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ORIGINAL ARTICLE

Heterotrophic carbon gain by the root hemiparasites, *Rhinanthus minor* and *Euphrasia rostkoviana* (Orobanchaceae)

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Abstract Hemiparasitic plants gain virtually all mineral nutrients and water from their host plant whilst organic carbon is provided, at least in part, by their own photosynthetic activity, although their rates of assimilation are substantially lower than that found in non-parasitic plants. Hence, hemiparasites must gain at least some of their organic carbon heterotrophically from the host plant. Despite this, heterotrophic carbon gain by root hemiparasites has been investigated only for a few genera. We investigated heterotrophic carbon gain by two root hemiparasites, *Rhinanthus minor* L. and *Euphrasia rostkoviana* Hayne (Orobanchaceae), using natural abundance stable isotope ($\delta^{13}C$) profiles of both parasites attached to C_3 (wheat) and C_4 (maize) hosts coupled to a linear two-source isotope-mixing model to estimate the percentage of carbon in the parasite that was derived from the host. Both *R. minor* and *E. rostkoviana* attached to maize hosts were significantly more enriched in ^{13}C than those attached to wheat hosts with *R. minor* becoming more enriched in ^{13}C than *E. rostkoviana*. The natural abundance ^{13}C profiles of both parasites were not significantly different from their wheat hosts, but were less enriched in ^{13}C than maize hosts. Using a linear two-source isotope-mixing model, we estimated that *R. minor* and *E. rostkoviana* adult plants derive c. 50 and 25% of their carbon from their hosts, respectively. In light of these results, we hypothesise that repeatedly observed negative effect of competition for light on hemiparasites acts predominantly in early ontogenetic stages when parasites grow unattached or the abstraction of host nutrients is less effective.

Keywords Heterotrophy · Maize · Parasitism · Wheat · $\delta^{13}C$

Introduction

Root hemiparasitic plants attack and withdraw resources from their host's vascular system through a specialised transfer organ called haustorium (Irving and Cameron 2009). Due to their often reduced root networks, hemiparasitic plants gain virtually all mineral nutrients and water from their host plant, whilst organic carbon is provided, at least in part, by their own photosynthetic activity (Watling and Press 2001). This heterotrophic strategy has evolved on multiple occasions, defined by differing physiological processes in several unrelated groups of angiosperms (Press and Graves 1995). The family Orobanchaceae is one of the most diverse plant families that comprise exclusively root hemiparasites or holoparasites, with the exception of a single non-parasitic genus, *Lindenbergia* (Wolfe et al. 2005; Bennett and Mathews 2006). In this group, the parasitic relationship only takes

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place below ground, whilst above ground the host–parasite interaction is underpinned by competition for light (Matthies 1995). The extent to which competition for light affects hemiparasite growth and development depends on the ability of a given hemiparasite to gain assimilates from the host plant. Consequently, hemiparasites acquiring large amounts of carbon from their host plant should be able to survive even under relatively severe shading from surrounding plants. Resolving the carbon budget of the host–hemiparasite relationship thus provides valuable insights into the dependency of individual hemiparasitic plants on the heterotrophic habit and their competitive ability.

Hemiparasitic plants are capable of photosynthesis (Press et al. 1987; Press 1989; Cameron et al. 2008), which is the source of some of their organic carbon. However, the rates of assimilation are substantially lower than those found in non-parasitic plants owing to high respiratory rates in most species of hemiparasites; thus, net photosynthetic carbon gain is usually negligible (Press et al. 1988; Press 1989). Moreover, net photosynthetic carbon gain may even be below the photosynthetic compensation point in some species that respire more CO_2 than they are able to assimilate (Press et al. 1987; Press 1989). Hence, hemiparasites must gain at least certain amount of organic carbon heterotrophically from their host plant. However, since hemiparasitic plants attack host xylem and not the phloem, they only have access to xylem-mobile organic compounds, such as organic nitrogen, most likely in the form of amino acids, and virtually no carbohydrates (Irving and Cameron 2009). Using $^{14}CO_2$ labelling of host plants, the host-to-parasite flux of assimilates has been directly demonstrated in parasitic relationships between *Odontites vernus* Dumort. and its hosts *Hordeum vulgare* L. and *Trifolium repens* L. (Govier et al. 1967). Although this approach was powerful in demonstrating the character of assimilates transferred via haustoria, it did not provide any quantitative assessment of the amount of organic carbon flux from the host to the parasite. Moreover, Hodgson (1973) applied similar radio-isotope tracer-based techniques to investigate the nature of host-to-parasite C transfers between *Euphrasia officinalis* agg., *Rhinanthus minor* L., *Pedicularis sylvatica* L., *Orthocarpus luteus* Nutt. and *Melampyrum pratense* L. and a range of host species, but again did not provide a quantitative C budget.

In a pioneering study, Press et al. (1987) demonstrated that it is possible to quantify the proportion of host-derived carbon in hemiparasite biomass using natural abundance stable isotope values of ^{13}C of the obligate root hemiparasite *Striga hermonthica* (Del.) Benth. attached to a C_4 host. Plants undertaking C_4 photosynthesis have a significantly enriched natural abundance $\delta^{13}C$ value compared to C_3 plants, owing to the differential discrimination of Ribulose-1,5-bisphosphate carboxylase oxygenase (Rubisco) and phosphoenolpyruvate carboxylase (PEPCase), the first enzymes of carbon fixation in C_3 and C_4 plants, respectively. Press et al. (1987) therefore harnessed these differential $\delta^{13}C$ values comparing $\delta^{13}C$ of C_3 parasite to that of its C_4 host and, by measuring the extent to which the C_3 parasite takes on C_4 $\delta^{13}C$ values, were able to estimate heterotrophic carbon gain. Using the same approach, Graves et al. (1990) found that almost 50% of the leaf biomass in *Striga hermonthica* was derived from its C_4 host, whilst Tennakoon and Pate (1996) revealed that 20–30% of the total biomass of the parasitic tree *Olax phyllanthi* (Labill.) R.Br. was host derived. However, such data for temperate hemiparasites in the Orobanchaceae family are currently absent in literature.

Whilst using such differences in the natural abundance of stable isotopes of carbon is a powerful tool for resolving the extent of parasitic plant heterotrophy, these cultivation experiments can be potentially biased by the fact that CO_2 respired by soil (i.e. by roots and soil microorganisms) bears the isotope signature of the host, the roots of which dominate the cultivation pots. Thus, the CO_2 respired by the C_4 host roots and shoots and by the microorganisms decomposing dead organic matter originating from the C_4 host can be re-fixed by the parasite, which would consequently lead to an overestimation of the host-derived carbon in the hemiparasite.

This study aims to assess the extent of heterotrophic carbon gain by the temperate hemiparasites, *Rhinanthus minor* L. and *Euphrasia rostkoviana* Hayne (Orobanchaceae), from their hosts through the application of the methods of Press et al. (1987) whilst addressing the problem of a potential bias caused by re-fixation of soil-respired CO_2 using paired partner plants. Resolving the extent to which re-fixation of respired CO_2 has the potential to influence the $\delta^{13}C$ values of the parasite in our experimental systems is important not only for the interpretation of our own results, but also has implications for previously published work (Press et al. 1987; Tennakoon and Pate 1996). Furthermore, the results presented here enhance our knowledge of heterotrophy in annual hemiparasitic forbs. This is important since a majority of studies estimating the percentage of host-derived carbon were conducted on mistletoes (e.g. Marshall et al. 1994; Bannister and Strong 2001) where the host–parasite interaction is quite different.

Materials and methods

Experimental species

Rhinanthus minor L. and *Euphrasia rostkoviana* Hayne are hemiparasitic Orobanchaceae displaying C_3 metabolism and were used as model species in our experiments. *Rhinanthus minor* is a relatively common species occurring on meadows across Western Eurasia (Meusel et al. 1978). It is able to parasitise a wide range of host species; however, parasite performance (in terms of growth and fecundity) is greatest when attached to grasses and legumes (Cameron et al. 2006; Rümer et al. 2007). It is by far the most widely used species in recent physiological and ecophysiological studies of hemiparasitic plants (see Irving and Cameron 2009 for a review). *Euphrasia rostkoviana* grows naturally on nutrient-poor meadows and displays a similar, albeit slightly more restricted, geographical range to *R. minor*. Its suitable host range can be considered relatively broad comprising not only grasses and legumes, but also certain forb species (Yeo 1964; Lammi et al. 1999). Maize (*Zea mays* L.) and wheat (*Triticum aestivum* L.) were used as host species in this study. *Plantago lanceolata* L. was introduced as a reference plant designed to capture the effect of the host identity on isotope signature value of hemiparasite assimilates caused by reassimilation of the soil-respired CO_2 (Fig. 1). Seeds of *E. rostkoviana* were collected from several hundred individuals in a wild population occurring on a meadow in the vicinity of the Horská Kvilda village (Bohemian Forest Mts., Czech Republic). *Rhinanthus minor* seeds were collected from several hundred individuals in a wild population occurring on a mesotrophic calcareous meadow in Lathkill Dale, near Monyash (Peak District National Park, Derbyshire, UK). Seeds of both host species were obtained from the school farm of the Faculty of Agriculture, University of South Bohemia. *Plantago lanceolata* seeds were obtained from Planta Naturalis Ltd. (Markvartice, Czech Republic).

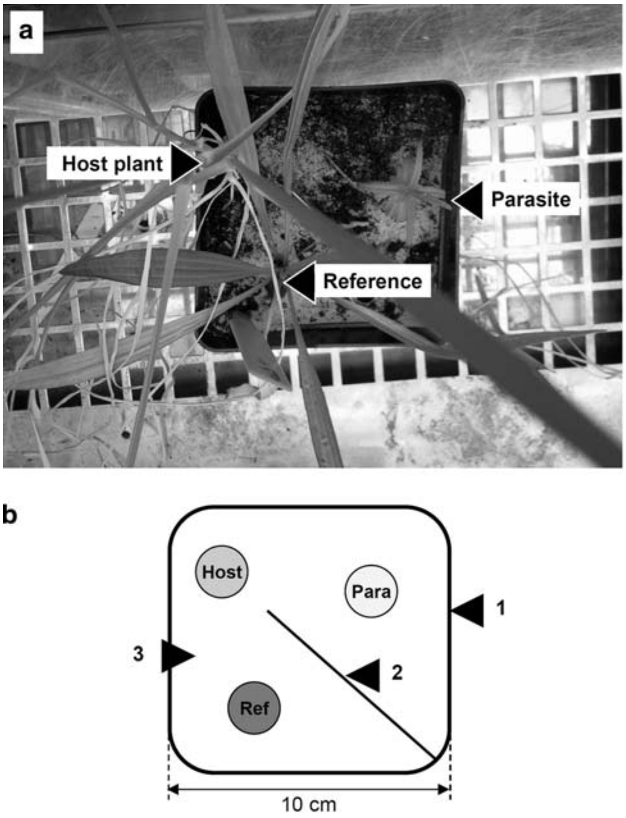

Fig. 1 **a** Photograph of the experimental split pot showing the host plant, parasite and reference plant (*Plantago lanceolata*). **b** Schematic diagram of the experimental split pot (*1*) showing host plant, parasite and reference plant, septum (*2*) and soil substrate (*3*)

Plant cultivation

Seeds of the hosts and *P. lanceolata* were germinated on Petri dishes on moist filter paper at 18°C. The seedlings were moved to 10 × 10 cm square pots containing substrate of Levington M3 compost and washed quartz sand (1:1, v/v) after successful germination. This growth medium contains only minimal reserves of inorganic nutrients, which corresponds to the composition of soil at the sites where the seeds of the experimental hemiparasitic species were obtained. The pots contained a septum that partially bisected the pot diagonally (Fig. 1). This design facilitated the separation of the parasite and *P. lanceolata* roots, whilst allowing the host roots to access the whole volume of the pots (Fig. 1). Although it has been demonstrated that *P. lanceolata* is resistant to *R. minor* (Cameron et al. 2006; Rümer et al. 2007) and the parasite is unable to abstract resources from this species (Cameron and Seel 2007), the design of the pot further decreases the probability of any interaction between *R. minor* and the reference plant. Pots were maintained in a controlled environment chamber with a cycle of 14-h light at 20°C and 10-h dark at 15°C (Conviron, Winnipeg, Canada). This diurnal light cycle corresponds to natural conditions assumed for the source populations of both hemiparasitic species growing in summer season at around 50° of the northern latitude. *Rhinanthus minor* seedlings (germinated on moist filter paper at 4°C) were sown at a density of one seedling per pot c. 3–4 cm from the host plant after 8 days of host development. The replicate pots ($n = 10$ per each host–parasite combination) were arranged in a randomised block design and watered every day until harvest. Hosts and the parasite *E. rostkoviana* were germinated in the same way and planted in simple, undivided pots containing a 1:1 (v/v) sand compost mixture. All replicate plants ($n = 10$ for each host–parasite combination) were cultivated in a growth cabinet at the Faculty of Science, University of South Bohemia with a cycle of 14-h light and 10-h dark at constant temperature of 18°C. After 14 weeks, leaf material was harvested and dried at 80°C for 48 h. Roots were washed and examined for the presence of haustoria to ensure attachment with the host plant. The basal leaves of the hemiparasites were excluded from the stable isotope analysis since they were produced autotrophically before the attachment to the host. The samples were homogenised separately and a 5-µg subset of each constituent part was analysed for ^{13}C content by continuous-flow mass spectrometry (PDZ Europa 2020 Isotope Ratio Mass Spectrometer (IRMS) coupled to a PDZ ANCA GSL preparation unit, SerCon Ltd, Crewe, Cheshire, UK). Data were collected as atom % ^{13}C and re-expressed as delta values relative to the Pee Dee Belemnite standard (δ) using Eq. 1

$$\delta^{13}\mathrm{C} = (R_{\mathrm{Sample}}/R_{\mathrm{Standard}} - 1) \times 1000[‰] \qquad (1)$$

where $R_{\mathrm{Sample}} = {}^{13}\mathrm{C}{:}{}^{12}\mathrm{C}$ ratio in the sample and $R_{\mathrm{Standard}} = {}^{13}\mathrm{C}{:}{}^{12}\mathrm{C}$ ratio in the Pee Dee Belemnite standard.

Assessment of host-derived carbon in hemiparasite biomass

Rhinanthus minor, E. rostkoviana and wheat perform C_3 photosynthesis, whilst maize performs C_4 photosynthesis. As a result of their photochemical processes, C_4 plants are usually significantly more enriched in ^{13}C than C_3 plants. Hence, it is possible to infer the extent of heterotrophic carbon gain of a hemiparasitic plant by measuring the relative change in the δ^{13}C value of the C_3 parasite attached to a C_4 host compared to when it is attached to a C_3 host. We used an adjusted form of a linear two-source isotope-mixing model (Marshall and Ehleringer 1990), adapted from Gebauer and Meyer (2003), to calculate the proportion of host-derived carbon in hemiparasite biomass (Eq. 2)

$$\%H = \left(\frac{\delta^{13}\mathrm{C}_{\mathrm{P}(\mathrm{C}_4)} - \delta^{13}\mathrm{C}_{\mathrm{P}(\mathrm{C}_3)}}{\delta^{13}\mathrm{C}_{\mathrm{H}(\mathrm{C}_4)} - \delta^{13}\mathrm{C}_{\mathrm{H}(\mathrm{C}_3)}}\right) \times 100[\%] \qquad (2)$$

where $\%H =$ the percentage of carbon in parasite biomass that is derived from the host, $\delta^{13}\mathrm{C}_{\mathrm{P(C3)}} = \delta^{13}$C of the parasite growing on the C_3 wheat host, $\delta^{13}\mathrm{C}_{\mathrm{P(C4)}} = \delta^{13}$C of the parasite growing on the C_4 maize host, $\delta^{13}\mathrm{C}_{\mathrm{H(C3)}} = \delta^{13}$C of the infected wheat host and $\delta^{13}\mathrm{C}_{\mathrm{H(C4)}} = \delta^{13}$C of the infected maize host. For each replicate, $\delta^{13}\mathrm{C}_{\mathrm{P(C4)}}$ and the $\delta^{13}\mathrm{C}_{\mathrm{H(C4)}}$ of the corresponding host plant were entered into the model calculation and the average values of $\delta^{13}\mathrm{C}_{\mathrm{H(C3)}}$ and $\delta^{13}\mathrm{C}_{\mathrm{P(C3)}}$ were used as the baseline reference value. The calculation was performed separately for each of the two hemiparasite species. The outcome of this model provides an estimate of proportion of heterotrophic carbon in hemiparasite biomass produced by an adult host-attached plant. This value integrates organic carbon flows from the host to the hemiparasite, the actual values of which can be variable in time. The model assumes that there is no fractionation during the assimilation of host-derived nutrients by the parasite or that the fractionation has the same effect on δ^{13}C in parasites cultivated with both maize and wheat. Such assumption is reasonable since direct luminal continuity exists in *R. minor* haustoria (Cameron et al. 2006). In the case of *E. rostkoviana*, absence of luminal continuity cannot be excluded; nonetheless, a study on *Olax phyllanthi*, which conducts nutrient transfer via contact interfacial parenchyma, does not report any substantial bias connected to isotope discrimination during the transfer (Tennakoon and Pate 1996). In addition, xylem δ^{13}C is assumed to be of the same value as the bulk leaf dry matter. Information on isotopic composition of xylem sap is unfortunately missing from literature, probably due to difficulty in measuring this value caused by low organic carbon concentration. Variation in δ^{13}C across main structures of a normal autotrophic plant, however, tends to be fairly restricted (Bowling et al. 2008). Moreover, if any significant fractionation effect occurred, it could be assumed to be equal in both host species, resulting in an equal effect on both terms in the mixing model numerator.

Statistical analysis

Differences between treatment means were analysed by ANOVA followed by Fisher's multiple comparison test or using student's *t* test. The statistical analysis was conducted using Minitab version 13 (Minitab Inc., State College, PA, USA). Where necessary, to satisfy the test assumptions, data were arcsine square root transformed. Welch's estimation of degrees of freedom was used for *t* tests performed on data with unequal samples sizes. Untransformed means and associated standard errors are presented. The isotope-mixing model outcomes were tested for significant difference from 0 (i.e. no carbon gain from heterotrophy) using student's *t* test.

Results

Carbon isotope composition of the host plants followed patterns expected for species displaying C_3 and C_4 metabolism (Fig. 2a, b). Experimental hemiparasitic plants attached to the wheat hosts also displayed δ^{13}C values typical for C_3 plants and were not significantly different from those of their hosts (one-way ANOVA; $P > 0.05$) (Fig. 2a, b). The δ^{13}C values of hemiparasites attached to maize were statistically significantly enriched compared to those attached to wheat (Fig. 2a, b), indicating contribution of host carbon to hemiparasite biomass. The difference in δ^{13}C between parasites attached to maize and wheat was more pronounced in *R. minor* than in *E. rostkoviana*. However, δ^{13}C values in *R. minor* displayed higher variation.

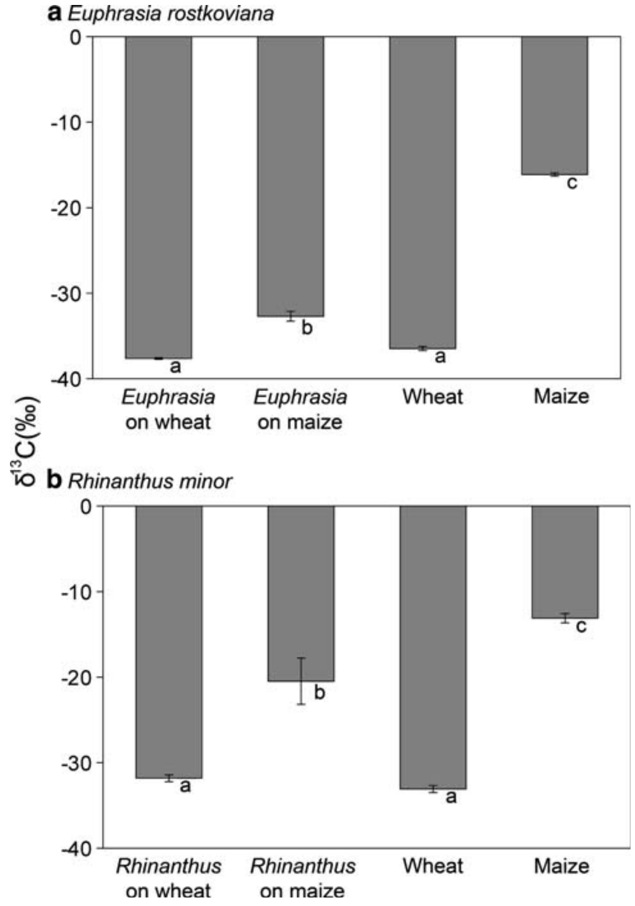

Fig. 2 **a** δ^{13}C values of wheat (C_3 photosynthesis) and maize (C_4 photosynthesis) hosts and *Euphrasia rostkoviana* (C_3 photosynthesis) or **b** *Rhinanthus minor* (C_3 photosynthesis) attached to each type of host plant. *Error bars* represent ±1 SE and *bars* with different letters are significantly different (1-way ANOVA–*E. rostkoviana*: *d.f.* = 3,30; $F = 1356$; $P < 0.001$ and *R. minor*: *d.f.* = 3,33; $F = 94.86$; $P < 0.001$)

These values of carbon isotope composition were used in the estimation of proportions of heterotrophic carbon in the biomass of the hemiparasites using a two-source linear isotope-mixing model. Mean values for the estimated percentage of heterotrophic carbon in biomass of the hemiparasites were 56% (ranging from 6.2 to 89.5%) for *R. minor* and 24% (ranging from 20.9 to 28.7%) for *E. rostkoviana* (Fig. 3). The proportions of host-derived carbon were significantly different from zero in both *R. minor* [student's *t* test (arcsine transformed data): *d.f.* = 6; $t = 4.70$; $P = 0.003$] and *E. rostkoviana* [student's *t* test (arcsine transformed data): *d.f.* = 2; $t = 18.56$; $P = 0.003$].

We used δ^{13}C values of the paired *P. lanceolata* reference plants to investigate the extent to which δ^{13}C values of the hemiparasites attached to the C_4 maize host could be influenced by recapturing of soil-respired CO_2. Carbon isotope ratio of the reference *P. lanceolata* plants did not significantly differ between the pots with maize and wheat (Fig. 4; Welch *t* test *d.f.* = 8.32; $t = 0.24$; $P = 0.82$). This implies that the recapturing of soil-respiratory CO_2 either does not occur, or occurs at such low rates that are not sufficient to bias the δ^{13}C value of the hemiparasites.

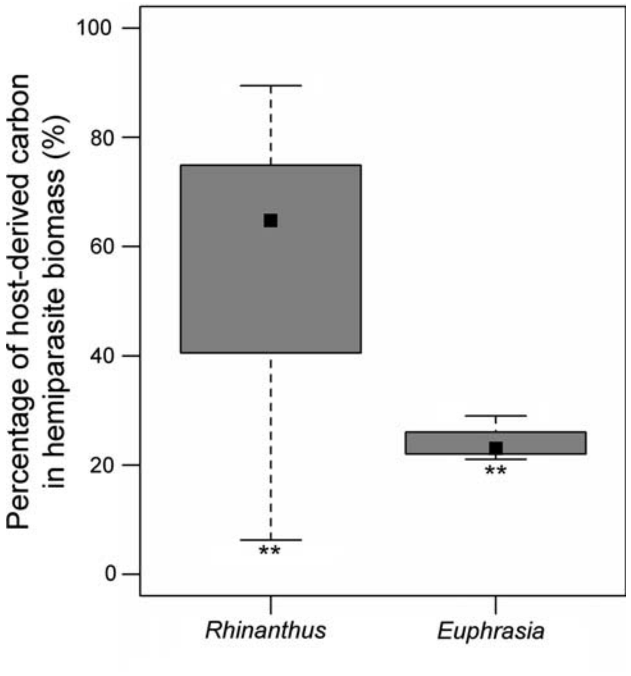

Fig. 3 Box plot representing the median (*symbol*), quartiles (*box*), maximum and minimum (*whiskers*) percentage of host-derived carbon in parasite biomass for *Euphrasia rostkoviana* and *Rhinanthus minor*. Double *asterisk* indicates that mean is significantly different from 0 (student's *t* test–*E. rostkoviana* (arcsine transformed data): *d.f.* = 2; $t = 18.56$; $P = 0.003$ and *R. minor* (arcsine transformed data): *d.f.* = 6; $t = 4.70$; $P = 0.003$)

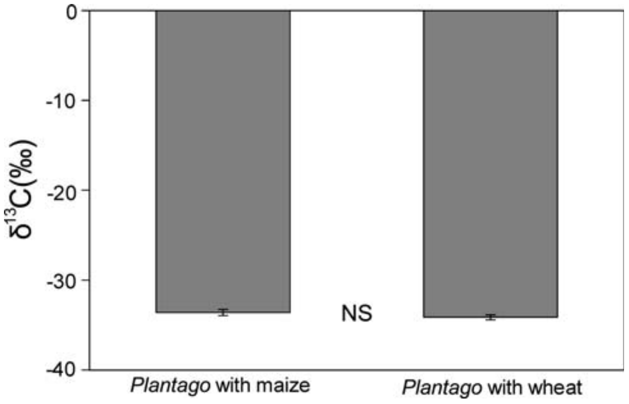

Fig. 4 δ^{13}C values of *Plantago lanceolata* (C_3 photosynthesis) reference plants growing with either wheat (C_3 photosynthesis) or maize (C_4 photosynthesis). *Error bars* represent ±1 SE. Treatment means were not significantly different (Welch *t* test *d.f.* = 8.32; $t = 0.24$; $P = 0.82$)

Discussion

The majority of parasitic plants display a hemiparasitic strategy for resource acquisition (Irving and Cameron 2009) and, whilst the photosynthetic process is active in these plants, potentially significant amounts of organic carbon can also be gained from the host in the form of xylem-mobile organic compounds (Govier et al. 1967; Hodgson 1973; Jiang et al. 2008b). Direct quantitative partitioning between the two sources of assimilates to the carbon budget of hemiparasite is virtually impossible given the complexity of the metabolic interactions between host and parasite (Hibberd and Jeschke 2001). Hitherto, the most robust method for the estimation of carbon heterotrophy by parasitic plants was presented by Tennakoon and Pate (1996), based on the comparison of the natural abundance δ^{13}C value of a hemiparasite attached to either a C_4 or C_3 host and the δ^{13}C value of the C_4 host itself. We further refine this technique and present a novel, more straightforward approach based on a two-source isotope-mixing model, modified from Marshall and Ehleringer (1990) and Gebauer and Meyer (2003), which includes δ^{13}C data of all experimental plants (i.e. C_3 and C_4 hosts together with parasites attached to these hosts). The calculation is then based on the excess of ^{13}C in hemiparasites attached to the C_4 host compared to those attached to the C_3 host related to the difference in isotope composition between the C_3 and C_4 hosts themselves. Furthermore, by using *P. lanceolata* as a reference plant, we ruled out the possibility of the ^{13}C enrichment observed in hemiparasites when grown with C_4 host being an artefact due to reassimilation of respired, ^{13}C-enriched CO_2.

This refined natural abundance stable isotope method allows a reliable estimation of heterotrophic carbon acquisition in two temperate species, *R. minor* and *E. rostkoviana*. We demonstrate that *R. minor* has a potential to acquire an extensive amount of organic carbon from its host. The mixing model calculations suggest that on average, over 50% of leaf biomass of the experimental individuals consists of host-derived carbon, although the model outputs are highly variable. *Rhinanthus minor* is known to be very efficient in the extraction of solutes of the host xylem (Jiang et al. 2003; Cameron and Seel 2007) due to an advanced haustorial structure (Cameron and Seel 2007; Rümer et al. 2007). Moreover, infection by *R. minor* is certainly more detrimental (in terms of host growth and fecundity reduction) than most other temperate hemiparasites (Gibson and Watkinson 1992; Matthies 1995). As a result of the extensive host damage, *Rhinanthus* species have been shown to exert significant effects on the structure and function of the communities they inhabit (Ameloot et al. 2005; Cameron et al. 2005; Cameron et al. 2009). The proportion of heterotrophic carbon in *Euphrasia rostkoviana* reached only up to 30%, corresponding to a relatively conservative hemiparasitic strategy (i.e. comparatively low dependence on resource supply from the host) reported, e.g. for *Olax phyllanthi*, which displays similar degree of carbon heterotrophy (Tennakoon and Pate 1996).

Our estimates of heterotrophic carbon gain by *R. minor* and *E. rostkoviana* are based on a cultivation experiment conducted under controlled conditions using host plants that are not native hosts for the two hemiparasitic species, although maize and wheat are highly susceptible to both hemiparasitic species. Therefore, these results must be taken with caution when inferring the consequences to plants growing in a natural environment. Nevertheless, this study is the first to provide a quantitative assessment of the potential for *R. minor* and *E. rostkoviana* to abstract and utilise heterotrophically derived carbon. Moreover, the extent of carbon acquisition in mature plants of *R. minor* recorded in our study is comparable to the values reported for *Striga* species (Press et al. 1987; Graves et al. 1990, 1992). Species of both genera, *Striga* and *Rhinanthus*, are also known to cause substantial damage to their hosts leading to significant reductions in host biomass and disruption of host metabolic processes (Watling and Press 2001; Cameron et al. 2008). Both *Rhinanthus* (Cameron et al. 2008) and *Striga* (Watling and Press 2001; Rank et al. 2004) suppress host growth and photosynthesis, a potential consequence of which is a reduction in host competition pressure. Nonetheless, this is much more prominent in *Striga*, which has been postulated to produce cytotoxic metabolites interfering with photosynthetic metabolic pathways in some host species (Musselman 1980). There are, however, also other substantial differences in their biology. *Striga* is an obligate hemiparasite requiring host–root induction to germinate. Its seedling produces a primary haustorium and is completely dependent on host carbon supply in the first stage of its development (Irving and Cameron 2009). In contrast, *Rhinanthus* is a facultative hemiparasite producing large seeds that germinate independently of the presence of a host plant. *Rhinanthus* seedlings are fully autotrophic and emerge from the soil prior to attachment to the host plant. After emergence, *Rhinanthus* seedlings produce roots that forage for a suitable host to which they attach via secondary haustoria (Irving and Cameron 2009). The early stage of development is evidently a critical part of facultative hemiparasite life cycle, as the seedlings are highly sensitive to competition for light due to slow development and inefficient photosynthesis. This may explain the observations that competition for light with the host has negative effect on *Rhinanthus* (Matthies 1995; Hejcman et al. 2007).

Both *R. minor* and *E. rostkoviana* are xylem-feeding hemiparasites. Direct luminal continuity between host and parasite vessel elements has been reported in *R. minor* haustoria (Cameron et al. 2006; Cameron and Seel 2007; Rümer et al. 2007). The uptake of xylem solutes is driven by mass flow because of the lower water potential of the parasite induced through the accumulation of osmotically active sugar alcohols (Jiang et al. 2008a) and elevated parasite transpiration rates facilitated by permanently open stomata that are insensitive to ABA-induced closure (Jiang et al. 2003). As a consequence of xylem–xylem continuity, the relative uptake of individual nutrients is directly related to their concentration in host xylem; the composition of mineral nutrients in the xylem sap of *R. minor* and its hosts is indeed very similar (Jiang et al. 2008b; Irving and Cameron 2009). This is also supported by the observation that the δ^{13}C values recorded in hemiparasites growing on a C_3 host are not significantly different from the δ^{13}C values recorded for the host itself (Fig. 2). Xylem sap is generally assumed to contain only a minimal amount of organic carbon, but a recent investigation has revealed that organic compounds can constitute ca 50% of xylem solutes (Alvarez et al. 2008) suggesting that the xylem is in fact a rich source of organic carbon for the parasite. Hence, the high rate of carbon heterotrophy in *R. minor* reported in our study is not surprising given its highly efficient abstraction of xylem solutes when attached to a suitable host (Jiang et al. 2003; Cameron and Seel 2007). Substantial variability in the degree of heterotrophy observed amongst individual plants of *R. minor* might be in part explained by a positive feedback relationship between hemiparasite growth and the amount of xylem sap that it is able to abstract from the host's vascular system. The mass flow of xylem sap from host to parasite, driven by a low parasite water potential, should increase with the size of a parasite and its leaf area, because large parasites represent greater sink strengths due to an increase in the total transpiration rate per individual plant. Such a positive feedback loop is likely to be more prominent in *R. minor* because of its larger leaf area, which would be in an agreement with the present experimental data. This hypothesis, however, requires further investigation.

Clearly, both *R. minor* and *E. rostkoviana* can abstract substantial amounts of organic carbon from their hosts; however, the nature of the mechanisms underlying the interplay between hemiparasite and host individuals in determining the degree of heterotrophy, hemiparasite growth and its effect on the host remain unclear and warrant further investigation.

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