Two sympatric root hemiparasitic *Pedicularis* species differ in host dependency and selectivity under phosphorus limitation

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Abstract. Parasitic biology of *Pedicularis* L. (Orobanchaceae) has been underinvestigated despite its wide distribution and potential ecological significance. To better understand the parasitic aspects of the root hemiparasites, host–parasite interactions were investigated with two sympatric *Pedicularis* species, *Pedicularis rex* C. B. Clarke and *Pedicularis tricolor* Hand.-Mazz., at two developmental stages. Plant DW, shoot phosphorus (P) content, root : shoot ratio and number of haustoria were measured in *Pedicularis* grown with either a host plant or a plant of its own species in pot experiments. In addition, effects of parasitism and intraspecific competition on growth and biomass allocation in four host species belonging to three major functional groups (grasses, legumes and forbs) were investigated. The two *Pedicularis* species showed obvious host preference, but preferred different host species. Interactions between *Pedicularis* and their hosts depended on both species identity and developmental stages of the partners. Overall, *P. rex* showed much weaker host dependency and less damage to hosts than *P. tricolor*. Interspecific variations were observed among different host species in their responses to intraspecific competition and parasitism. We concluded that different *Pedicularis*-host pairs showed different interaction patterns. Sympatric *Pedicularis* may have differential influence on plant community structure and productivity.

Additional keywords: host–parasite association, lousewort, plant parasitism.

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Introduction

Pedicularis L. (Orobanchaceae) is a large lineage of root hemiparasitic plants consisting of ~600 described species widely distributed in the frigid and alpine zones of the northern hemisphere and best represented (352 species) in the mountains of SW China (Yang *et al.* 1998). Although the pollination biology of this genus has received much attention and is well characterised (Macior 1968, 1973, 1986; Wang and Li 2005; Tang *et al.* 2007; Liao *et al.* 2011), the parasitic biology is poorly understood.

The parasitic habit of *Pedicularis* has been known since 1847 (Piehl 1963). However, investigation of parasitic biology of *Pedicularis* has been rare compared with its parasitic European counterpart genus *Rhinanthus*, which has been extensively studied physiologically and ecologically (Gibson and Watkinson 1992; Joshi *et al.* 2000; Press and Phoenix 2005; Bardgett *et al.* 2006; Cameron *et al.* 2009; Tšitel *et al.* 2011). To our knowledge, the small number of studies (mostly very preliminary) has been conducted exclusively on European or American species (Piehl 1963; Lackney 1981; Nilsson and

Svensson 1997; Hedberg *et al.* 2005). Parasitic biology of Chinese *Pedicularis* species has hardly been documented (Li and Guan 2008; Ren *et al.* 2010).

Accumulating evidence has shown that root hemiparasitic plants play significant ecological roles in regulating above- and below-ground biodiversity and community structure in their ecosystems (Joshi *et al.* 2000; Quested *et al.* 2003; Press and Phoenix 2005; Bardgett *et al.* 2006; Spasojevic and Suding 2011). Although ecological roles of *Pedicularis* spp. remain largely unknown, one study on *Pedicularis canadensis* Hadač suggested that it can influence prairie community composition (Hedberg *et al.* 2005). In addition, a growing number of reports suggest that grassland productivity is strongly reduced by *Pedicularis* species in China (Qiu *et al.* 2006; Liu *et al.* 2008; Zhang *et al.* 2009), leading us to postulate that these root hemiparasites have significant ecological roles. In view of their wide distribution in the northern hemisphere and potential influence on their ecosystems, there is a clear need to have a better knowledge of the parasitic biology of *Pedicularis*.

Parasitism occurs to varying degrees in root hemiparasitic plants (Irving and Cameron 2009). Some can grow without a host (but mostly grow better when attached to a host; facultative parasites), whereas others depend on a host to survive (obligate parasites). In all circumstances, compatible host–parasite combinations are essential for successful establishment of parasitic associations, characterised by formation of several functional connecting structures (known as haustoria) between the root vascular systems of the two partners (Irving and Cameron 2009; Westwood *et al.* 2010). Despite the fact that root hemiparasites generally have wide host ranges (Piehl 1963; Nilsson and Svensson 1997; Irving and Cameron 2009), host selectivity has been observed for several species irrespective of their extent of parasitism (Lackney 1981; Matthies 1997; Hedberg *et al.* 2005; Cameron *et al.* 2006; Ren *et al.* 2010). Among the three functional groups of hosts, grasses are the most common and legumes are consistently good for stimulating growth of *Pedicularis* species (Piehl 1963; Lackney 1981; Hedberg *et al.* 2005; Liu *et al.* 2008; Ren *et al.* 2010). Forbs are generally poor hosts for *Rhinanthus minor* (Cameron *et al.* 2006; Cameron and Seel 2007; Rümer *et al.* 2007), but *Solidago canadensis* L. (Asteraceae) appeared to be a satisfactory host for *P. canadensis* (Hedberg *et al.* 2005). To our knowledge, forbs have rarely been experimentally tested regarding their host quality for *Pedicularis*, though they frequently occur in the same natural habitats (AR Li, pers. obs.).

Apart from host identity, other factors may affect host–root hemiparasite interactions, such as developmental stage (Graves 1995) and nutrient supply (Cechin and Press 1994; Jiang *et al.* 2010). To our knowledge, studies on parasitic biology of root hemiparasites have been conducted either at the early seedling stage (Lackney 1981; Tomilov *et al.* 2004) or over a whole growing season (Ren *et al.* 2010; Rowntree *et al.* 2011). Variations in host–parasite interactions between different developmental stages have been scarcely addressed. Nitrogen (N) has been the focus of investigation of effects of nutrient supply (Jiang *et al.* 2010), whereas effects of phosphorus (P) have received comparatively little attention (but see Davies and Graves 2000). As parasitism in *Pedicularis* was suggested to be an adaptation to P deficiency (Lackney 1981), it is relevant to test the host–parasite interactions using a P-limited growth medium.

In this work we report the interactions between two sympatric Chinese *Pedicularis* species and four potential host species representing different functional groups (grasses, legumes and forbs). The plants were grown in a sterilised growth medium with limited P but sufficient N in a greenhouse pot experiment and harvested at two developmental stages. We addressed the following specific questions. First, do growth responses, shoot P content and haustorium formation in the root hemiparasites differ when grown with different plant species? Second, do the host–parasite interactions vary at different growth stages? Third, how strong is the effect of parasitism on the host compared with intraspecific competition by the host on host growth? Knowledge obtained will help us better understand the parasitic biology and potential ecological roles of this group of underinvestigated root hemiparasites.

Materials and methods

Experimental design

Two *Pedicularis* species (*Pedicularis rex* C. B. Clarke and *Pedicularis tricolor* Hand.-Mazz.) and four host species were used. *P. rex* has a much wider distribution but is sympatric to *P. tricolor* in some habitats in Shangri-la, Yunnan Province, China. Plant species that are susceptible to infection by other closely related root parasitic plants (Matthies 1995; Ren *et al.* 2010) and comply with the Australian quarantine laws were used as hosts (the study was conducted in Australia). The host species were: one grass species (barley (*Hordeum vulgare* L. cv. Fleet)), two legumes (barrel medic (*Medicago truncatula* L.) and subterranean clover (*Trifolium subterraneum* L.)) and one forb (tomato (*Solanum lycopersicum* L.)). A single plant of each *Pedicularis* species was grown with either a second plant of its own species or with one plant of each host species. In addition, two individuals of each host species were planted into one pot for determination of intraspecific competition. A fixed distance (~3 cm) was set between the two plants in each pot to reduce distance effects. Each plant combination had 10 replicate pots, with a total of 140 pots for the experiment.

Plant materials

Seeds of *P. rex* and *P. tricolor* were collected from Shangri-la, Yunnan Province, People's Republic of China, in September 2008 and stored in paper bags at 4°C until used, except for transport to Australia. To promote germination, seeds were surface-sterilised in 4.5% commercial sodium hypochlorite for 10 min, rinsed thoroughly with running reverse osmosis (RO) water and soaked in $1\,g\,L^{-1}$ gibberellic acid for 2 h and then stratified at 4°C for one week. Germination was conducted on moist filter papers at 20°C in the dark for 6 days.

Seeds of the host species were surface-sterilised in 4.5% commercial sodium hypochlorite for 10 min, rinsed with RO water and germinated on moist filter papers at 25°C in the dark for 2–4 days (depending on species).

Planting and growth conditions

Uniform seedlings of *Pedicularis* and their host plants were transplanted simultaneously to white plastic pots containing 1.4 kg mix of 10% soil and 90% fine sand. Soil was collected from the Waite Arboretum, University of Adelaide, Australia. Soil was sieved through a 2-mm sieve, autoclaved at 121°C (twice on separate days, 1 h each time) and then mixed with autoclaved fine sand. The soil mix had 2.6 mg kg^{-1} plant-available P by the resin extraction method (McLaughlin *et al.* 1994) and its pH (in 0.01M CaCl$_2$ solution) was ~6.0.

The surface of the soil–sand mix was covered with autoclaved polyethylene beads to retain moisture. Pots were watered to weight with RO water whenever necessary to maintain water content around 10% oven-dry soil. Long Ashton nutrient solution minus P but with increased N (Li *et al.* 2012) was applied weekly (15 mL per pot) after transplanting. Pots were fully randomised and re-randomised at each watering to reduce position effects.

The experiments were conducted from mid May to early August (Autumn–Winter in the southern hemisphere) in an environmentally controlled glasshouse at the Waite Campus,

University of Adelaide. Night–day temperature range in the glasshouse was 15.8–28.1°C. During cloudy days, supplementary lights were turned on to increase irradiance, which was in the range of 237–1000 $\mu mol\,m^{-2}\,s^{-1}$.

Harvest and sampling

Two harvests were conducted to examine the interactions between the *Pedicularis* and their hosts at different developmental stages, one at 6 weeks after planting (early seedling stage for the parasites and vigorous vegetative growth stage for hosts) and the other at 14 weeks (late seedling stage for the parasites and fruiting stage for all host species). Some tomato seedlings died because of a plant disease 6 weeks after planting, data are missing for tomato at the second harvest.

Survival of both *Pedicularis* and host plants was recorded each week after planting. At harvest, shoots were cut at the soil surface and separated from roots. Shoot DW per plant was determined after oven drying at 85°C for 48 h. Roots were washed thoroughly and FW were determined after blotting with paper towels. *Pedicularis* roots were separated from those of their host plants. Haustoria tightly attached to host roots were carefully cut off under a stereomicroscope with as little host tissue as possible and pooled with *Pedicularis* roots. A weighed subsample of *Pedicularis* root material was taken and stored in 50% ethanol for later assessment of haustorium formation in different plant combinations. The remainder of each root sample was oven-dried at 85°C for 48 h and DW determined. DW of the subsample used for checking haustorium formation was obtained from the FW : DW ratio of the remainder and the FW of the subsample. Total DW per plant and root : shoot (R : S) ratio were calculated using DW of corresponding materials. The root systems of the same species proved to be impossible to separate from each other, so roots from the same pot were treated as one sample for those pots with two individuals of a single species. Accordingly, R : S ratio was calculated per pot rather than per plant for those treatments.

To facilitate examination of internal structures of haustoria, sampled roots were washed free of ethanol, cleared in 10% KOH and stained in a 5% ink-vinegar solution (Vierheilig *et al.* 1998). Number of haustoria (H) in the subsample was counted under a brightfield microscope at 40× magnification. Incidence of haustorium formation was recorded as number of H mg^{-1} root DW. Haustoria with distinct xylem bridges were recorded as presumably functional haustoria (PFH; Li and Guan 2008). Total numbers of H and PFH per plant were calculated from the number mg^{-1} dry root multiplied by the total root DW.

Growth response of *Pedicularis* to host is presented as percentage growth response, based on shoot dry weight (SDW) per *Pedicularis* plant. Calculation was done using the equation: HGR (%) = 100 × (SDW with a host – mean SDW without a host)/mean SDW without a host, where HGR is host growth response.

Shoot P concentrations in the root hemiparasites were determined following digestion of dried material in a concentrated HNO_3 (69.8 weight %) and analysis using the phosphovanado-molybdate method (Hanson 1950). Shoot P content per plant was calculated from the shoot P concentration multiplied by shoot DW.

Statistical analyses

One-way analysis of variance was performed using Statistical Product and Service Solutions (SPSS) software (ver. 16.0; SPSS China Ltd, Shanghai, China) for most of the data. Data for R : S ratios were arcsine transformed and number of haustoria and PFH were square-root transformed before analysis in order to meet ANOVA assumptions of normality and homogeneity. Duncan's multiple range test was used for comparison of the means. Spearman rank correlations were calculated between numbers of haustoria and shoot biomass as well as shoot P content per plant of *Pedicularis*, using the same SPSS software. PERMANOVA, a non-parametric method for analysis of variance, was used for data that did not fulfil the assumptions of either normality (shoot DWs of *P. rex* at 14 weeks) or homogeneity of variances (root DWs of the *Pedicularis* species at 6 weeks) for a parametric ANOVA. Pair-wise *a posteriori* comparisons of the means were conducted wherever necessary according to the User's Guide of this program (Anderson 2005).

Results

Root hemiparasites

Survival, DW and R : S ratio

Both *Pedicularis* species survived well (no mortality) without a host plant. A few individuals of *P. rex* grown with a second plant of its own species had flower buds at the second harvest. The root hemiparasites grew very slowly during the early seedling stage, but much faster between 6 and 14 weeks (Fig. 1). P deficiency symptoms were observed in both *Pedicularis* species – as purple leaves in *P. rex* in the presence of barley; and necrotic spots on leaves of *P. tricolor* grown with its own species.

Total DWs of *P. rex* were not different between any species pairs at 6 weeks (Table 1; Fig. 1*a*). *P. rex* grown with a second plant of its own species did not differ in shoot DW from those grown with a host plant. However, plants grown with barley had significantly smaller shoots than those grown with barrel medic. Root DW and R : S ratio were significantly smaller when grown with barley or tomato for 6 weeks compared with plants grown with its own species (Table 1; Fig. 1*a*). The reduction of root biomass allocation was not significant when attached to barrel medic or subterranean clover. After 14 weeks, the total DW was significantly greater when grown with barrel medic than in other species pairs. Shoot DW was significantly increased by the presence of barrel medic, but not by barley and subterranean clover (Table 1; Fig. 1*b*). Biomass allocation to roots in *P. rex* was reduced when grown with barley or subterranean clover rather than when grown with its own species. The reduction in R : S ratio was not significant grown with barrel medic for 14 weeks.

Hosts showed no obvious effects on total DWs of *P. tricolor* at 6 weeks (Table 1; Fig. 1*c*). Shoot DW of *P. tricolor* was significantly increased by the presence of tomato. Other host species stimulated shoot growth to some extent, but the increases were not statistically significant. Root DW did not differ among species pairs, but the reduction in R : S ratio of the root hemiparasite was significant in the presence of all
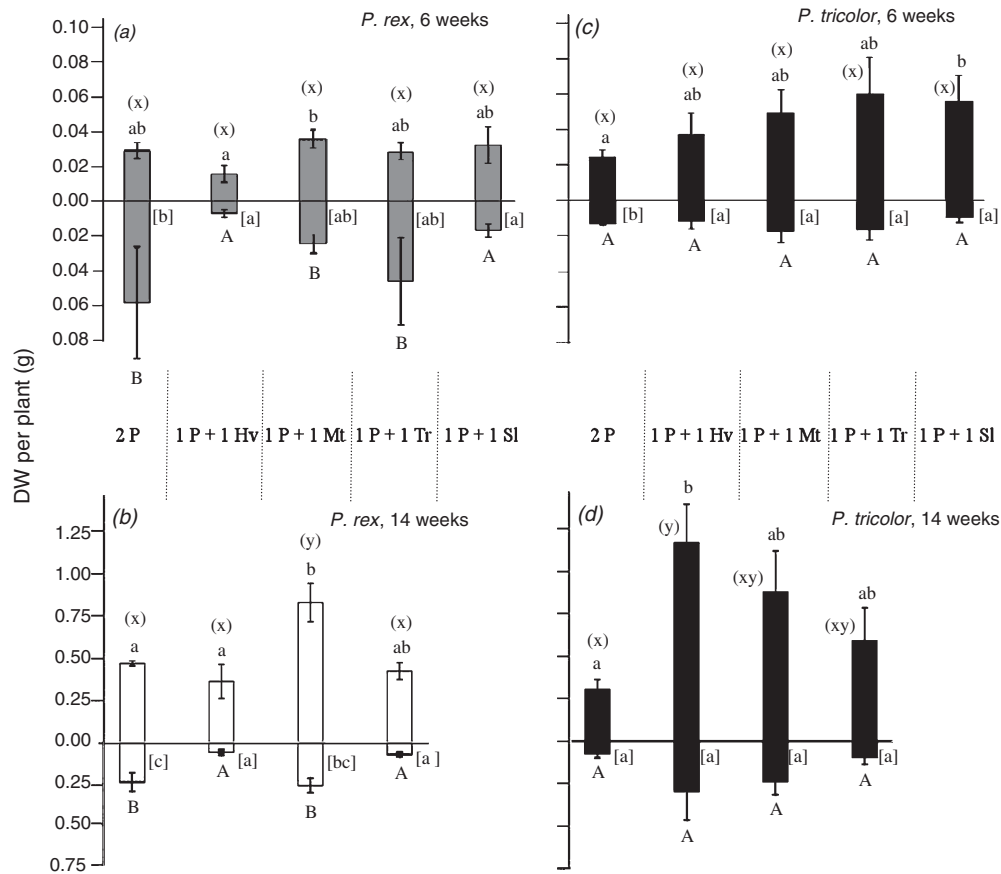
Fig. 1. Dry weight (DW, g) per plant of *Pedicularis rex* (grey bars) and *Pedicularis tricolor* (black bars) when grown with different plant species after 6 (*a*, *c*) and 14 weeks (*b*, *d*) of planting. Data are presented as mean ± s.e. of five replicate pots. Values for pots with two *Pedicularis* plants are means of the individuals. Statistics were conducted separately for shoot (above the line), root (below the line), total DW and root : shoot (R : S) ratio of each plant as well as time. Bars for shoots (lower case), roots (upper case), total DWs (in round brackets) and R : S ratios (in square brackets) with different letters indicate statistically significant difference at $P < 0.05$ level. Treatments (host–parasite combinations): 2 P, two *Pedicularis* of the same species; 1 P + 1 Hv, one *Pedicularis* and one *Hordeum vulgare*; 1 P + 1 Mt, one *Pedicularis* and one *Medicago truncatula*; 1 P + 1 Tr, one *Pedicularis* and one *Trifolium subterraneum*; 1 P + 1 Sl, one *Pedicularis* and one *Solanum lycopersicum*. Data are missing for *Solanum lycopersicum* at 14 weeks because of an outbreak of fatal plant disease 6 weeks after planting.

Table 1.   ANOVA results (*F*-values and significance levels) for the effects of different host species on shoot DW (SDW), root DW (RDW), total DW (TDW), root : shoot ratio (R : S), shoot concentration (SPConc), total number of haustoria (H) and number of presumably functional haustoria (PFH) of *Pedicularis rex* and *Pedicularis tricolor*

Significance levels after *F*-values are indicated: ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$. Data transformation before ANOVA: [†], arcsine; [‡], square-root

Growth stage	*Pedicularis*	SDW	RDW	TDW	R : S[†]	SPConc	No. H[‡]	No. PFH[‡]
Early seedling (6 weeks), d.f. = (4, 20)	*P. rex*	1.401	3.645*	1.557	1.908	192.972***	2.289*	1.793
	P. tricolor	1.081	0.202	0.607	4.628**	325.546***	1.491	2.090*
Late seedling (14 weeks), d.f. = (3, 16)	*P. rex*	6.689**	8.561***	8.298***	7.440**	6.200**	5.301***	5.601***
	P. tricolor	3.742*	1.248	3.282*	0.867	1.729	3.001**	2.539*

tested hosts compared with two plants of *P. tricolor*. After 14 weeks, total and shoot DW of *P. tricolor* attached to barley was significantly increased (Table 1; Fig. 1*d*). Total and shoot DWs of the root hemiparasite grown with barrel medic and subterranean clover did not differ statistically from either those

grown with barley or those grown with a second plant of its own species. No difference was observed in R : S ratios between any plant combinations after 14 weeks.

Overall, *P. rex* showed lower growth responses to all the tested hosts than *P. tricolor*. The largest average increase in

shoot DW of *P. rex* following attachment to a host was only 76%, when grown with barrel medic for 14 weeks (Fig. 1*b*). Shoot DW of *P. tricolor* increased by an average of 287 or 190% when grown for 14 weeks with barley or barrel medic respectively (Fig. 1*d*). Growth response of *P. rex* and *P. tricolor* to different host species varied greatly. *P. rex* showed a negligible growth response to tomato and subterranean clover and a negative response to barley, whereas *P. tricolor* showed a positive growth response to all the tested host species, particularly barley. Both *Pedicularis* species showed a positive growth response to barrel medic at both growth stages.

Percentage growth responses of both *Pedicularis* species increased with time (data not shown). The directionality of the responses (positive or negative) to different host species was quite consistent between the two harvests (Fig. 1).

Shoot P concentration and content per plant

At 6 weeks, shoot P concentrations of *P. rex* (µg mg^{-1} DW) were in the following order: (2 *P. rex* plants together) = (1 *P. rex* + 1 barley) < (1 *P. rex* + 1 subterranean clover) < (1 *P. rex* + 1 barrel medic) < (1 *P. rex* + 1 tomato) (Fig. 2*a*). Taking into account differences in shoot DW among treatments and large variability (Fig. 1*a*), shoot P content (µg plant^{-1}) of *P. rex* was in the following order: (1 *P. rex* + 1 barley) < (2 *P. rex* plants together) = (1 *P. rex* + 1 barrel medic) = (1 *P. rex* + 1 subterranean clover) < (1 *P. rex* + 1 tomato) (data not shown). Thus, tomato

gave the greatest benefit on the bases of both P concentration (Fig. 2*a*) and content.

At 14 weeks, shoot P concentrations when two *P. rex* were grown together were significantly lower than those in other treatments, which showed no significant differences between them (Table 1; Fig. 2*b*). Again, taking into account differences and variability in DW (Fig. 1*b*), only *P. rex* plus barrel medic had significantly higher shoot P content than treatments when two *P. rex* plants were grown together or with barley or subterranean clover, which showed no significant differences between them (data not shown). In the absence of a treatment with tomato, barrel medic gave the largest benefit in terms of shoot P content, reflecting shoot size (Fig. 1*b*) but no longer a high P concentration (cf. Fig. 2*b*).

At 6 weeks, shoot P concentrations in *P. tricolor* were in the following order: (2 *P. tricolor* plants together) = (1 *P. tricolor* + 1 barrel medic) = (1 *P. tricolor* + 1 subterranean clover) < (1 *P. tricolor* + 1 tomato) < (1 *P. tricolor* + 1 barley) (Fig. 2*c*). Using the data for DW (Fig. 1*c*), *P. tricolor* only had significantly higher shoot content when grown with tomato, although effects of other hosts were not significantly different from those of tomato (data not shown). Both barley and tomato gave large benefits on the bases of P concentration (Fig. 2*c*) and content.

At 14 weeks there were no significant differences in shoot P concentrations in P. *tricolor* (Table 1; Fig. 2*d*) and P content followed the following order: (2 *P. tricolor* plants together) < (1 *P. tricolor* + 1 barrel medic) = (1 *P. tricolor* + 1 subterranean
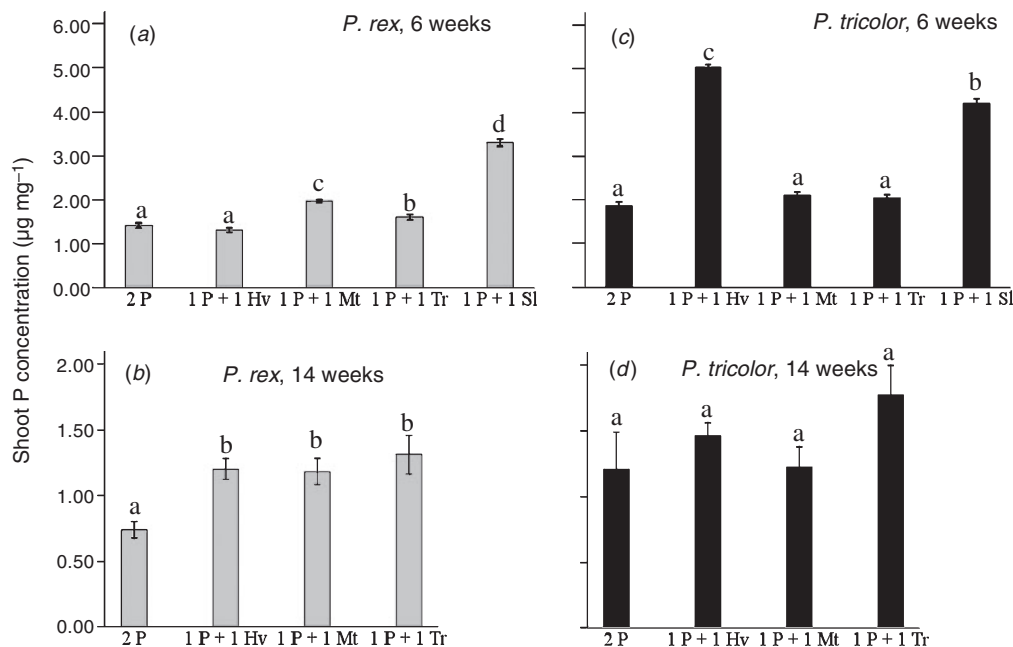

Fig. 2. Shoot phosphorus concentration (µg mg^{-1}) of *Pedicularis rex* (grey bars) and *Pedicularis tricolor* (black bars) when grown with different plant species after 6 (*a, c*) and 14 weeks (*b, d*) of planting. Data are presented as mean ± s.e. of five replicate pots. Statistics are done separately for each plant as well as time. Different letters indicate statistically significant difference at *P* < 0.05 level. Treatments (host–parasite combinations): 2 P, two *Pedicularis* of the same species; 1 P + 1 Hv, one *Pedicularis* and one *Hordeum vulgare*; 1 P + 1 Mt, one *Pedicularis* and one *Medicago truncatula*; 1 P + 1 Tr, one *Pedicularis* and one *Trifolium subterraneum*; 1 P + 1 Sl, one *Pedicularis* and one *Solanum lycopersicum*. Data are missing for *Solanum lycopersicum* at 14 weeks because of an outbreak of fatal plant disease 6 weeks after planting.

clover) < (1 *P. tricolor* + 1 barley) (data not shown). In this case, again in the absence of a treatment with tomato, barley gave the biggest benefit in terms of shoot P content, reflecting shoot size (Fig. 1*d*) but no longer a high P concentration (compare Fig. 2*d*).

Despite differences in shoot P concentration among treatments, shoot P content of both hemiparasites positively correlated with shoot DW ($P < 0.001$) at both growth stages (Table 2).

Haustorium formation

Both *Pedicularis* species formed haustoria when grown with a second plant of its own species (Fig. 3).

Neither number of total H nor number of PFH per plant of *P. rex* was significantly affected by presence of a host plant at 6 weeks after planting (Table 1; Fig. 3*a*, *b*). However, the number of total H per plant was significantly higher when grown with barrel medic than with barley (Fig. 3*a*). After 14 weeks, *P. rex* grown with barrel medic produced significantly more H and PFH than those grown with the other two hosts or a second plant of the root hemiparasite (Fig. 3*c*, *d*). The presence of subterranean clover increased number of PFH, but had no significant effect on number of H (Table 1). Barley had no influence on either total H or PFH per plant.

After 6 weeks, *P. tricolor* produced significantly more H with barrel medic but more PFH with barley than with other hosts or another individual of its own species (Table 1; Fig. 3*e* and *f*). Subterranean clover and tomato had no significant influence on either total number of H or number of PFH at this stage (Table 1). After 14 weeks, all three host species increased the numbers of H and PFH (Fig. 3*g*, *h*), although the increased number of PFH with subterranean clover was not significant (Table 1).

At both developmental stages, *P. tricolor* produced more H and PHF (up to 5-fold) than *P. rex* when grown with a host plant (Fig. 3). Numbers of H and PFH per plant positively correlated with both shoot DW and shoot P content of both root hemiparasites. In most cases the correlation was significant at $P < 0.01$ level (Table 2). The positive correlations became more significant with time.

Hosts

All hosts survived well (no mortality) except that some tomato plants died in all species pairs 6 weeks after planting due to a break

out of a plant disease. P deficiency symptoms were observed in tomato plants (with distinct purpling of the under sides of the leaves), but not in other host species.

At 6 weeks, total DWs per host plant did not differ among different species pairs (Table 3), except that total DW per barley plant was higher when grown with *P. rex* than with *P. tricolor* (Fig. 4*a*). Shoot DWs per plant of barley and barrel medic were higher when grown with either *Pedicularis* species than with a second plant of the host species. *P. tricolor* demonstrated stronger parasitic effects (shown by a reduction in DW) than *P. rex* on barley, but not on barrel medic. No significant difference was observed in shoot DW of subterranean clover and tomato among different plant combinations at 6 weeks (Table 3). However, root DW of subterranean clover was significantly higher when grown with a second plant of its own species than with either *Pedicularis* species. For all the host species, R : S ratio was significantly higher when grown with another plant of its own species than with either *Pedicularis* species at 6 weeks (Table 3; Fig. 4*a*).

At 14 weeks, barley grew significantly better in the presence of *P. rex* than with either *P. tricolor* or a second barley plant (Fig. 4*b*). Total DW of barley grown with *P. tricolor* was higher than grown with a second barley plant, but shoot DWs were the same. No significant differences were observed in either total DWs or shoot DWs of barrel medic among different plant combinations (Table 3). Subterranean clover produced higher total DW when grown with *P. rex* than with a second plant of its own species, but showed no difference in shoot DWs among different species pairs (Fig. 4*b*). Root DWs of barley and subterranean clover were higher when grown with the root hemiparasites than with a second plant of a host. However, R : S ratio was significantly higher only in barley grown with *P. tricolor* than when grown with *P. rex* or another barley plant (Table 3).

Total DW per pot

With the exception of barrel medic, all pots with two host plants had about twice the total DW (shoots plus roots) per pot as in host–*Pedicularis* combinations after 6 weeks (Fig. 5*a*). The root hemiparasites made negligible contributions to the total DW at this stage. After 14 weeks, total DW per pot was similar among all plant combinations with the same host species (Fig. 5*b*). Total DW in host–*P. rex* pairs was slightly higher than that with two host plants for barley and subterranean clover. Host–*P. tricolor* pairs produced lower total DW for all tested hosts. The contributions of the root hemiparasites to total DW were higher at this stage, but were still small when compared with that of the hosts.

Discussion

Root hemiparasites

The two *Pedicularis* species used in this study (particularly *P. rex*) showed growth and development in the absence of a host, indicating that they are facultative root hemiparasites. This finding contrasts with other tested *Pedicularis* spp. that did not develop beyond the seedling stage in the absence of a host (Lackney 1981; Ren *et al.* 2010). Since *Pedicularis* spp. do not require host signals for seed germination (Li *et al.* 2007), their
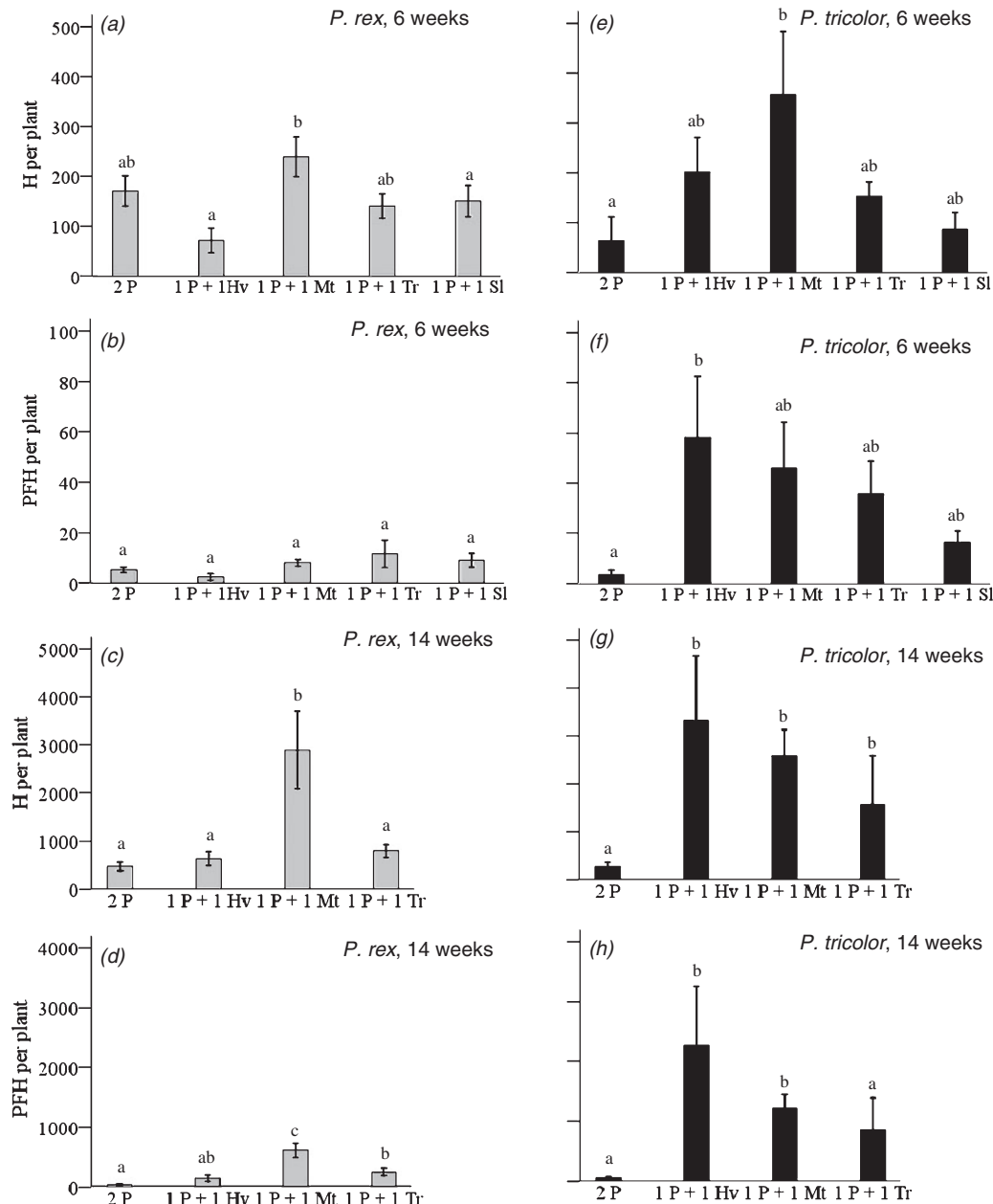
Table 2. Spearman correlation coefficient between shoot DW (SDW), shoot P content (SPC) and total number of haustoria (H) or presumably functional haustoria (PFH) per plant in two *Pedicularis* species
Correlation significance is indicated: ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$

Growth stage	*Pedicularis*	Item	SDW	No. H	No. PFH
Early	*P. rex*	SDW		0.445*	0.422*
seedling		SPC	0.691***	0.446*	0.522*
(6 weeks)	*P. tricolor*	SDW		0.565**	0.616**
		SPC	0.805***	0.387	0.543**
Late	*P. rex*	SDW		0.701**	0.626**
seedling		SPC	0.818***	0.672**	0.863***
(14 weeks)	*P. tricolor*	SDW		0.748***	0.785***
		SPC	0.866***	0.639**	0.663**

Fig. 3. Haustoria (H) and presumably functional haustoria (PFH) formed per plant of *Pedicularis rex* (*a–d*) and *Pedicularis tricolor* (*e–h*) when grown with different plant species after 6 (*a, b, e, f*) and 14 weeks (*c, d, g, h*) of planting. Data are presented as mean ±s.e. of five replicate pots. Statistics were conducted separately for shoot and root of each plant as well as time. Different letters indicate a statistically significant difference at *P* < 0.05 level. Treatments (host–parasite combinations): 2 P, two *Pedicularis* of the same species; 1 P + 1 Hv, one *Pedicularis* and one *Hordeum vulgare*; 1 P + 1 Mt, one *Pedicularis* and one *Medicago truncatula*; 1 P + 1 Tr, one *Pedicularis* and one *Trifolium subterraneum*; 1 P + 1 Sl, one *Pedicularis* and one *Solanum lycopersicum*. Data are missing for *Solanum lycopersicum* at 14 weeks because of an outbreak of fatal plant disease 6 weeks after planting.

autotrophic ability may contribute to fitness when host environments are unpredictable.

Pedicularis rex benefited less when grown with a host and hence showed weaker host dependency than *P. tricolor* in terms of shoot growth and P content. These findings may be partially explained by the stronger capacity for autotrophic

growth in *P. rex* than in *P. tricolor* (Fig. 1). Negative correlations between host dependency and autotrophic ability have been observed previously in other root hemiparasites (Matthies 1995, 1997), but mechanisms were not revealed. In this study we found that while *P. rex* showed a late and small response to the presence of a host plant in terms of

Table 3.   ANOVA results (*F*-values and significance levels) for the effects of parasitism by *Pedicularis rex* and *Pedicularis tricolor* and of competition from a second plant of the same host species on host shoot DW (SDW), root DW (RDW), total DW (TDW) and root : shoot ratio (R : S)

Significance levels of *F*-values are indicated: ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$. Data transformation before ANOVA: [†], arcsine

Growth stage	Host species	SDW	RDW	TDW	R : S[†]
Vegetative stage	*Hordeum vulgare*	34.475***	1.025	3.375	9.494**
(6 weeks), d.f. = (2, 12)	*Medicago truncatula*	5.345*	0.125	1.604	45.225***
	Trifolium subterraneum	0.047	16.94***	2.030	71.124***
	Solanum lycopersicum	0.223	0.802	0.089	8.296**
Reproductive stage	*Hordeum vulgare*	20.741***	7.725**	15.158***	11.450**
(14 weeks), d.f. = (2, 12)	*Medicago truncatula*	0.504	1.450	1.557	0.776
	Trifolium subterraneum	2.208	5.263*	4.518*	2.460

haustorium (particularly PFH) formation, *P. tricolor* responded at the early seedling stage and produced up to 5-fold more haustoria (particularly PFH) than *P. rex* in the presence of a host plant. Since haustoria are the exclusive connections responsible for nutrient transfer from a host to a parasitic plant (Westwood *et al.* 2010), a larger number of haustoria may facilitate nutrient extraction and hence there is more host dependency. Nevertheless, further investigation is required to determine if autotrophic ability of *Pedicularis* spp. is always negatively correlated with their capability for haustorium formation.

The two *Pedicularis* species used in this study showed obvious host preferences in terms of DW, shoot P concentration and shoot P content, but preferred different host species. Barley was a good host for *P. tricolor*, which had the highest P concentration at 6 weeks and highest growth at 14 weeks. However, its only stimulatory effect on *P. rex* was an increase in P concentration at 14 weeks. This disagrees with previous results showing that grasses are good hosts for virtually all root hemiparasitic plants (Lackney 1981; Hedberg *et al.* 2005; Irving and Cameron 2009; Ren *et al.* 2010). Unlike *Rhinanthus minor* that does not effectively parasitise forbs (Cameron *et al.* 2006; Cameron and Seel 2007; Rümer *et al.* 2007), both of the *Pedicularis* species tested here benefited from growing with tomato, at least during early growth stage.

Host preference of the root hemiparasites was reflected in number of haustoria (particularly PFH), since both *Pedicularis* species produced higher numbers of haustoria when grown with those hosts that were most beneficial in increasing growth. However, the number of haustoria was not the only determinant of host effects, as the hemiparasites with the highest number of haustoria did not always perform the best and *vice versa*.

Considering the large difference in plant sizes of the partners and the limited pot space and P supply in the soil, competition from the host may have played a significant role in some species pairs, as observed for other host–parasite pairs (Matthies 1995; Keith *et al.* 2004). Strong competition for nutrients mediated by large root systems of barley (Fig. 4*a*) may also have influenced growth of the root hemiparasites during early seedling growth regardless of potential nutrient uptake via haustoria, which were small in number. As the number of haustoria increased with time, the competition-dominated interaction shifted to a parasitism-dominated one. As a result, inhibitory effects of barley on *P. rex* became

weaker and stimulatory effects of barley on *P. tricolor* were much stronger after 14 weeks. Similarly, competition for light by the vigorously growing subterranean clover but not by barrel medic between 6 and 14 weeks might partially explain the slight difference in their relative performance as host plants between the two growth stages.

Reduction in root biomass allocation of root hemiparasites attached to a host has been suggested to be a strategy for saving energy, as such plants invest less into their root systems than when grown autotrophically (Matthies 1995, 1997). Our findings suggested that patterns of biomass allocation in *Pedicularis* were influenced by host identity. However, the extent of reduction in root allocation in the presence of a host in either species did not correlate with growth stimulation by the host. For example, *P. rex* showed significant reduction in root allocation even when grown with barley, which was the worst host species in terms of growth of the parasite in this study. Reduction in root biomass allocation in root hemiparasites is therefore not always a reliable indicator of host dependency for nutrients, but may also be a result of competition from its host (as mentioned in the barley-*P. rex* associations). Therefore, caution must be used in interpreting R : S ratios in terms of benefits to parasites attached to different hosts.

Our results clearly demonstrated that host–*Pedicularis* interactions were not only species-specific, but also depended, to some extent, on developmental stages. Therefore, in investigations addressing host–*Pedicularis* interactions, a single sampling may be insufficient to get a complete picture of the complex interactions.

Hosts

When space and P was limited by the size of the pots, intraspecific competition had a stronger influence on growth of unattached plants of barley and barrel medic than parasitism by either *Pedicularis* species (as indicated by lower DW per plant and higher R : S ratios when there were two host plants; Fig. 4). This effect was particularly clear during vigorous vegetative growth of the hosts when parasite DW was low. Compared with *P. rex*, the presence of *P. tricolor* eventually resulted in lower shoot DW in barley, to an extent similar to competition from a much bigger barley plant (~10 times of the size of the hemiparasite), suggesting significant influence of the root hemiparasite on growth of its host in this species pair.

Parasitism by *P. tricolor* greatly increased R : S ratio in barley, as observed for other effective root parasites (Matthies 1997;
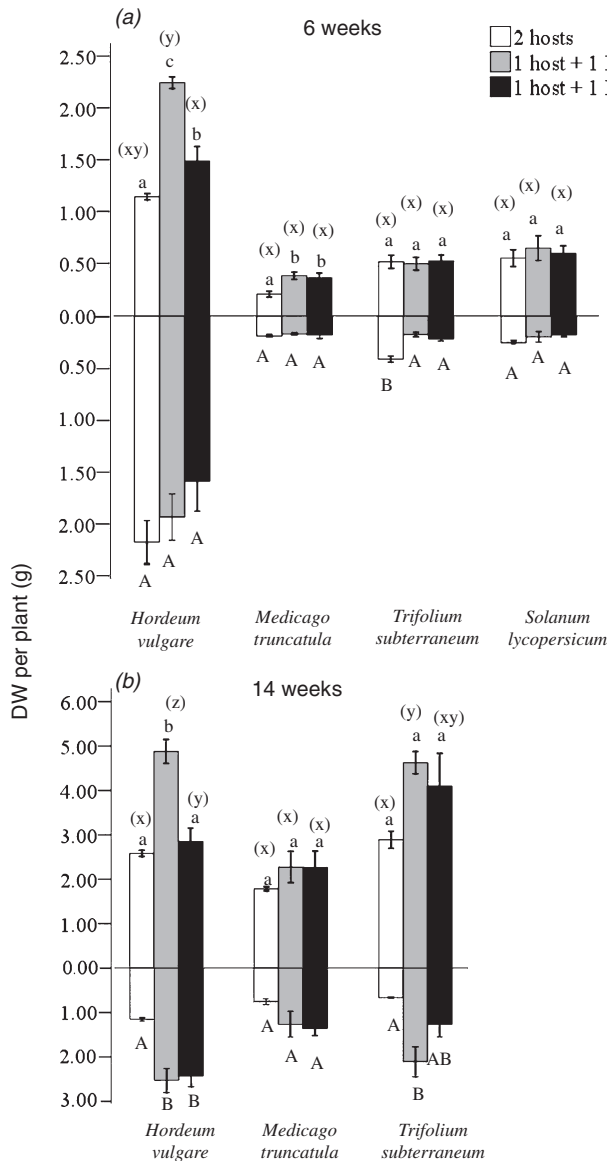
Fig. 4. Effects of *Pedicularis rex*, *Pedicularis tricolor* and a second plant of the same host species on host DW (g) per plant at 6 (*a*) and 14 weeks (*b*) after planting. Data are presented as mean ±s.e. of five replicate pots. Values for pots with two host plants are means of the individuals. Statistics are done separately for shoot (above the line), root (below the line) and total DW of each plant as well as time. Bars for shoots (lower case), roots (upper case) and total DWs (in round brackets) with different letters within each host species indicate statistically significant difference at *P* < 0.05 level. Treatments (host–parasite combinations): 2 hosts, two plants of the same host species; 1 host + 1 Pr, one host and one *Pedicularis rex*; 1 host + 1 Pt, one host and one *P. tricolor*. Data are missing for *Solanum lycopersicum* at 14 weeks because of an outbreak of fatal plant disease 6 weeks after planting.

Fig. 5. Total DW (shoot DW plus root DW, g) per pot with different host–*Pedicularis* combinations at 6 (*a*) and 14 weeks (*b*) after planting. Data are presented as means of five replicate pots, with stacked bars showing values of hosts and the root hemiparasites separately. Treatments (host–parasite combinations): 2 hosts, two plants of the same host species; 1 host + 1 Pr, one host and one *Pedicularis rex*; 1 host + 1 Pt, one host and one *P. tricolor*. Note the small contribution of *Pedicularis* (black bars) to total DW cannot be shown diagrammatically at 6 weeks. Data are missing for *Solanum lycopersicum* at 14 weeks because of an outbreak of fatal plant disease 6 weeks after planting.

Irving and Cameron 2009). However, biomass allocation in hosts of other host–parasite combinations investigated here was not affected by the presence of a parasite. This may be due to either relatively weak parasitism in associations with *P. rex*, or stronger tolerance to parasitism in some host species (Joshi *et al.* 2000; Irving and Cameron 2009). Underlying mechanisms for lack of
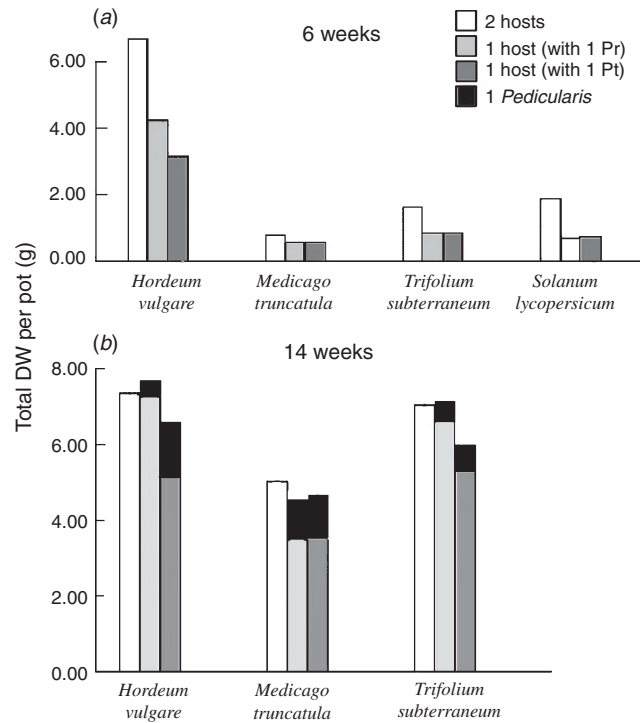
biomass allocation responses to parasitism in other hosts investigated here require further investigations.

The results indicated that *P. tricolor* may reduce aboveground productivity in grass–parasite combinations, which agrees with the general observation that root hemiparasites have strong influences on grass-dominated plant communities (Hedberg *et al.* 2005; Qiu *et al.* 2006; Zhang *et al.* 2009; Hellström *et al.* 2011). Parasitic effects of *Pedicularis* spp. at community level are, therefore, determined not only by the effectiveness of the parasite, but also by the plant community structure where the hemiparasite occurs.

Unlike *P. rex*, *P. tricolor* consistently reduced total DW per pot, compared with DW when there were two host plants (Fig. 5). Where the combination of one host plus one *Pedicularis* plant gives total DW per pot below maximum DW (e.g. with two hosts), this must be due to a parasitic effect not compensated for by growth of the host or parasite. Reduction in host DW caused by effective parasitism is often not compensated for by the DW produced by the parasites (Matthies 1997; Joshi *et al.* 2000; Irving and Cameron 2009), leading to reduced productivity per pot, as here in the presence of *P. tricolor*.

It has been suggested that host growth depression caused by parasitism can be alleviated by increased N supply in some

cases (Cechin and Press 1994; Jiang *et al.* 2010). This hypothesis was not experimentally tested in this study. However, we could not exclude the possibility that higher N supply in the nutrient solution may have alleviated the growth reductions of hosts in the presence of *Pedicularis* in some host–parasite pairs. It will be worthwhile testing how N availability affects host–*Pedicularis* interactions along N gradients.

This study was conducted in a P-deficient growth medium that may have stressed some species pairs. Nevertheless, both *Pedicularis* species showed significantly increased P uptake and improved growth performance when attached to appropriate hosts, suggesting substantial P transfer from hosts to the root hemiparasites. Phosphorus should therefore also be considered in investigations of effects of nutrient supply on host–*Pedicularis* interactions.

Conclusion

Both *P. rex* and *P. tricolor* were capable of autotrophic growth but growth was stimulated by attachment to an appropriate host. However, *P. tricolor* showed a much stronger growth response to the presence of a host and decreased host growth to a greater extent than *P. rex*. *P. tricolor* may, therefore, like other effective root hemiparasites (Gibson and Watkinson 1992; Cameron *et al.* 2009; Hellström *et al.* 2011), influence competition between host species and potentially affect the plant community structure and diversity of the habitats where it occurs. However, the results of this study suggest that a generalisation of consequences of host–parasite interactions is impossible, because even sympatric root hemiparasites (as used here) can affect and respond very differently to the same host species. Nevertheless, since grasses are generally the most susceptible hosts to infection by root hemiparasites (Ameloot *et al.* 2005), priority should be directed to management of grass-dominated ecosystems in which abundant root hemiparasites occur. In addition, since *Pedicularis*-host interactions vary among different developmental stages, multi-samplings over the growing season are encouraged to understand the complex host–parasite interactions.

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