Title

Nutrient mediation of sink strength in the Orobanche minor — Red clover association

Journal

Environmental and Experimental Botany, 228

ISSN

0098-8472

Authors

Hattori, Mao

Frederica, Clarissa Frances

Irving, Louis John

Publication Date

2024-12-01

DOI

10.1016/j.envexpbot.2024.106041

Peer reviewed

Environmental and Experimental Botany 228 (2024) 106041

Contents lists available at ScienceDirect

Environmental and Experimental Botany

journal homepage: www.elsevier.com/locate/envexpbot

Research paper

Nutrient mediation of sink strength in the *Orobanche minor* – Red clover association

Mao Hattori, Clarissa Frances Frederica [1], Louis John Irving [*,2]

Institute of Life and Environmental Sciences, School of Science and Technology, University of Tsukuba, Tennodai 1-1-1, Tsukuba 305-8577, japan

ARTICLE INFO

Keywords:
Orobanche minor
Red clover
Nutrients
Sink strength
Light
Growth allocation

ABSTRACT

Holoparasites are non-photosynthetic plants which derive all their growth requirements from their host plant and are thought to act as a very strong sink for host resources. Here, we grew red clover plants in split-root boxes to explore the effect of nutrient supply to *Orobanche minor* parasitized or unparasitized host roots. Where nutrients were supplied to parasitized roots, parasite growth was strongly promoted at the expense of the host. Conversely, host growth did not differ significantly from unparasitized controls where nutrients were supplied to unparasitized roots. While ^{15}N labelling suggested both strong parasitic ammonium abstraction and reduced nitrate uptake in parasitized roots, the total N content of systems where nutrients were fed to parasitized roots was approximately 26 % higher than control plants, suggesting that changes in host and parasite growth rates were due to changes in sink strength, rather than nutrient uptake. Parasitism and nutrient supply had strong effects on leaf carbohydrate metabolism but did not affect photosynthetic rates or leaf N concentration. In the second experiment, we investigated the importance of light level on the host – parasite relationship, concluding that parasitism had a diminished effect on host growth under low light conditions. Total system mass was unaffected by the apparent sink strength of the parasite. Our results suggest a dynamic relationship between host shoot and parasite sink strengths, mediated by changes in nutrient status.

1. Introduction

Parasitic plants abstract nutrients and other resources from their host plant and are a major cause of food insecurity worldwide (Rodenburg et al., 2016). Holoparasites, such as members of the genus *Orobanche*, are non-photosynthetic and rely on a host plant for all their growth needs. There are over 200 species of *Orobanche* (World Flora Online, 2024), with several species being important agricultural weeds (Habimana et al., 2014). Despite their importance, there are relatively few studies investigating the mature host – holoparasite relationship, and we lack a clear understanding of how environmental factors may influence the relationship.

Orobanche connect to the host plant via an organ known as the haustorium (Musselman, 1980), which allows the formation of direct symplastic phloem connections between the parasite and its host (Dörr and Kollmann, 1995; Krupp et al., 2019). *Orobanche* is thought to act as a very strong sink for phloem resources, able to outcompete the host meristems and completely suppress host growth (Barker et al., 1996; Fernández-Aparicio et al., 2016a; Hibberd et al., 1998). The average mass of *Orobanche* parasites has been noted to decline exponentially with the number of individuals per host plant (Fernández-Aparicio et al., 2016a; Hibberd et al., 1998), which has been interpreted as the parasites competing for a common photoassimilate pool (Hibberd and Jeschke, 2001). In addition to carbon (C), *Orobanche* is thought to derive all of its nutrients from the host vascular system and are reported to lack functional roots (Fernández-Aparicio et al., 2016b). While it is generally thought that C abstraction is the main mechanism by which the parasite causes damage, Mauromicale et al. (2008) found that declines in host biomass were primarily due to reductions in host photosynthetic capacity, with the parasite accounting for only 4 % of the lost biomass.

The relative growth rates of autotrophic plants strongly correlate with whole plant C gain (Kruger and Volin, 2006). For non-photosynthetic holoparasites such as *Orobanche*, growth rates would be contingent on both the rate of host photosynthesis and the transfer of photoassimilates to the parasite. Hibberd et al. (1999) reported an increase in whole plant C fixation in tobacco plants parasitized

* Corresponding author.
E-mail address: irving.louis.fb@u.tsukuba.ac.jp (L.J. Irving).
[1] **ORCID:** 0000–0003-1238–8025
[2] **ORCID:** 0000–0002-9735–2648

https://doi.org/10.1016/j.envexpbot.2024.106041
Received 27 July 2024; Received in revised form 30 October 2024; Accepted 12 November 2024
Available online 13 November 2024

by *O. cernua*, which was attributed to a suppression of senescence and the maintenance of photosynthesis in older leaves. However, other studies have reported either no effect (Dale and Press, 1998), or a decline (Jokinen and Irving, 2019; Mauromicale et al., 2008) in host photosynthetic rates in response to parasitism by *Orobanche*. In *O. cumana* infected sunflower plants, declines in photosynthetic rates were associated with a severe reduction in mesophyll thickness (Pincovici et al., 2018).

The growth of new tissues – including holoparasites – is facilitated by the transport of sugars and amino acids from source tissues to sink organs by the phloem (Lalonde et al., 2003). As the main source of photoassimilates, leaves are the primary site of phloem loading in plants. N taken up by roots is transported to the shoot in the xylem, where it can be loaded into the phloem as amino acids (Tegeder and Hammes, 2018). Phloem unloading of resources by *Orobanche* is facilitated by the accumulation of mannitol and hexose in parasite tissues (Delavault et al., 2002), although active transport of sugars also appears to be important (Péron et al., 2017). The source – sink dynamics of a mature plant are complex, with resource allocation determined by a combination of sink strength (Minchin and Lacointe, 2005) and proximity to the source organs (Osaki et al., 2004). It has been hypothesized that root holoparasites and N fixation by rhizobia may compete for C (Watling and Press, 2001), with root nodules representing a strong sink for photoassimilates given the high energetic cost of N fixation (Gordon et al., 1987; Schuize et al., 1999). We attempted to test this hypothesis in a previous paper by our group by manipulating host C and N status (Jokinen and Irving, 2019). However, contrary to expectations, we did not find evidence of changes in the competitive balance between the host, parasite, and rhizobium. This led us to speculate that nutrient assimilation and parasitism may occur on different roots, with nutrient uptake suppressed only on parasitized roots.

In our first experiment, we investigated the effect of nutrient supply to parasitized or unparasitized roots on the host – parasite relationship. We hypothesized that 1) host mass would be lower in plants supplied nutrients to the parasitized root, due to suppressed nutrient uptake, 2) parasite mass would not significantly differ between treatments, assuming that parasite growth is largely determined by C supply, and that fed and unfed roots have equal access to C from the shoot, and 3) C abstraction by the parasite would lead to a reduction in host leaf C status, resulting in increased photosynthetic rates. Our second experiment investigated the effects of fed root and light level on the host – parasite relationship, with the goal of manipulating plant source: sink dynamics. We hypothesized that 4) parasite mass would be greater in plants grown in strong light, resulting in greater host damage.

2. Material and methods

2.1. Fed-root experiment

Our first experiment explored the effects of nutrient supply to *Orobanche minor* parasitized vs. unparasitized roots (fed root). We grew red clover (*Trifolium pratense* L.) host plants in split root boxes with two chambers, each approximately 68 mm wide, 160 mm deep and 8 mm thick (Supplementary Figure 1). In half the boxes, *O. minor* seeds were mixed with vermiculite 1 cm from the top of the separator in the left-side chamber, while the other half of the boxes acted as parasite-free controls. This resulted in four treatments, where the plants were designated either as parasitized (P) or control (C) and fed nutrients to either the left (L) or right (R) chamber. *Orobanche minor* seeds were derived from a natural population on the campus of the University of Tsukuba, Japan.

Red clover seeds (Takii seeds, Kyoto, Japan) were germinated in vermiculite filled straws (12 mm diameter) for one week before transplanting into the split-root boxes. At transplanting, the tip of the tap root was excised to promote lateral root growth into both chambers. The plants were established in the boxes for two weeks, during which they were provided with 10 ml of Broughton and Dilworth nutrient solution supplemented with 10 mM N as ammonium nitrate (Broughton and Dilworth, 1971). Following this, the plants were supplied 20 ml of the same nutrient solution without phosphate every two days for a further two weeks to promote parasite seed germination (Yoneyama et al., 2007). The plants were supplied the standard nutrient solution for a further two weeks before starting treatments. The composition of the nutrient solution may be found in the supplemental materials.

The plants received approximately 450 μmol photons m^{-2} s^{-1} from LED growth lights with a 16/8 photoperiod. The temperature in the growth chamber was approximately 25°C during the day and 22°C at night. There were 13 replicates in each group.

2.1.1. Nutrient treatments

During the treatment period, 30 ml of a modified Broughton and Dilworth nutrient solution containing 10 mM of N as ammonium nitrate was supplied either to the left or right chamber of each box every two days for six weeks, with water supplied to the other chamber. The nutrient supplied roots were designated as the fed roots. One day before harvest, 1 mg of 99 at% ^{15}N was injected 2 cm from the bottom of the fed chamber. Seven boxes in each treatment had ^{15}N supplied as $(^{15}NH_4)_2SO_4$, while the other six boxes received $K^{15}NO_3$.

2.1.2. Photosynthetic measurements

Two days before harvest, leaf photosynthetic properties were measured using a MultispeQ v2.0 running the Photosynthesis RIDES 2.0 protocol (PhotosynQ Inc., Michigan, USA). The measured leaf was harvested, with one leaflet imbibed in methanol for 24 hours in the dark in a refrigerator at 4°C for chlorophyll quantification using the method of Porra et al. (1989).

2.1.3. Harvest

At harvest, the plants were separated into shoots, tap root, left and right roots and parasites. Host leaves were scanned using a Canon LiDE 220 flatbed scanner and their area quantified using imageJ (Schneider et al., 2012). The tissues were oven-dried at 70°C for at least five days before being weighed. The host leaves and parasite tissues were ground to powder using a ball mill, and the tissue N concentrations and ^{15}N abundances were quantified by an elemental analyzer coupled to a mass spectrometer, with glycine used as a standard (EA Isolink CN IRMS System, ThermoFisher Scientific, Japan). Host leaf alcohol soluble sugars were quantified using the phenol-sulfuric acid method for total carbohydrates, while starch contents were determined following amylase digestion using the PGO enzyme method, according to the protocol of Chow and Landhausser (2004).

2.2. Light level experiment

This experiment aimed to study the influence of light intensity on the host – parasite relationship. The plants were established as in the fed-root experiment. All plants in this experiment were parasitized. During the treatment period, half the plants were grown under strong light (S) and the other half under weak light (W) conditions (500 and 100 μmol photons m^{-2} s^{-1} respectively), with the assumption that the host plants would be C-deficient under weak light conditions. Within each group, half the plants were supplied nutrients to the parasitized root (left: L) and the other half were supplied nutrients to the unparasitized roots (right: R). The experimental duration and biomass, leaf area and chlorophyll measurements were conducted as in the fed root experiment. Tissue nitrogen concentration was quantified following acid digest using Nessler reagent. There were ten replicates in each group except SL which had nine.

2.3. Statistical analysis

Host data was analyzed by two-way ANOVA in JASP version 0.18.3 (JASP Team, 2024). Specific pairwise comparisons were conducted

using planned contrasts. Paired root data was analyzed by Repeated Measures ANOVA. In the fed-root experiment, starch data was cube-root transformed prior to analysis, while parasite mass data was compared by Welch's t-test. In light-level experiment, the leaf N concentration was analyzed by Kruskal – Wallis test.

3. Results

3.1. Fed-root experiment

3.1.1. Biomass

The goal of this experiment was to test whether host growth would be suppressed where nutrients are supplied to parasitized roots due to reduced nutrient uptake. The system mass (host plus parasite) was 17 % lower ($t_{(48)} = 2.67$, $P = 0.010$; Table 1) in PL plants compared with the control and PR plants, which formed a single group. PL host stem mass was approximately 40 % lower than the other groups, while PL and PR leaf dry mass were 44 % and 14 % lower than controls ($P < 0.001$ and 0.028, respectively, Fig. 1). Parasitism had little influence on the partitioning of shoot matter between leaves and stems.

Nutrient effects on the host – parasite relationship were more pronounced belowground. PL plants achieved a root mass 44 % that of the other three groups, with the left and right root masses 43 and 31 % those of the CL plants. In PR and control plants, the mass of fed roots was approximately 2.90 (±0.17) times greater than unfed roots. In PL plants, the fed root mass was approximately 5.11 (±0.71) times the unfed roots, primarily due to the low mass of the right chamber roots. PL parasites achieved a mass 2.50 times greater than the PR treatment ($t_{(22.41)} = 4.77$, $P < 0.001$).

3.1.2. ^{15}N distribution

One day before harvest, the plants were supplied 1 mg of ^{15}N either as ammonium sulphate or potassium nitrate to the bottom of the fed chamber to quantify the uptake and transfer of N within the system. Host leaf and parasite ^{15}N content were measured. No difference in system ^{15}N (host + parasite) content was found between treatments in the ammonium-fed systems (Fig. 2a). In the PL systems, the host leaves and parasite contained approximately equal amounts of ^{15}N, while PR parasites accounted for only 18.2 % (±2.4) of system ^{15}N. Where ^{15}N was supplied as nitrate (Fig. 2b), system ^{15}N content was 40 % lower in the PL systems compared with control plants, while the PR systems exhibited a 49 % increase in ^{15}N content ($t_{(44)} = 2.20$, $P = 0.033$ and $t_{(20)} = 2.68$, $P = 0.010$, respectively). In the PL systems, host leaf ammonium and nitrate ^{15}N contents were 45 and 62 % lower than control plants, while the leaf ammonium and nitrate ^{15}N contents of PR host plants were 96 and 131 % of controls.

3.1.3. Tissue characteristics

The total leaf area was 12 % lower in PR plants compared with controls ($t_{(48)} = 2.02$, $P = 0.049$), and 41 % lower in the PL plants ($t_{(48)} = 6.56$, $P < 0.001$; Fig. 3a). Individual leaflet area was 11 % lower in parasitized plants ($F_{(1,48)} = 4.17$, $P = 0.047$) and 11 % lower in the left-fed than right-fed plants ($F_{(1,48)} = 4.11$, $P = 0.048$), and although no interaction was noted ($P = 0.212$) this seemed to be mainly due to a reduction in PL leaf area. No significant treatment effects were noted on electron transport rates (Fig. 3b) or other leaf fluorescence parameters, suggesting that parasitism had no effect on leaf photosynthesis.

The leaf chlorophyll concentration was significantly higher in parasitized plants compared to controls ($F_{(1,48)} = 4.92$, $P = 0.031$; Fig. 3c), which may have been due to the reduced average leaf area in parasitized plants. Leaf N concentration did not differ significantly between treatments (Fig. 3d). Parasite N concentration was 2.7 % (±0.6) with no difference between treatments ($P = 0.795$; data not shown).

Parasitism caused a 35 % decrease in leaf soluble sugar concentration ($F_{(1,48)} = 22.22$, $P < 0.001$), with no effect of fed root (Fig. 3e). Starch levels were 48 % and 77 % lower in the PR and PL plants than in controls ($t_{(48)} = 2.75$, $P = 0.008$ and $t_{(48)} = 6.47$, $P < 0.001$ respectively; Fig. 3 f).

Table 1;
Biomass (mg) of the total system (host + parasite), host shoot, host root and parasite of red clover plants grown in a split root box in the presence (P) or absence (C) of a *Orobanche minor* parasite seeds in the left-side chamber, where nutrients were fed to the left (L) or right (R) roots. Values in parentheses represent standard errors. $n = 13$.

	PL	PR	CL	CR
Total system	1436.1 (90.6)	1788.7 (83.8)	1720.1 (115.2)	1657.4 (77.8)
Host shoot	558.7 (35.5)	866.7 (45.3)	990.8 (61.9)	956.4 (50.5)
Host root	315.4 (35.4)	697.3 (50.0)	729.5 (66.5)	701.0 (37.1)
Parasite	562.0 (56.2)	224.7 (42.0)	-	-

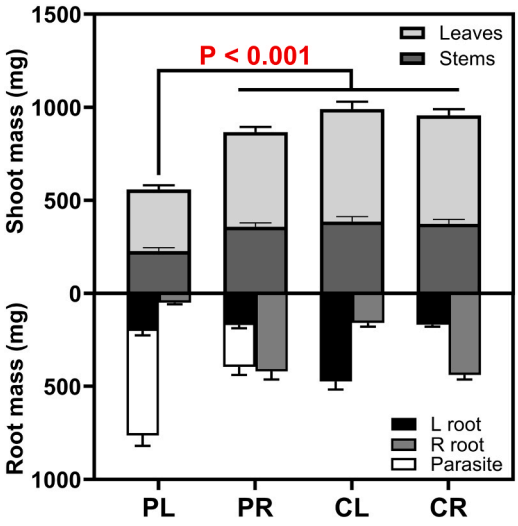

Fig. 1. Shoot and root biomass of *O. minor* parasitized (P) and control (C) red clover plants grown in split root boxes. Nutrients were fed to the left (L) or right (R) roots. $n = 13$. Error bars represent standard errors. PL host shoot mass was significantly reduced relative to PR and control plants.

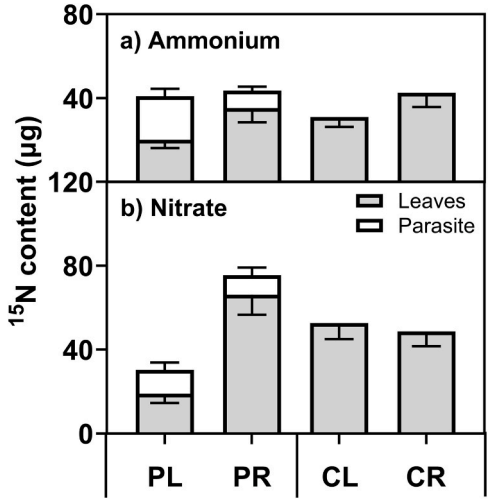

Fig. 2. Host leaf and parasite ^{15}N content of *O. minor* parasitized clover plants, supplied 1 mg of ^{15}N as a) ammonium sulphate or b) potassium nitrate to the left (L) or right (R) roots of parasitized (P) or control (C) plants one day before harvest. For ammonium, $n = 7$, for nitrate $n = 6$. Error bars represent standard errors.

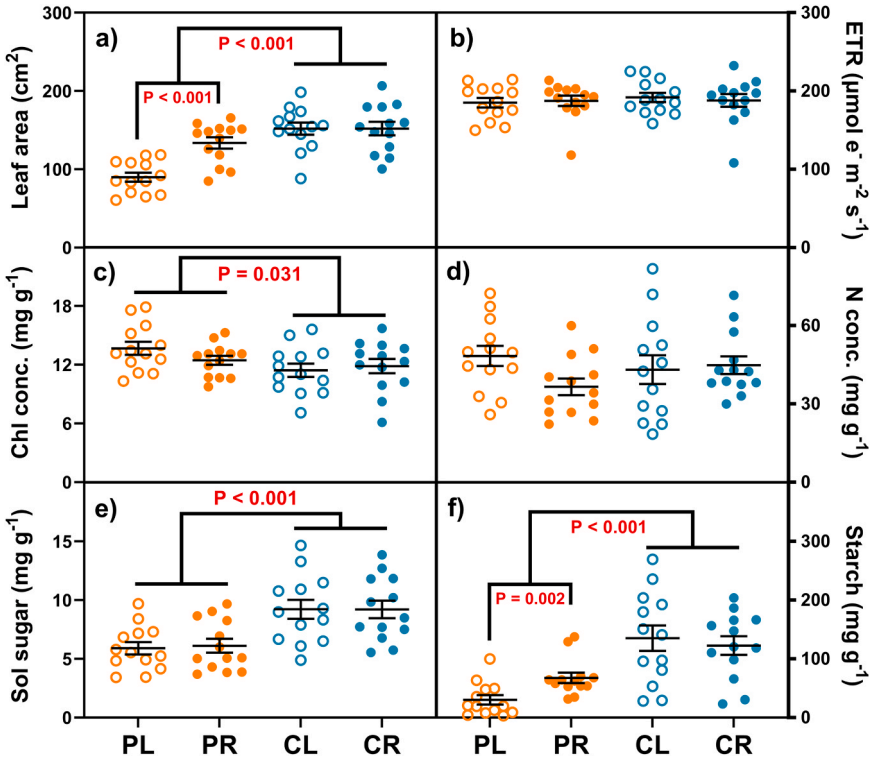

Fig. 3. a) leaf area, b) electron transport rate, c) chlorophyll concentration, d) N concentration, e) alcohol soluble sugar concentration, f) starch concentration of *O. minor* parasitized (P, orange) and control (C, blue) red clover leaves. The plants were grown in a split root box and provided nutrients to either the left (L, open symbols) or right (R, closed symbols) roots. $n = 13$. Error bars represent standard errors.

3.1.4. System N content

Leaf N content was 31 % lower in parasitized host plants relative to controls ($F_{(1,48)} = 19.83$, $P < 0.001$; Fig. 4) with no effect of fed root. In PL plants, this reduction was associated with a lower leaf mass, while in PR plants the reduction in N content was associated with a combination of non-significant declines in both host leaf mass and tissue N concentration. Where the parasite and host leaf N contents were summed, the control and PR plants were almost identical (24.7 mg ±1.1), while the PL plants had an N content approximately 26 % higher (30.1 mg ±2.5; $t_{(48)} = 2.58$, $P = 0.013$).

3.2. Light level experiment

In this experiment, we aimed to explore the influence of plant C balance on the host – parasite system, with the hypothesis that parasitic damage will be greater under high light conditions. Total system mass was approximately 2.1-times greater under strong light conditions ($F_{(1,35)} = 53.75$, $P < 0.001$; Fig. 5), with no effect of fed root ($P = 0.753$). Under weak light conditions, fed root had no effect on either host or parasite growth. SL plants achieved shoot masses similar to the weak light plants ($P = 0.528$), while the SR plants achieved significantly higher shoot and root masses ($P < 0.001$) than the other groups. Similarly, parasite masses did not differ between SR and weak light plants, while the SL parasites achieved masses approximately 3.8-times greater than the SR parasites.

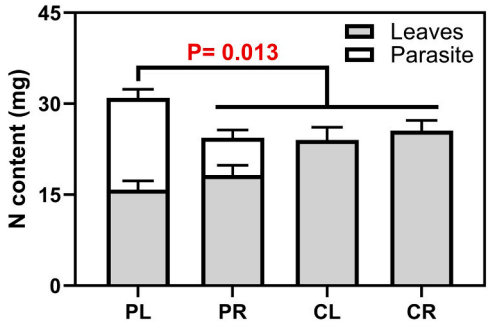

Fig. 4. Host leaf and parasite N content of parasitized (P) or control (C) plants, supplied nutrients to the left (L) or right (R) root chamber. $n = 13$. Error bars represent standard errors.

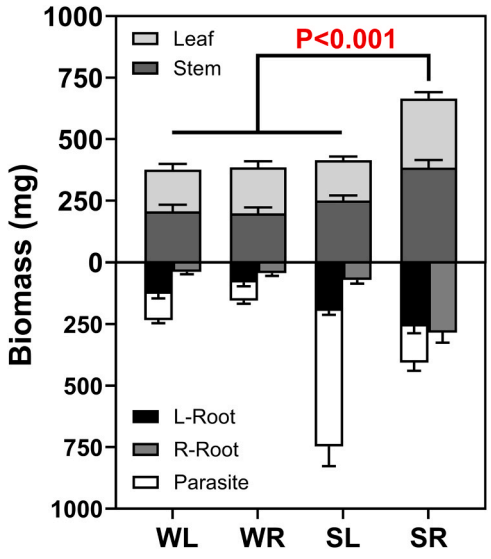

Fig. 5. Shoot and root biomass of *O. minor* parasitized red clover plants grown under weak or strong light (W / S: 100 / 500 µmol photons m^{-2} s^{-1}), and supplied nutrients to the left (L) or right roots (R). $n = 10$. Error bars represent standard errors.

Right fed plants had a 40 % higher leaf area than left-fed plants ($F_{(1,36)} = 10.56$, $P = 0.003$; Fig. 6a), with no effect of light level ($P = 0.394$). The specific leaf mass was 37 % higher in strong light plants ($F_{(1,34)} = 70.38$, $P < 0.001$: Fig. 6b), with no effect of fed root ($P = 0.559$). The chlorophyll concentration was 81 % higher in weak light plants ($F_{(1,34)} = 99.73$, $P < 0.001$: Fig. 6c), with no effect of fed root ($P = 0.546$). Leaf N concentrations tended to be higher under weak light ($H_{(1)} = 7.61$, $P = 0.006$) and in left-fed plants ($H_{(1)} = 6.89$, $P = 0.009$; Fig. 6d).

4. Discussion

In the fed-root experiment, PL host plants exhibited significantly reduced biomass while PR plants did not differ from controls (Fig. 1). Our ^{15}N data shows that PL parasites strongly abstracted ammonium from their host root and caused a suppression of nitrate uptake, while the PR parasites had no negative effect on N uptake (Fig. 2). Despite this, while leaf N content was lower in parasitized hosts relative to controls, it did not differ between PL and PR plants. This suggests that contrary to both our hypothesis and the ^{15}N data, reduced N uptake was not responsible for differences in host shoot biomass. Indeed, the total N content (host leaf + parasite) was 26 % higher in PL plants relative to PR and control plants (Fig. 4), suggesting higher N uptake rates by parasitized roots over the entire treatment period (Hibberd et al., 1999).

Contrary to expectations, PL parasite mass was approximately 2.5-times greater than the PR systems. Along with the higher N content of PL systems, PL parasites accounted for a greater proportion of that N (49 %) compared with PR systems (24 %). Our data suggests that nutrient-replete parasites are a strong sink for host carbohydrate, with increased parasite growth explaining the greater host growth suppression in PL systems. Conversely, PR parasites were seemingly a weak sink for phloem resources, unable to redirect sufficient nutrients to support rapid growth. This is consistent with the ^{15}N data, which indicate that the parasite has little access to N supplied to unparasitized roots. While the mass of PR parasites could be explained by non-significant declines in host mass, this was not true in the PL plants, suggesting that our data cannot be explained by source: sink dynamics alone. Parasite respiration may explain the lower total biomass in PL systems.

Although there was no difference in linear electron flow rates between treatments, PL plants exhibited decreased leaf area, suggesting that whole plant C gain may have been lower than in control plants (Dale and Press, 1998). Parasitism caused a significant reduction in host leaf carbohydrate levels (Abbes et al., 2009), although other studies have reported increases in leaf soluble sugars (Singh et al., 1972; Jokinen and Irving, 2019). Carbohydrate abstraction by *Orobanche* seemingly lowered host C status to a level where starch accumulation was suppressed (Singh et al., 1968), with the greater declines in the PL treatment being consistent with our hypothesis that nutrient supply influences parasitic C sink strength. Contrary to some previous studies (Amri et al., 2021; Mauromicale et al., 2008), chlorophyll levels were slightly higher in parasitized plants, while N concentrations were unaffected.

Under weak light conditions there was no difference in either host or parasite biomass between WL or W*R* plants, presumably due to C-insufficiency. Under strong light conditions, the total system biomass did not differ between groups, although the parasite accounted for 43.2, and 10.7 % of system biomass in SL and SR treatments, with the higher figure broadly similar to previous reports (Fernández-Aparicio et al., 2016a; Lins et al., 2007). Given that plant relative growth rates correlate with plant photosynthesis (Kruger and Volin, 2006), the lack of difference in system biomass between left and right-fed plants at each light level suggests that the source strength of the leaves was not strongly affected by fed root. This is consistent with the lack of difference in ETR between treatments in the fed-root experiment. It should be noted that neither photosynthetic rates nor leaf sugar levels were measured in the light level experiment, and further investigation is required to confirm these conclusions. As in the fed-root experiment, the leaf area tended to be lower in left-fed plants, although a higher leaf N concentration may have compensated for this.

N uptake is strongly dependent on shoot photoassimilates (Lejay et al., 2003). In our experiments, host roots tended to be considerably thinner below the point of parasite attachment (Fig. 7), likely due to parasitic C abstraction (Dale and Press, 1998). Contrary to expectations, photoassimilate withdrawal had no effect on ammonium uptake, while nitrate uptake was strongly suppressed. Although the total amount of ^{15}N supplied (1 mg ml^{-1}; 71 mM) was in-keeping with the nutrient solution used, the concentration of ammonium around the injection site

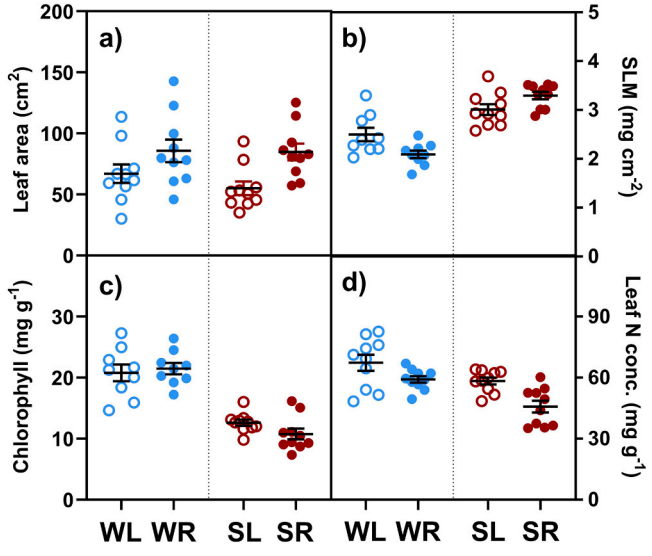

Fig. 6. a) leaf area, b) specific leaf mass, c) chlorophyll concentration, d) leaf N concentration of *O. minor* parasitized red clover plants grown under weak or strong light (W, blue / S, red: 100 / 500 μmol photons m^{-2} s^{-1}) and supplied nutrients to the left (L, open symbols) or right roots (R, closed symbols). $n = 10$. Error bars represent standard errors.

Fig. 7. *Orobanche minor* attached to a red clover root. The upper arrow indicates the point of attachment of a secondary haustoria. The lower arrow indicates the attachment point of the primary haustoria. The host root is noticeably thicker above the point of secondary haustorium attachment.

may have been sufficiently high for passive low-affinity transport to occur (Britto et al., 2001; Coskun et al., 2013; Yuan et al., 2007), potentially rendering root C-status irrelevant. Conversely, nitrate uptake is always active (Hachiya and Sakakibara, 2017), with the noted declines in nitrate uptake in PL plants presumably due to C insufficiency.

It is likely that some of the roots in the left-chamber were not parasitized in the PL group, and our observation that ^{15}N supplied as ammonium was split approximately equally between the parasites and the host leaves may potentially underestimate of the magnitude of ammonium abstraction from individual parasitized roots. Ammonium is primarily assimilated to glutamine in the root (Tobin and Yamaya, 2001), and host to parasite transfer of ammonium-N was presumably as amino acids, although ammonium can also be transported in the xylem (Husted et al., 2000). *Orobanche* is generally considered to be phloem-feeding and although it retains a xylem bridge with the host plant, this is not considered to be a major conduit for resources (Hibberd et al., 1999). Instead, it has been proposed that holoparasitism may cause the rapid transfer of xylem-borne resources to the host phloem (Jeschke et al., 1994), and direct abstraction of nitrate from nutrient-supplied host roots by *O. minor* has been demonstrated (Kawachi et al., 2008). Although it is thought that the parasite is incapable of direct nutrient uptake (Fernández-Aparicio et al., 2016b), we cannot discount it as a possibility.

Although we have focused on N transfer, the roots were supplied a complete nutrient solution to one side and water to the other, and some other component of the nutrient solution may have been more important in explaining parasite growth than N (Southwood, 1971). Furthermore, the nutrient solutions were supplied from the top of the box during the treatment period, while ^{15}N was injected to the bottom of the fed chamber. It is not clear whether nutrient uptake rates differ above and below the point of parasite attachment. In the PR treatment, we assume that ^{15}N recovered in the parasite resulted from phloem transport within the plant, but nutrient leakage or root crossover between chambers cannot be ruled out.

The ecological and agronomic implications of our findings will depend on the specific responses of both the host and parasite to nutrients. Changes in host mass and leaf area have obvious implications for competition, as do the more severe reductions in root mass. Where rhizobia are present, the situation becomes yet more complex. Assuming that parasites and rhizobia on different roots do not compete for C, the proportion of parasitized roots may have a strong influence on host growth. Nutrient supply to unparasitized roots may represent a metabolically "cheap" source of N for the plant relative to fixation (Kaschuk et al., 2009), and although exogenous N application may suppress nodule formation, it has also been found to have a suppressive effect on *Orobanche* spp. (Habimana et al., 2014).

In conclusion, in the fed-root experiment, parasitized plants achieved lower shoot and root masses where nutrients were supplied to parasitized roots, which was seemingly due to an increase in parasite sink strength. Parasites attached to nutrient-fed roots achieved higher masses than those attached to unfed roots, which we speculate was primarily due to nutrient-replete parasites acting as a strong sink for host C. In the light level experiment, we concluded that the parasite had little effect on host growth under low-light conditions, while at high light, parasite growth was strongly contingent on local nutrient supply. Our data reveals a more dynamic relationship between *Orobanche minor* and its red clover host than has been previously suggested, and calls into question assumptions about the sink strength of the parasite.

Funding information

This work was supported by a Japan Society for the Promotion of Science grant, award number 23K05916.

CRediT authorship contribution statement

Mao Hattori: Writing – original draft, Visualization, Investigation, Formal analysis, Data curation, Conceptualization. **Clarissa Frances Frederica:** Writing – review & editing, Visualization, Investigation, Formal analysis, Conceptualization. **Louis Irving:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Louis John Irving reports financial support was provided by Japan Society for the Promotion of Science. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors would like to thank Dr. Shigeki Wada (University of Tsukuba) for assistance with mass spectrometry. We would like to thank three anonymous referees for their kind comments which strengthened the manuscript.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.envexpbot.2024.106041.

Data Availability

Data is available at: Zenodo https://doi.org/10.5281/zenodo.11631254.

References

Abbes, Z., Kharrat, M., Delavault, P., Chaïbi, W., Simier, P., 2009. Nitrogen and carbon relationships between the parasitic weed *Orobanche foetida* and susceptible and tolerant faba bean lines. Plant Physiol. Biochem. 47, 153–159. https://doi.org/10.1016/j.plaphy.2008.10.004.

Amri, M., Abbes, Z., Trabelsi, I., Ghanem, M.E., Mentag, R., Kharrat, M., 2021. Chlorophyll content and fluorescence as physiological parameters for monitoring *Orobanche foetida* Poir. Infection in faba bean. PLoS One 16. https://doi.org/10.1371/journal.pone.0241527.

Barker, E.R., Press, M.C., Scholes, J.D., Quick, W.P., 1996. Interactions between the parasitic angiosperm *Orobanche aegyptiaca* and its tomato host: Growth and biomass allocation. N. Phytol. 133, 637–642. https://doi.org/10.1111/j.1469-8137.1996.tb01932.x.

Britto, D.T., Siddiqi, M.Y., Glass, A.D.M., Kronzucker, H.J., 2001. Futile transmembrane NH_4^+ cycling: A cellular hypothesis to explain ammonium toxicity in plants. Proc. Natl. Acad. Sci. 98, 4255–4258. https://doi.org/10.1073/pnas.061034698.

Broughton, W.J., Dilworth, M.J., 1971. Control of leghaemoglobin synthesis in snake beans. Biochem. J. 125, 1075–1080. https://doi.org/10.1042/bj1251075.

Chow, P.S., Landhausser, S.M., 2004. A method for routine measurements of total sugar and starch content in woody plant tissues. Tree Physiol. 24, 1129–1136. https://doi.org/10.1093/treephys/24.10.1129.

Coskun, D., Britto, D.T., Li, M., Becker, A., Kronzucker, H.J., 2013. Rapid ammonia gas transport accounts for futile transmembrane cycling under NH3/NH4+ toxicity in plant roots. Plant Physiol. 163, 1859–1867. https://doi.org/10.1104/pp.113.225961.

Dale, H., Press, M.C., 1998. Elevated atmospheric CO_2 influences the interaction between the parasitic angiosperm *Orobanche minor* and its host *Trifolium repens*. N. Phytol. 140, 65–73. https://doi.org/10.1046/j.1469-8137.1998.00247.x.

Delavault, P., Simier, P., Thoiron, S., Véronési, C., Fer, A., Thalouarn, P., 2002. Isolation of mannose 6-phosphate reductase cDNA, changes in enzyme activity and mannitol content in broomrape (*Orobanche ramosa*) parasitic on tomato roots. Physiol. Plant 115, 48–55. https://doi.org/10.1034/j.1399-3054.2002.1150105.x.

Dörr, I., Kollmann, R., 1995. Symplasmic sieve element continuity between Orobanche and its host. Bot. Acta 108, 47–55. https://doi.org/10.1111/j.1438-8677.1995.tb00830.x.

Fernández-Aparicio, M., Flores, F., Rubiales, D., 2016a. The effect of *Orobanche crenata* infection severity in faba bean, field pea, and grass pea productivity. Front Plant Sci. 7, 1–8. https://doi.org/10.3389/fpls.2016.01409.

Fernández-Aparicio, M., Reboud, X., Gibot-Leclerc, S., 2016b. Broomrape weeds. Underground mechanisms of parasitism and associated strategies for their control: a review. Front Plant Sci. https://doi.org/10.3389/fpls.2016.00135.

Gordon, A.J., Mitchell, D.F., Ryle, G.J.A., Powell, C.E., 1987. Diurnal production and utilization of photosynthate in nodulated white clover. J. Exp. Bot. 38, 84–98. https://doi.org/10.1093/jxb/38.1.84.

Habimana, S., Nduwumuremyi, A., Chinama R, J.D., 2014. Management of *Orobanche* in field crops: a review. J. Soil Sci. Plant Nutr. 14, 43–62. https://doi.org/10.4067/S0718-95162014005000004.

Hachiya, T., Sakakibara, H., 2017. Interactions between nitrate and ammonium in their uptake, allocation, assimilation, and signaling in plants. J. Exp. Bot. 68, 2501–2512. https://doi.org/10.1093/jxb/erw449.

Hibberd, J.M., Jeschke, W.D., 2001. Solute flux into parasitic plants. J. Exp. Bot. 52, 2043–2049. https://doi.org/10.1093/jexbot/52.363.2043.

Hibberd, J.M., Quick, W.P., Press, M.C., Scholes, J.D., 1998. Can source-sink relations explain responses of tobacco to infection by the root holoparasitic angiosperm *Orobanche cernua*? Plant Cell Environ. 21, 333–340. https://doi.org/10.1046/j.1365-3040.1998.00272.x.

Hibberd, J.M., Quick, W.P., Press, M.C., Scholes, J.D., Jeschke, W.D., 1999. Solute fluxes from tobacco to the parasitic angiosperm *Orobanche cernua* and the influence of infection on host carbon and nitrogen relations. Plant Cell Environ. 22, 937–947. https://doi.org/10.1046/j.1365-3040.1999.00462.x.

Husted, S., Hebbern, C.A., Mattsson, M., Schjoerring, J.K., 2000. A critical experimental evaluation of methods for determination of NH4+ in plant tissue, xylem sap and apoplastic fluid. Physiol. Plant 109, 167–179. https://doi.org/10.1034/j.1399-3054.2000.100209.x.

JASP Team, 2024. JASP (Version 0.18.3) [Computer software].

Jeschke, W.D., Baumel, P., Rath, N., Czygan, F.C., Proksch, P., 1994. Modelling of the flows and partitioning of carbon and nitrogen in the holoparasite *Cuscuta reflexa* Roxb. and its host *Lupinus albus* L. II. Flows between host and parasite and within the parasitized host. J. Exp. Bot. 45, 801–812.

Jokinen, J.I., Irving, L.J., 2019. Effects of light level and nitrogen supply on the red clover-*Orobanche minor* host-parasite interaction. Plants 8. https://doi.org/10.3390/plants8060146.

Kaschuk, G., Kuyper, T.W., Leffelaar, P.A., Hungria, M., Giller, K.E., 2009. Are the rates of photosynthesis stimulated by the carbon sink strength of rhizobial and arbuscular mycorrhizal symbioses? Soil Biol. Biochem 41, 1233–1244. https://doi.org/10.1016/j.soilbio.2009.03.005.

Kawachi, N., Fujimaki, S., Sakamoto, K., Ishioka, N., Matsuhashi, S., Sekimoto, H., 2008. Analysis of NO_3 interception of the parasitic angiosperm Orobanche spp. using a positron-emitting tracer imaging system and $^{13}NO_3$: A new method for the visualization and quantitative analysis of the NO_3 interception ratio. Soil Sci. Plant Nutr. 54, 408–416. https://doi.org/10.1111/j.1747-0765.2008.00252.x.

Kruger, E.L., Volin, J.C., 2006. Reexamining the empirical relation between plant growth and leaf photosynthesis. Funct. Plant Biol. 33, 421–429. https://doi.org/10.1071/FP05310.

Krupp, A., Heller, A., Spring, O., 2019. Development of phloem connection between the parasitic plant *Orobanche cumana* and its host sunflower. Protoplasma 256, 1385–1397. https://doi.org/10.1007/s00709-019-01393-z.

Lalonde, S., Tegeder, M., Throne-Holst, M., Frommer, W.B., Patrick, J.W., 2003. Phloem loading and unloading of sugars and amino acids. Plant Cell Environ. 26, 37–56. https://doi.org/10.1046/j.0016-8025.2002.00847.x.

Lejay, L., Gansel, X., Cerezo, M., Tillard, P., Müller, C., Krapp, A., Von Wirén, N., Daniel-Vedele, F., Gojon, A., 2003. Regulation of root ion transporters by photosynthesis: Functional importance and relation with hexokinase. Plant Cell 15, 2218–2232. https://doi.org/10.1105/tpc.013516.

Lins, R.D., Colquhoun, J.B., Mallory-Smith, C.A., 2007. Effect of small broomrape (*Orobanche minor*) on red clover growth and dry matter partitioning. Weed Sci. 55, 517–520. https://doi.org/10.1614/WS-07-049.1.

Mauromicale, G., Monaco, A., Lo, Longo, A.M.G., 2008. Effect of Branched broomrape (*Orobanche ramosa*) infection on the growth and photosynthesis of tomato. Weed Sci. 56, 574–581. https://doi.org/10.1614/ws-07-147.1.

Minchin, P.E.H., Lacointe, A., 2005. New understanding on phloem physiology and possible consequences for modelling long-distance carbon transport. N. Phytol. 166, 771–779.

Musselman, L.J., 1980. The biology of *Striga*, *Orobanche*, and other root-parasitic weeds. Annu Rev. Phytopathol. 18, 463–489. https://doi.org/10.1146/annurev.py.18.090180.002335.

Osaki, M., Shinano, T., Yamada, M., Yamada, S., 2004. Function of node unit in photosynthate distribution to root in higher plants. Photosynthetica 42, 123–131. https://doi.org/10.1023/B:PHOT.0000040580.22939.55.

Péron, T., Candat, A., Montiel, G., Veronesi, C., Macherel, D., Delavault, P., Simier, P., 2017. New Insights into phloem unloading and expression of sucrose transporters in vegetative sinks of the parasitic plant *Phelipanche ramosa* L. (Pomel). Front. Plant Sci. 7, 2048. https://doi.org/10.3389/fpls.2016.02048.

Pincovici, S., Cochavi, A., Karnieli, A., Ephrath, J., Rachmilevitch, S., 2018. Source-sink relations of sunflower plants as affected by a parasite modifies carbon allocations and leaf traits. Plant Sci. 271, 100–107. https://doi.org/10.1016/j.plantsci.2018.03.022.

Porra, R.J., Thompson, W.A., Kriedemann, P.E., 1989. Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls a and b extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. Biochim Biophys. Acta 975, 384–394. https://doi.org/10.1016/S0005-2728(89)80347-0.

Rodenburg, J., Demont, M., Zwart, S.J., Bastiaans, L., 2016. Parasitic weed incidence and related economic losses in rice in Africa. Agric. Ecosyst. Environ. 235, 306–317. https://doi.org/10.1016/j.agee.2016.10.020.

Schneider, C.A., Rasband, W.S., Eliceiri, K.W., 2012. NIH Image to ImageJ: 25 years of image analysis. Nat. Methods 9, 671–675. https://doi.org/10.1038/nmeth.2089.

Schuize, J., Adgo, E., Merbach, W., 1999. Carbon costs associated with N_2 fixation in *Vicia faba* L and *Pisum sativum* L. over a 14-day period. Plant Biol. 1, 625–631. https://doi.org/10.1111/j.1438-8677.1999.tb00273.x.

Singh, Jagdish Narain, Rai, T.B., Singh, Jai Narain, 1972. Studies on the physiology of host-parasite relationship in *Orobanche*. III. Carbohydrate and nitrogen metabolism of host and parasite. Physiol. Plant 27, 347–353. https://doi.org/10.1111/j.1399-3054.1972.tb03626.x.

Singh, M., Singh, D.V., Misra, P.C., Tewari, K.K., Krishnan, P.S., 1968. Biochemical aspects of parasitism by the angiosperm parasites: starch accumulation. Physiol. Plant 21, 525–538. https://doi.org/10.1111/j.1399-3054.1968.tb07278.x.

Southwood, O.R., 1971. The effect of superphosphate application, 2,4-db and grazing on broomrape (*Orobanche minor* sm.) In a subterranean clover pasture. Weed Res 11, 240–246. https://doi.org/10.1111/j.1365-3180.1971.tb01006.x.

Tegeder, M., Hammes, U.Z., 2018. The way out and in: phloem loading and unloading of amino acids. Curr. Opin. Plant Biol. 43, 16–21. https://doi.org/10.1016/j.pbi.2017.12.002.

Tobin, A.K., Yamaya, T., 2001. Cellular compartmentation of ammonium assimilation in rice and barley. J. Exp. Bot. 52, 591–604. https://doi.org/10.1093/jexbot/52.356.591.

Watling, J.R., Press, M.C., 2001. Impacts of infection by parasitic angiosperms on host photosynthesis. Plant Biol. 3, 244–250. https://doi.org/10.1055/s-2001-15195.

World Flora Online, 2024. Orobanche L. [WWW Document]. URL https://www.worldfloraonline.org/taxon/wfo-4000027206 (accessed 6.2.24).

Yoneyama, Kaori, Yoneyama, Koichi, Takeuchi, Y., Sekimoto, H., 2007. Phosphorus deficiency in red clover promotes exudation of orobanchol, the signal for mycorrhizal symbionts and germination stimulant for root parasites. Planta 225, 1031–1038. https://doi.org/10.1007/s00425-006-0410-1.

Yuan, L., Loqué, D., Kojima, S., Rauch, S., Ishiyama, K., Inoue, E., Takahashi, H., Von Wiréna, N., 2007. The organization of high-affinity ammonium uptake in Arabidopsis roots depends on the spatial arrangement and biochemical properties of AMT1-type transporters. Plant Cell 19, 2636–2652. https://doi.org/10.1105/tpc.107.052134.