Suppression of Host Photosynthesis by the Parasitic Plant Rhinanthus minor

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INTRODUCTION

Parasitic plants have significant, deleterious effects on their host in terms of photosynthetic rate and/or total canopy photosynthesis over the life of the plant (Watling and Press, 2001). Broadly, these effects can be classified in terms of direct effects of resources abstraction (source–sink interactions) and indirect, non-source–sink interactions (Watling and Press, 2001; Cameron et al., 2005). For example, tobacco (Nicotiana tabacum), in response to infection with the holoparasite Orobanche cernua, exhibits suppressed leaf senescence, with the net effect of increasing canopy photosynthesis over the life of the host by 20%, although in the study it did not completely compensate for the carbon lost to the parasite (Hibberd et al., 1998). In contrast, in the Striga hermonthica–Zea mays association, only 20% of the host biomass reduction effected by the parasite is attributable to the direct effects of resource abstraction with the remainder associated with parasite-induced suppression of host photosynthesis (Graves et al., 1989). The mechanisms through which S. hermonthica induces these deleterious effects on host photosynthesis are not fully elucidated (Gurney et al., 1995); however, host plants parasitized by S. hermonthica typically show elevated abscisic acid (ABA) levels (Taylor et al., 1996; Frost et al., 1998). Increased ABA concentrations result in a reduction in host stomatal conductance (Frost et al., 1998), increasing the effective sink strength of the parasite by reducing competition with the host for xylem sap (Taylor et al., 1996; Watling and Press, 2001). Xylem sap is drawn through the haustorium, the organ of attachment providing vascular continuity between Striga and its host, and into the parasite via cohesion due to elevated parasite transpiration (Press and Graves, 1995).

The negative effects of the obligate hemiparasitic weeds on host photosynthesis are well documented (Watling and Press, 2001); there are, however, virtually no such data for the effect of non-weedy, facultative hemiparasitic plants on host photosynthesis or, indeed, for the effect of the identity of the host on parasite photosynthesis. Cameron et al. (2005) reviewed the direct and indirect effects of the facultative hemiparasite Rhinanthus minor (Orobanchaceae) on its hosts providing some evidence that the parasite was able to lower the steady-state quantum yield of PSII (ΦPSII) in host leaves. Moreover,
the negative effects of *R. minor* on its hosts – in excess of 20 species (Gibson and Watkinson, 1989) – in terms of growth and reproduction, are highly variable. In general, graminoid and leguminous hosts are significantly damaged by attachment of the parasite whilst, in contrast, forbs remain undamaged (Cameron et al., 2005) due to their ability to express successful defence responses against the invading parasite haustorium (Cameron et al., 2006; Rümer et al., 2007). Similarly, graminoid and legume species represent the best hosts for *R. minor* in terms of growth and reproduction (Cameron et al., 2006) as the parasite is able to abstract significantly more of the host’s resources from grasses than forbs (Cameron and Seel, 2007). This differential success in colonization of potential host species represents a potent tool to investigate the effects of facultative hemiparasites on host photosynthesis and the reciprocal effect of host identity on parasite photochemistry.

Here, using chlorophyll fluorescence techniques, the effect of *R. minor* on the photosynthetic capacity of the host is investigated and the effect of host species on the photosynthetic capacity of the parasite is reciprocally investigated with two potential host species at opposing ends of the ‘quality’ spectrum.

MATERIALS AND METHODS

Plant material

Twenty individual 6-week-old seedlings (hosts) of *Phleum bertolonii* (henceforth *Phleum*) and *Plantago lanceolata* (henceforth *Plantago*) were grown in 15-cm-diameter pots (one plant per pot) containing 50:50 sand:John Innes No. 3 compost. *Rhinanthus minor* seeds were germinated according to Keith et al. (2004). Briefly, seeds were surface-sterilized for 5 min in 3 % sodium hypochlorite solution, washed in distilled water and preconditioned on moist filter paper at 4 °C until germination (approx. 8 weeks). Four seedlings of *Rhinanthus minor* were transplanted into five of the pots containing the host species. The parasites were subsequently reduced to one per host when the first parasite showed morphological changes associated with attachment (Klaren and Janssen, 1978). Water was supplied to the soil daily and the pots were arranged in a randomized block design. The hosts together with the parasites were then grown for a further 14 weeks (after parasite attachment) in a glasshouse (temperature range 16–28 °C).

Chlorophyll fluorescence

One or two of the youngest fully expanded leaves were removed from three individuals of *Rhinanthus* growing on *Phleum* (a good host) and three *Rhinanthus minor* individuals growing on *Plantago* (a bad host) 14 weeks after parasite attachment. The fresh weight was recorded and, after fluorescence measurements had been conducted, the leaves were oven-dried (80 °C for 2 d) and the weight was recorded and added to the total parasite dry weight measured. The maximum and steady-state quantum yields (*Fv/Fm* and *ΦPSII*, respectively) of the detached leaves were measured using a pulse-modulated fluorimeter (MFMS; Hansatech Ltd, King’s Lynn, UK) as per the manufacturer’s directions. Samples were dark-adapted for 15 min prior to measurements of *Fv/Fm* and the intensity of the 0.7-s light pulse was varied to obtain *Fv/Fm* light-response curves. Leaves were adapted to an actinic beam of variable intensity for 15 min prior to measurement, or until *Fv*/F0 stabilized, to obtain *ΦPSII* light response curves. In the *ΦPSII* measurements the pulse had an intensity of 3200 μmol photons m−2 s−1 for 0.7 s. Different irradiances were obtained by interrupting the light beam with a combination of neutral density filters. All fluorescence parameters were estimated as per the manufacturer’s instructions.

Chlorophyll content

The second two youngest leaves were removed from all parasites and the fresh biomass recorded. Dry weights were estimated using the fresh weight/dry weight ratio of leaves harvested for chlorophyll fluorescence. Leaves were ground in a mortar and pestle with a small amount of acid-washed sand (as an abrasive) and 5 ml of 80 % ice-cold acetone. The mortar and pestle was washed out twice with a further 2 mL of acetone and transferred to a centrifuge tube. The samples were centrifuged at 8000 g for 5 min and the supernatant diluted to 10 mL total volume with 80 % ice-cold acetone. The optical density of the supernatant was measured at 645 nm and 663 nm using a Hitachi U-2001 spectrophotometer.

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\text{Chlorophyll } a (\text{mg L}^{-1}) = (12 \times 7 \times \text{OD}_{665}) - (2 \times 69 \times \text{OD}_{645})
\]  

(1)

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\text{Chlorophyll } b (\text{mg L}^{-1}) = (22 \times 9 \times \text{OD}_{645}) - (4 \times 68 \times \text{OD}_{663})
\]  

(2)

The chlorophyll concentration (mg L−1 of extract) was calculated according to Arnon (1949) using eqns (1) and (2) above and re-expressed as milligrams of chlorophyll per gram of tissue fresh weight (mg g−1).

Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) content

Following the protocol described by Irving and Robinson (2006), fresh leaf material was weighed, then ground in five times its mass of 50 mM sodium phosphate buffer (pH 7.5) containing 5 % glycerol, 0.8 % 2-mercaptoethanol and 3.5 % (w/v) iodoacetic acid. A 200-μL aliquot of the homogenate was diluted with a further 200 μL of extraction buffer. After adding 2 μL of 25 % Triton X-100, the solution was vortexed, followed by centrifugation for 5 min at 10 000 g. Then 8.6 μL of 25 % (w/v) lithium dodecyl sulfate and a further 5.3 μL of 2-mercaptoethanol were added. The sample was inverted twice before being boiled at 100 °C for 90 s, then pulse centrifuged. Samples were
stored at −20 °C before being applied to an SDS–PAGE gel (5 % stacking gel, 12.5 % separating gel), along with a suitable wheat Rubisco standard, for protein separation. Gels were stained using Coomassie Brilliant Blue R250, the Rubisco containing band excised, and the protein concentration determined spectrophotometrically at 595 nm, after elution of the stain in formamide for 12 h.

**Statistical analysis**

Differences between treatment means were analysed by ANOVA and Fisher's multiple comparison test using Minitab 13 (Minitab Inc., Pennsylvania, USA).

**RESULTS**

Host biomass and total biomass (host + parasite) of infected *Phleum bertolonii* was significantly lower than the control plants (*P* > 0.05, two-way ANOVA, *n* = 3–5) by the end of the experimental period (Fig. 1). However, in *Plantago lanceolata*, no significant differences in host or total biomass were noted, while the parasite biomass was significantly lower than the biomass of *Rhinanthus* growing on *Phleum* (*P* < 0.05).

Maximum quantum yield (*F*$_{v}$/*F*$_{m}$) was measured for the two host species, either parasitized or un-parasitized, and for the parasite, *Rhinanthus minor*, attached to either host plant (Fig. 2). *F*$_{v}$/*F*$_{m}$ was significantly lower for parasitized *Plantago* then non-parasitized plants, using pooled data from 310 µmol photons m$^{-2}$ s$^{-1}$ up (*P* < 0.001), whilst in *Phleum* the mean separation approached significance (*P* = 0.092). *F*$_{v}$/*F*$_{m}$ was 18 ± 2 % higher in *Rhinanthus* plants parasitizing *Phleum* than *Plantago* (*P* < 0.001). *F*$_{v}$/*F*$_{m}$ values for *Rhinanthus* parasitizing *Plantago* appeared to decrease with increasing flash irradiance; however, the decrease in mean values was coupled with an increase in the variance, and no significant change in the mean range was noted with increasing irradiance. However, this small decrease in mean *F*$_{v}$/*F*$_{m}$ values may be the result of increased quenching at higher irradiances. The light-adapted quantum efficiency (*Φ*$_{PSII}$) was determined for each host species, both parasitized and unparasitized, and for the parasite on each host (Fig. 3). In *Phleum*, *Φ*$_{PSII}$
was significantly lower in infected than uninfected plants ($P < 0.05$) at each light intensity except 750 and 1170 $\mu$mol photons m$^{-2}$ s$^{-1}$, which approached significance ($P = 0.156$ and 0.128, respectively). In *Plantago*, $\Phi_{PSII}$ was numerically lower in infected plants than uninfected plants at all light intensities, and significantly lower from 40 to 310 $\mu$mol photons m$^{-2}$ s$^{-1}$ ($P < 0.005$ at all points). At light intensities higher than 310 $\mu$mol photons m$^{-2}$ s$^{-1}$, $\Phi_{PSII}$ was not significantly different ($P > 0.05$) between treatments. *Rhinanthus* exhibited significantly lower values of $\Phi_{PSII}$ when grown on *Plantago* than *Phleum* at every light intensity from 40 to 500 $\mu$mol photons m$^{-2}$ s$^{-1}$, and zero values for *Plantago* cultured plants above 500 $\mu$mol photons m$^{-2}$ s$^{-1}$ (Fig. 3C).

In *Phleum*, whilst parasitism led to significant decreases in the total chlorophyll concentration (Fig. 4A), the chlorophyll $a:b$ ratio did not alter significantly. A non-significant reduction in Rubisco concentration was also noted (Fig. 4B). Conversely, in *Plantago*, parasitism did not lead to a reduction in either chlorophyll content or in Rubisco concentration. *Rhinanthus* grown on *Phleum* had a higher Rubisco concentration in its leaves than *Rhinanthus* grown on

Fig. 3. $\Phi_{PSII}$ for host plants either infected or uninfected by the parasitic plant *Rhinanthus minor*: (A) *Phleum bertolonii* and (B) *Plantago lanceolata*; and (C) $\Phi_{PSII}$ for the parasite growing with these species. The actinic beam was of variable intensity to obtain $\Phi_{PSII}$ light-response curves. Error bars represent ± s.e. Part (A) is redrawn from Cameron et al. (2005).

Fig. 4. (A) Chlorophyll concentration and (B) Rubisco concentration in infected and uninfected host plants *Phleum bertolonii* and *Plantago lanceolata* parasitized by *Rhinanthus minor*, and for the parasite growing with these species. Error bars represent ± s.e. Chlorophyll $a:b$ ratios are given above the corresponding bar in (A) with the corresponding ± s.e. given in parentheses.
Photoinhibition in these plants.

Infection of *Phleum* by *R. minor* led to significant reductions in plant biomass, the quantum efficiency of PSII ($\Phi_{PSII}$), and chlorophyll concentration, and a non-significant reduction in Rubisco concentrations. However, no such effects were noted in *Plantago* plants infected by *R. minor*, with the exception of significant decreases in $\Phi_{PSII}$ at low light intensities, below 310 μmol photons m$^{-2}$ s$^{-1}$, and in the maximum quantum yield ($F_v/F_m$) at high light intensities, above 310 μmol photons m$^{-2}$ s$^{-1}$. Although the decrease in *Plantago* $F_v/F_m$ was not large, it may be indicative of mild light stress and the deactivation of some of the reaction centres at the growth irradiances used. Likewise, $F_v/F_m$ values for *R. minor* parasitizing *Plantago* were significantly lower than the 0.83 multi-species average expected for healthy plants (Maxwell and Johnson, 2000), and significantly lower than the values from *R. minor* parasitizing *Phleum*, suggesting significant photoinhibition in these plants.

In infected *Phleum* plants, significant decreases in $\Phi_{PSII}$ were noted at all light intensities, except the very highest light levels to which the plants were exposed. Reductions in the electron transport rate (ETR), and thus $\Phi_{PSII}$, may be a result of three processes; the sink strength of the Calvin cycle for photosynthetic reductants, ATP and NADPH (CO$_2$ limitation); limitation by light interception and leaf area; or limitation by the thylakoids' electron transport capacity (Buckley and Farquhar, 2006). As $\Phi_{PSII}$ was measured under saturating irradiances light interception at the leaf surface can effectively be ruled out, and decreases in $\Phi_{PSII}$ must be the result of either decreased intracellular CO$_2$ concentrations, potentially as a result of stomatal closure, or of diminished thylakoid capacity, characterized by the large decrease in chlorophyll concentration. Given the reduction in host leaf chlorophyll and Rubisco concentrations, and the fact that parasitism does not increase host ABA levels (Jiang, 2004), which prompt stomatal closure, the latter seems more likely. Potential differences in stomatal aperture should, however, be tested in future investigations. ETR (and hence $\Phi_{PSII}$) is known to strongly correlate with photosynthetic rates, and may represent one factor explaining the growth repression suffered by *Phleum*.

Conversely, any reductions in $\Phi_{PSII}$ in parasitized *Plantago* relative to unparasitized controls occurred at low irradiances, between 40 and 310 μmol photons m$^{-2}$ s$^{-1}$, suggesting that reductions in ETR at these irradiances are due to reduced light harvesting by the PSI complex, which corresponds to the small but significant noted decrease in $F_v/F_m$.

It is important, however, to note that vascular continuity between *Rhinanthus* and *Plantago* (Cameron et al., 2006). The signalling mechanism between host and parasite facilitating such suppression of host photosynthesis is unclear, especially given the lack of vascular continuity between *Rhinanthus* and *Plantago* (Cameron et al., 2006).
is not essential for abstraction of host solutes by parasitic plants; it has been shown recently that there is no vascular continuity between the haustorium of Santalum album and its host Tithonia diversifolia, instead the host–parasite interface was characterized by the presence of significant amounts of interfacial parenchyma (Tennakoon and Cameron 2006). The occurrence of solute transfers between host and parasite in the absence of vascular continuity coupled with the observation of Cameron and Seel (2007) that the resistance mechanisms induced by Plantago significantly impede but do not absolutely prevent solute transfer, leaves a physiological mechanism for host–parasite signalling. The signal-transduction pathway, however, remains unclear but it appears, in contrast to other hemiparasites such as Stiga, that this is not a function of ABA biosynthesis in either host or parasite (Jiang, 2004).

It has been long known that infection by parasitic Rhinanthus spp. can suppress the biomass of the associated host plant and, reciprocally, host identity strongly influences parasite success (Hwangbo, 2000). Differences in defence characteristics have recently been shown to underpin these differences in host quality and the associated parasite-induced host damage (Cameron et al., 2006; Cameron and Seel, 2007; Rümer et al., 2007). The degree of Rhinanthus-induced suppression of host biomass cannot, however, be explained in terms of source–sink relationships alone as the biomass achieved by the parasite is less than the biomass lost by the host as a result of infection. This ‘missing biomass’ may be a function of a number of factors, e.g. inefficient assimilation of host-derived solutes, or a result of solute ‘leakage’ from the haustoria. We show, however, for the first time in any facultative hemiparasitic plant, that at least a component of this ‘missing biomass’ is a result of host photosynthetic repression by R. minor.

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LITERATURE CITED