of the hemi-parasites and covered these with a cage. Cages were constructed from a transparent plastic pipe (20cm height by 4.5cm diameter) with a window (4cm by 2cm) cut in the side to facilitate airflow. The ends of the tubes and the windows were sealed with a fine mesh. Cages allowed the aphids to move freely around the parasitic plants, but prevented them from colonizing the host plants. Aphids were left to multiply for 14 days, and counted. Then we harvested the above-ground portions of all plants. All plant material was dried at 60°C for two days and weighed. We determined nitrogen to carbon (N:C) ratios of the above ground biomass of *R. minor* from approximately 1.5 mg of ground, homogenised plant material using an elemental combustion system (Costech Instruments, Milan, Italy), calibrated against a standard compound ($C_{26}H_{26}N_2O_2S$). We planted 20 replicates per treatment (N = 180) but not all *R. minor* plants survived until the end of the experiment. See Table S1 for final replicate numbers. We calculated N:C ratios rather than the more commonly used C:N ratios as we were specifically interested in the effect of nitrogen on the aphids. We used a standard picrate paper assay (Egan, Yeoh & Bradbury 1998) to determine if the *L. corniculatus* plants used were cyanogenic, and whether any cyanogens that were passed to the hemi-parasite were degraded to release hydrogen cyanide (HCN). The picrate assay is a rapid but extremely sensitive technique to measure HCN and has a detection limit of around 10mg/kg (10ppm) (Siegler 1991). Each test required the destructive use of 100mg fresh plant material, so we prepared ten additional replicates of the *R. minor-L. corniculatus* treatment using seed from the same batches as the experiment.

Experiment 3: effect of multiple host species on R. minor *and its aphid herbivores*

We compared the performance of *R. minor* and its aphid herbivores when grown on single or mixed host treatments to test whether a "generalist" attachment strategy benefitted the parasite and/or the herbivore. Plants were sourced and prepared as previously, except that two host plants and one *R. minor* were grown per pot. We used a subset of the previous host plants and chose the legume *O. repens*, the non-leguminous forb *S. minor* and the grass *D. glomerata* as representative species from each functional group that the aphids had not had prior exposure to. We used the legume *L. corniculatus* as the partner species with each of the other hosts for the mixed host treatment as the previous experiment showed it to have a large negative effect on the performance of the aphid herbivores. We wanted to test whether this adverse impact could still be detected with the addition of other host species or whether these somehow "diluted" the negative effect of this host on the aphids. We conducted three separate sub-experiments where we compared each single non-*Lotus* host treatment (*O. repens*, *S. minor* or *D. glomerata)* with the respective mixed host treatment (*L. corniculatus*-*O. repens, L. corniculatus*-*S. minor* or *L. corniculatus*-*D. glomerata*) and the single *Lotus* treatment. Due to space limitations, the treatments including *O. repens* were conducted at a different time from those containing *D. glomerata* and *S. minor*. Single host *L. corniculatus* treatments were shared for *D. glomerata* and *S. minor*.

Twenty aphids were added as in Experiment 2 and counted after 14 days on the hemi-parasite. Then the above ground portions of all plants were harvested and dried at 60°C for two days before weighing. We planted 20 replicates per treatment (N = 60 per functional group) but not all *R. minor* plants survived until the end of the experiment. See Table S2 for final replicate numbers.

Statistical Analysis

Experiment 1: *R. minor* biomass and haustorial number were natural log transformed prior to analysis. Data were analysed using a linear mixed model (REML) with *R. minor* biomass as the response variable; block as a random factor; haustorial number as a continuous factor; host functional group and host species nested within host functional group as fixed factors. Differences among host species were explored with *post hoc* Tukey tests with significance values set at p=0.05. Host biomass was not included in the model as initial analysis showed it to have no significant effect and the model fit was improved with its removal (AICc 283.95 vs 286.98).

Experiment 2: Aphid abundance and *R. minor* biomass were natural log transformed prior to analysis. Data were analysed using a linear mixed model (REML) with aphid abundance as the response variable; block as a random factor; *R. minor* biomass as a continuous factor; host species as a fixed factor. N:C ratio (Table S3) and host functional group were not included in the model as initial analyses showed them to have no significant effect and model fit was improved with their removal (AICc 327.17 vs 333.75). Differences among host species were explored with *post hoc* Tukey tests with significance values set at p=0.05.

Experiment 3: For each host functional group – *L. corniculatus* combination, *R. minor* biomass was natural log transformed prior to analysis. Aphid abundance data were analysed using separate linear mixed models (REML) with aphid abundance as the response variable; block as a random factor; *R. minor* biomass as a continuous factor; host combination as a fixed factor. *Rhinanthus minor* biomass data were analysed using separate linear mixed models (REML) with *R. minor* biomass as the response

variable; block as a random factor; host combination as a fixed factor. Differences among host combinations were explored with *post hoc* Tukey tests with significance values set at p=0.05. All statistical analyses were carried out using JMP® 9.0.2 (SAS Institute Inc.).

Results

Experiment 1

The fitted model explained 58% of the variation in the *R. minor* biomass data (R^2_{adj} = 0.58). Of this, 0.07% of the variation was attributed to block. There was a significant positive relationship between haustorial number and *R. minor* biomass ($F_{1, 95.52}$ = 47.07, $p < 0.0001$; Figure 1a). Biomass was also significantly affected by host functional group ($F_{2, 88.34} = 5.47$, $p = 0.0057$) and by host species nested within functional group ($F_{6, 88.54} = 5.00$, $p = 0.0002$). On average among functional groups, biomass of *R. minor* grown on legumes was significantly greater than when the plants were attached to non-leguminous forbs ($p < 0.05$), but there were no significant differences between grass hosts and legumes or grass hosts and non-leguminous forbs ($p > 0.05$; Figure 1b). There was considerable variation in quality of species as hosts for *R. minor* that did not reflect the average response of the functional groups. Although the highest biomass of *R. minor* was achieved when attached to the legume *L. corniculatus*, the only hosts on which the parasite biomass was significantly lower than these were the grass *B. minor* and the non-leguminous forb *P. lanceolata* ($p < 0.05$). The lowest biomass of *R. minor* was achieved by the parasites with *P. lanceolata* as a host, but there were no significant differences between these plants and those attached to the legumes *O. repens* and *T. pratense*, the grass *B. media*, or the non-leguminous forb *A. millefolium* ($p > 0.05$; Figure 1c).

Experiment 2

The fitted model explained 60% of the variation in the aphid abundance data (R^2_{adj} = 0.60). Of this, 10% of the variation was attributed to block. There was a significant positive relationship between aphid abundance and *R. minor* biomass ($F_{1, 138}$ = 47.59, $p < 0.0001$; Figure 2a). There was also a significant effect of host species on aphid abundance ($F_{8, 123.9}$ = 13.94, $p < 0.0001$). The main species level effect was caused by *L. corniculatus*, as aphid abundance was significantly reduced when this was the host of *R. minor* compared to all other host species ($p < 0.05$). Aphid abundance on *R. minor* attached to *H. lanatus* and *A. millefolium* was also significantly reduced compared to numbers on the parasites with *O. repens* hosts ($p < 0.05$; Figure 2b). Colour changes in the picrate papers confirmed the presence of HCN in the *L. corniculatus* plants, but no HCN was detected from the *R. minor* attached to these hosts.

Experiment 3

When the legume *O. repens* was the partner species to *L. corniculatus*, the model fitted to aphid abundance explained 41% of the variation in the data (R^2_{adj} = 0.41). Of this, 4.6% of the variation was attributed to block. There was a significant positive relationship between aphid abundance and *R. minor* biomass ($F_{1, 42.06}$ = 9.70, p = 0.003; Figure 3a) and a significant effect of host combination ($F_{2, 33.41}$ = 11.99, $p < 0.0001$). Aphid abundances on *R. minor* were significantly different between all host combinations ($p < 0.05$), with the highest number of aphids on the *R. minor* growing only on *O. repens* and the least number of aphids on the *R. minor* growing only on *L. corniculatus* (Figure 3b). The model fitted to *R. minor* biomass explained 34% of the variation in the data (R^2_{adj} = 0.34). Of this, 21% of the variation was attributed to

block. There were no significant effects of host combination on *R. minor* biomass ($F_{2, 27.96} = 2.17$, $p = 0.13$; Figure 4a)

When the grass *D. glomerata* was the partner species to *L. corniculatus*, the model fitted to aphid abundance explained 70% of the variation in the data ($R^2_{adj} = 0.70$). Of this, 21% of the variation was attributed to block. There was no significant relationship with *R. minor* biomass ($F_{1, 48.36} = 1.56$, $p = 0.22$; Figure 3c), but there was a significant effect of host combination ($F_{2, 34.54} = 40.47$, $p < 0.0001$). Removing *R. minor* biomass did not improve the model fit. Aphid abundances on *R. minor* were significantly different between all host combinations ($p < 0.05$) with the highest number of aphids on the *R. minor* growing only on *D. glomerata* and the least number of aphids on the *R. minor* growing only on *L. corniculatus* (Figure 3d). The model fitted to *R. minor* biomass explained 41% of the variation in the data ($R^2_{adj} = 0.41$). Of this, 13% of the variation was attributed to block. There was a significant effect of host combination on *R. minor* biomass ($F_{2, 35.65} = 10.93$, $p = 0.0002$). Biomass for the mixed host combination was significantly greater than for the single grass host ($p < 0.05$), but not for the single *L. corniculatus* host ($p > 0.05$; Figure 4b).

When the non-leguminous forb *S. minor* was the partner species of *L. corniculatus*, the model fitted to aphid abundance explained 75% of the variation in the data ($R^2_{adj} = 0.75$). Of this 23% was attributed to block. There was a significant negative relationship between aphid abundance and *R. minor* biomass ($F_{1, 46.72} = 4.92$, $p = 0.03$; Figure 3e) and a significant effect of host combination ($F_{2, 32.2} = 52.04$, $p < 0.0001$). Aphid abundances on *R. minor* were significantly different between all host combinations ($p < 0.05$) with the highest number of aphids on the *R. minor* growing only on *S. minor* and the least number of aphids on the *R. minor* growing only on *L.*

corniculatus (Figure 3f). The model fitted to *R. minor* biomass explained 5% of the variation in the data ($R^2_{adj} = 0.05$). Of this, 2% of the variation was attributed to block. There were no significant effects of host combination on *R. minor* biomass ($F_{2, 37.13} = 1.54$, $p = 0.23$; Figure 4c).

Discussion

This study is the first assessment of how attachment to different combinations of multiple host species affects both *R. minor* and its associated aphid herbivores. Our data shows that when attached to a single host, *R. minor* biomass was best explained by the number of haustorial attachments to the host rather than the size, or biomass, of the host plant. While it is known that not all haustoria produce functional attachments (Cameron & Seel 2007), our results demonstrate that the investment in such structures by the parasite reflects the quality of the host. They also support the idea that differential resistance among a variety of host species across functional groups (Cameron, Coats & Seel 2006; Cameron & Seel 2007) is related to the number and effectiveness of haustorial connections in addition to any effects of host growth rate (Hautier *et al.* 2010). Certainly haustorial connections were a more important determinant of host quality for the parasitic plant in our study, since host biomass had no influence on *R. minor* performance.

There was considerable variation in host suitability at the species level, which was not predictable from the species' functional groups. For example, although when analysed at the level of functional group, legumes and grasses were better hosts for *R. minor* than non-leguminous forbs (see also Seel, Cooper & Press 1993; Seel & Press 1993); when species level effects were considered *R. minor* actually performed no worse on the non-leguminous forbs *S. minor* and *A. millefolium* than on the best host

the legume *L. corniculatus*. In fact, the poor performance of *P. lanceolata* as a host, which has been previously well documented (Cameron, Coats & Seel 2006; Cameron & Seel 2007), is the predominant factor in reducing the performance of the non-leguminous forbs as hosts overall. Similarly, the performance of the functional group legumes as hosts is predominantly influenced by the high biomass attained when *R. minor* is growing on *L. corniculatus*, whilst the other legumes, *O. repens* and *T. pratense* were no better hosts than *P. lanceolata*. The grasses *H. lanatus* and *D. glomerata* were as good hosts as *L. corniculatus*, but overall, the grasses were no better as hosts than the non-leguminous forbs and no worse than the legumes. In light of this species level variation, we caution against making generalisations of *R. minor* host performance based only on information about plant functional group.

Aphid abundances feeding on *R. minor* attached to a single host increased with *R. minor* biomass, but were not affected by the N:C ratio of the parasite. This suggests that the size of the available resource rather than its quality, at least when expressed in relation to nitrogen, is of greater importance to aphid fitness. Host functional group was not important in determining aphid abundance, but host species was. The aphids used in this experiments had prior exposure to *R. minor* attached to two of our host species (*T. repens* and *H.lanatus*). It is possible, although not necessarily the case (Via 1991), that prior exposure enabled the aphids to better tolerate any negative effects of these hosts. Numbers of aphids were reduced on *R. minor* attached to *H. lanatus* and *A. millefolium* compared to those attached to *O. repens*, but by far the greatest effect was seen when the parasite was attached to *L. corniculatus*. On these plants, numbers of aphids were considerably reduced compared to all other host species, possibly because of anti-herbivore secondary metabolites associated with this

species. Previous studies on other parasitic plant species have demonstrated that compounds with anti-herbivory properties can be transferred from the host to the parasitic plant (Marvier 1996; Adler & Wink 2001). In *L. corniculatus*, cyanogenic glycosides are the principle toxic metabolites, known to be effective against herbivores (Scriber 1978). These interact with degradation enzymes within the plant to release HCN when plants are under attack. The picrate assay that we used detects the release of HCN as a result of this degradation process and, while we did detect this compound in *L. corniculatus*, there was no evidence of HCN production in the parasitic plant. We cannot, however, rule out the transfer of cyanogenic glycosides themselves between the host and parasite as it is possible that the compounds were present in *R. minor*, but not degraded to release HCN.

Our third experiment investigated the relationship between host species, in particular *L. corniculatus,* and *R. minor* aphid herbivores, by testing mixtures of species including *L. corniculatus* against single host plantings. For all host combinations tested, aphid abundance decreased as the proportion of *L. corniculatus* plants increased. Whilst this does not reveal the specific mechanisms by which the host plants were influencing the parasite's aphid herbivores, it does demonstrate that *L. corniculatus* confers resistance to herbivores on *R. minor*. The most likely mechanism for this is via the transfer of secondary metabolites across the haustoria (Adler & Wink 2001), although we found no evidence of this mechanism here (see above). These results also suggest that the propensity of *R. minor* to attach to multiple host species in the field is likely to benefit the parasite's herbivores by diluting any negative effects of particular host species.

When attached to multiple host species the relationship between *R. minor* biomass and aphid abundance breaks down and depends entirely on host species identity. When *O. repens* was paired with *L. corniculatus*, larger *R. minor* plants supported greater numbers of aphids, but when *S. minor* and *L. corniculatus* were paired as hosts, smaller plants supported more aphids. This discrepancy in the impact of host combinations is likely due, in part, to the negative effect of *L. corniculatus* on the aphids, but also its suitability as a host for *R. minor*.

Attachment to multiple host species had little discernable effect on *R. minor* biomass, except when *D. glomerata* was paired with *L. corniculatus*. In this case, the parasite was larger when attached to both host species compared to when it was only attached to the grass. Previous work has produced contradictory conclusions on whether attachment to a single host species (Matthies 1996) or multiple host species (Marvier 1998) is the most beneficial for parasitic plants. Our results suggest that the host identity within mixtures is likely to be critical in explaining these apparent contradictions on *R. minor* performance. Further, the responses of *R. minor* to host mixtures did not predict the response of the aphids since the relationship between *R. minor* biomass and aphid abundance is different for each combination of hosts.

In conclusion, host species identity has considerable effects on the performance of both *R. minor* and its aphid herbivores, and this variation between species cannot be explained solely by plant functional group. Furthermore, this effect holds for interactions with single and multiple host species. Investment in haustorial structures is a good indication of host suitability for the parasite, whilst parasite biomass is a good predictor of the size of aphid populations feeding on *R. minor*, but only when the parasite is attached to a single host. When attached to multiple hosts,

the relationship between host plant and parasite herbivore is more complex. The "generalist" nature of *R. minor* is likely to benefit its aphid herbivores by diluting any negative effects of a single host species thereby significantly influencing herbivore population dynamics. With this work we demonstrate that the interactions between the keystone parasitic plant *R. minor* and its hosts extend beyond the plants themselves and have significant consequences for the wider ecological community.

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Supporting information

Additional supporting information may be found in the online version of this article.

Table S1 Treatments and replicate numbers for Experiment 1 & 2.

Table S2 Treatments and replicate numbers for Experiment 3.

Table S3 *Rhinanthus minor* N:C values from Experiment 2.

FIGURE LEGENDS

Figure 1. (a) *Rhinanthus minor* biomass plotted against haustorial number for all plants. Natural log scaled axes are used to demonstrate the relationship between the model response variable and covariate. The analysis showed a significant positive relationship between *R. minor* biomass and haustorial number ($p < 0.0001$) (b) Back transformed least squares means of *R. minor* biomass when grown without aphids on non-leguminous forb [F, filled bars], grass [G, hatched bars] and legume [L, open bars] hosts. Error bars are 95% confidence intervals. Different letters on the bar graphs denote significant differences

among treatments calculated from *post-hoc* Tukey tests ($p < 0.05$). (c) Back transformed least squares means of *R. minor* biomass when grown without aphids on nine species of host plants. Filled bars show the non-leguminous forbs *Achillea millefolium* [AM], *Plantago lanceolata* [PL] and *Sanguisorba minor* [SM]. Hatched bars show the grasses *Briza media* [BM], *Dactylis glomerata* [DG] and *Holcus lanatus* [HL]. Open bars show the legumes *Lotus corniculatus* [LC], *Ononis repens* [OR] and *Trifolium pratense* [TP]. Error bars are 95% confidence intervals. Different letters on the bar graphs denote significant differences among treatments calculated from *post-hoc* Tukey tests ($p < 0.05$).

Figure 2. (a) Aphid abundance per *R. minor* plant plotted against *R. minor* biomass. Natural log scaled axes are used to demonstrate the relationship between the model response variable and covariate. The analysis showed a significant positive relationship between aphid abundance and *R. minor* biomass ($p < 0.0001$). (b) Back transformed least squares means of aphid abundance per *R. minor* plant grown on nine species of host plants. Filled bars show the non-leguminous forbs *Achillea millefolium* [AM], *Plantago lanceolata* [PL] and *Sanguisorba minor* [SM]. Hatched bars show the grasses *Briza media* [BM], *Dactylis glomerata* [DG] and *Holcus lanatus* [HL]. Open bars show the legumes *Lotus corniculatus* [LC], *Ononis repens* [OR] and *Trifolium pratense* [TP]. Error bars are 95% confidence intervals. Different letters on the bar graphs denote significant differences among treatments calculated from *post-hoc* Tukey tests ($p < 0.05$).

Figure 3. (a, c, e) Aphid abundance plotted against *R. minor* biomass. The x axis is natural log scaled to demonstrate the relationship between the model response variable and covariate. Different host combinations are shown by circles [(a) *O. repens*; (c) *D. glomerata*; (e) *S. minor*], crosses [mixed hosts] and triangles [*L. corniculatus*]. Analyses showed a significant positive relationship between aphid abundance and *R. minor* biomass for host *O. repens* (a; $p = 0.003$), no significant relationship between aphid abundance and *R. minor* biomass for host *D. glomerata* (c; $p = 0.22$) and a significant negative relationship between aphid abundance and *R. minor* biomass for host *S. minor* (e; $p = 0.03$). (b, d, f) Least squares means of aphid abundance per *R. minor* plant when grown on single or mixed host treatments. Host plants are (a, b) *Ononis repens* [O] and *Lotus corniculatus* [L]; (c, d) *Dactylis glomerata* [D] and *Lotus corniculatus* [L]; (e, f) *Sanguisorba minor* [S] and *Lotus corniculatus* [L]. Error bars are 95% confidence intervals. Different letters on the bar graphs denote significant differences among treatments calculated from *post-hoc* Tukey tests ($p < 0.05$).

Figure 4. Back transformed least squares means of *R. minor* biomass when grown on single or mixed host treatments. Host plants are (a) *Ononis repens* [O] and *Lotus corniculatus* [L]; (b) *Dactylis glomerata* [D] and *Lotus corniculatus* [L]; (c) *Sanguisorba minor* [S] and *Lotus corniculatus* [L]. Error bars are 95% confidence intervals. Different letters on the bar graphs denote significant differences among treatments calculated from *post-hoc* Tukey tests ($p < 0.05$).