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Does light influence the relationship between a native stem hemiparasite and a native or introduced host?

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● **Background and Aims** There have been very few studies investigating the influence of light on the effects of hemiparasitic plants on their hosts, despite the fact that hemiparasites are capable of photosynthesis but also access carbon (C) from their host. In this study we manipulated light availability to limit photosynthesis in an established hemiparasite and its hosts, and determined whether this affected the parasite's impact on growth and performance of two different hosts. We expected that limiting light and reducing autotrophic C gain in the parasite (and possibly increasing its heterotrophic C gain) would lead to an increased impact on host growth and/or host photosynthesis in plants grown in low (LL) relative to high light (HL).

● **Methods** The Australian native host *Leptospermum myrsinoides* and the introduced host *Ulex europaeus* were either infected or not infected with the native stem hemiparasite *Cassytha pubescens* and grown in either HL or LL. Photosynthetic performance, nitrogen status and growth of hosts and parasite were quantified. Host water potentials were also measured.

● **Key Results** *In situ* midday electron transport rates (ETRs) of *C. pubescens* on both hosts were significantly lower in LL compared with HL, enabling us to investigate the impact of the reduced level of parasite autotrophy on growth of hosts. Despite the lower levels of photosynthesis in the parasite, the relative impact of infection on host biomass was the same in both LL and HL. In fact, biomass of *L. myrsinoides* was unaffected by infection in either HL or LL, while biomass of *U. europaeus* was negatively affected by infection in both treatments. This suggests that although photosynthesis of the parasite was lower in LL, there was no additional impact on host biomass in LL. In addition, light did not affect the amount of parasite biomass supported per unit host biomass in either host, although this parameter was slightly lower in LL than HL for *U. europaeus* ($P = 0.073$). We also found no significant enhancement of host photosynthesis in response to infection in either host, regardless of light treatment.

● **Conclusions** Despite lower photosynthetic rates in LL, *C. pubescens* did not increase its dependency on host C to the point where it affected host growth or photosynthesis. The impact of *C. pubescens* on host growth would be similar in areas of high and low light availability in the field, but the introduced host is more negatively affected by infection.

Key words: Biomass, *Cassytha pubescens*, gas exchange, hemiparasite–host association, *Leptospermum myrsinoides*, light, nitrogen, photosynthesis, *Ulex europaeus*, water potential.

INTRODUCTION

Parasitic plants are of global importance as they are found in almost all ecosystems and can have substantial effects on landscape processes, plant community structure and host populations (Pennings and Callaway, 1996; Press and Phoenix, 2005; Quested, 2008). For example, in a model European grassland the presence of the root hemiparasite *Rhinanthus minor* can increase nutrient cycling (likely through indirect means) and plant diversity, but also decrease community biomass (Bardgett *et al*., 2006). Such decreases can be explained by *R. minor* restricting the dominance of grasses, which thereby releases forbs from competitive exclusion and changes community structure (Bardgett *et al*., 2006; Mudrák and Lepš, 2010). Such outcomes may depend on some hosts showing resistance to infection, while others show a varying degree of tolerance (Press and Graves, 1995; Press and Phoenix, 2005). For instance, some forb species show resistance to *R. minor*

(Cameron *et al*., 2006; Cameron and Seel, 2007; Rümer *et al*., 2007). Tolerance of infection by parasitic plants is often greater in native hosts infected with native parasites compared with introduced hosts (Li *et al*., 2012). For example, in Australia the native host *Leptospermum myrsinoides* shows greater tolerance of infection with the native stem hemiparasite *Cassytha pubescens* than the introduced host, *Cytisus scoparius* (Prider *et al*., 2009).

Hemiparasites often affect less tolerant hosts via a combination of resource removal and impacts on host photosynthesis (Graves *et al*., 1989; Press *et al*., 1999; Shen *et al*., 2006). While hemiparasites are capable of photosynthesis, they are also known to remove significant amounts of carbon (C) from the xylem of their host(s) (Marshall and Ehleringer, 1990; Press *et al*., 1991; Seel *et al*., 1992; Marshall *et al*., 1994; Těšitel *et al*., 2010). Restricting parasite photosynthesis may change this balance and result in increased dependency on host C. For example, Cechin and Press (1993) found that as nitrogen (N)

supply decreased from 3 mol m^{-3} to 0·5 mol m^{-3}, photosynthesis of *Striga hermonthica* decreased by around 50 % while the proportion of host C found in leaves of the parasite increased by 21 %. Another way of manipulating parasite photosynthesis is to change light availability. Těšitel *et al.* (2011) found that, when shaded, *Rhinanthus alectorolophus* had lower rates of photosynthesis and a significantly higher percentage of host C in its biomass, relative to unshaded *R. alectorolophus*. They also found that, relative to controls, shading the young parasite had no impact or a positive effect on host biomass. The latter was presumably a result of shaded parasites being much smaller and representing a smaller carbon sink for the host than unshaded parasites. Studies by Těšitel *et al.* (2011, 2015) investigating carbon relations of associations involving *R. alectorolophus* and subsequent effects on host growth were conducted over a relatively short term (∼1·5 months), using juvenile seedlings of an annual parasite with determinate growth. In fact, dry mass of *R. alectorolophus* was only 0·5–1·0 g even in unshaded plants, and in shaded seedlings was <0·1 g. Unlike *R. alectorolophus*, many hemiparasites are perennial, have indeterminate growth and can have much higher biomass that can represent a significant C sink for hosts (Marshall and Elheringer, 1990; Marshall *et al.*, 1994). In this latter case, it is reasonable to speculate that when established parasites are shaded to an extent that results in lower photosynthesis (and thus autotrophic C gain), they may become more dependent on the host for C, and that this could be a sufficiently large enough demand to have an impact on the host's growth and photosynthesis, particularly if host growth is also limited, e.g. by low light. Additionally, hosts that show some tolerance of infection may be less impacted than more susceptible ones, as parasites typically grow more vigorously on the latter (Prider *et al.*, 2009) and thus should represent a larger sink for C on these hosts. However, to our knowledge there have been no studies on the influence of light on host:parasite systems such as these.

Here we report results of experiments investigating the effect of light on the performance of the Australian native stem hemiparasite *C. pubescens* and its effect on growth and physiology of the tolerant, native host *L. myrsinoides* and the more susceptible, introduced host *Ulex europaeus* (Prider *et al.*, 2009). It was hypothesized that parasite photosynthesis would be lower in low light compared with high light and that this would increase the dependence of the parasite on its host. As a consequence, it was speculated that the parasite would have a greater relative effect on host photosynthesis and growth in low light than in high light.

MATERIALS AND METHODS

Study species

Cassytha pubescens (Lauraceae) is a perennial hemiparasitic coiling vine native to Australia (Kokubugata *et al.*, 2012). It has indeterminate growth with photosynthetic stems that are 0·5−1·5 mm in diameter with reduced scale-like leaves. *Cassytha pubescens* spreads over its hosts and attaches to stems and leaves via multiple haustoria (McLuckie, 1924). *Leptospermum myrsinoides* (Myrtaceae) is a perennial evergreen shrub native to south-eastern Australia (Harden, 1991). It is abundant in open woodland and is a common, but tolerant,

host for *C. pubescens* (Prider *et al.*, 2009). *Ulex europaeus* (Fabaceae) is a perennial evergreen shrub native to central and western Europe and North Africa (Clements *et al.*, 2001) that was introduced to Australia in the 19th century (Parsons and Cuthbertson, 2001). *Ulex europaeus* is frequently parasitized by *C. pubescens*, which has significant negative impacts on growth of this host (Britton, 2002).

Growth conditions and experimental design

In Experiment 1, 10-month-old *L. myrsinoides* plants were obtained from a local commercial nursery. They were individually transplanted into 140 mm diameter (1·65 L) pots containing sandy/loam (60/40) in early May 2010. Three months later they were individually re-potted into 200 mm diameter (4·7 L) pots of sandy/loam (60/40). Plants were supplied with slow-release fertilizer (Osmocote; Scotts-Sierra Horticultural Products, Marysville, OH, USA) for the remainder of the experiment according to the manufacturer's recommended dosage.

In Experiment 2, *U. europaeus* (∼15 cm in height) were collected from the field in the Adelaide Hills (35°27′41″ S, 138°43′91″ E). Plants were excavated and individually potted in 140 mm diameter (1·65 L) pots containing sandy/loam (60/40) in mid-January 2011. Eleven months later they were individually transplanted into 200 mm diameter (4·7 L) pots of sandy loam (60/40). Throughout, they were provided with liquid fertilizer (Nitrosol; Rural Research Ltd, Auckland, New Zealand; NPK 8:3:6) in accordance with the manufacturer's directions.

Both experiments were carried out in the same glasshouse (University of Adelaide) at a similar time of year, using the same shade cloth structures, and plants were well watered throughout each experiment. Synchronous infection with *C. pubescens* of randomly selected host individuals was achieved using the technique of Shen *et al.* (2010). Briefly, infected *U. europaeus* (donor plants) were placed next to the experimental plants. *C. pubescens* stems extending from the donor plant were allowed to coil and attach to stems of experimental hosts. After *C. pubescens* had successfully attached to the new hosts, the connection with the donor host was severed. The infection process of *C. pubescens* on hosts took 3 months for *L. myrsinoides* and 5 months for *U. europaeus*. Plants were monitored for a further week to ensure that *C. pubescens* had successfully established on the new hosts. Light treatments were implemented around 1 month after the infection process for both experiments.

Infected and non-infected plants were randomly arranged into two light treatments, high light (HL) or low light (LL), and two blocks, with each block on a separate bench (replicate numbers are mentioned under each parameter measured). Plants in the LL treatment were housed in a frame (2 m high × 1·5 m deep × 1·2 m wide) completely covered by neutral density shade cloth that allowed 35 % light penetration. Adjacent HL plants were grown in ambient light and plant position within treatment blocks was re-randomized fortnightly. Light treatments for the *L. myrsinoides* and *U. europaeus* experiments were imposed in mid-January 2011 and early January 2012 and ran until early May 2011 and mid-May 2012, respectively. Mean midday photosynthetic photon flux density (PPFD) was

recorded with a quantum sensor (LI-190SA; LI-COR, Lincoln, NE, USA) and data logger (LI-1400) on sunny days during each experiment. The PPFDs for the HL treatment blocks were $1182 \pm 66 \, \mu\text{mol m}^{-2}\text{s}^{-1}$ (± 1 s.e.) in Experiment 1 and $1159 \pm 11 \, \mu\text{mol m}^{-2}\text{s}^{-1}$ in Experiment 2. For the LL treatment blocks they were $351 \pm 22 \, \mu\text{mol m}^{-2}\text{s}^{-1}$ in Experiment 1 and $300 \pm 5 \, \mu\text{mol m}^{-2}\text{s}^{-1}$ in Experiment 2.

Physiological and growth measurements

As we were not evaluating acclimation in this experiment, but rather were interested in the *in situ* photosynthesis, we measured gas exchange and chlorophyll fluorescence under growth light conditions. Nevertheless, rapid light response curves were measured for parasite and hosts (Supplementary Data Fig. S1) using a chlorophyll fluorometer (MINI-PAM; Walz, Effeltrich, Germany) fitted with a leaf clip (2030-B; Walz, Effeltrich, Germany). Midday electron transport rates (ETRs) were obtained *in situ* using the chlorophyll fluorometer and were calculated as follows:

$$\text{ETR} = \text{yield} \times \text{PAR} \times 0.5 \times 0.84$$

where yield is the photochemical efficiency of photosystem II (PSII) in the light, PAR is photosynthetically active radiation (measured as photon flux density in $\mu\text{mol quanta m}^{-2}\text{s}^{-1}$), 0.5 is included as absorption of two quanta are needed to transport an electron, and 0.84 is a standard absorption factor for higher plants (White and Critchley, 1999; Strong *et al.*, 2000). Measurements were made on a single fully mature leaf of *L. myrsinoides* and spine of *U. europaeus*, and also 15 cm from the growing tip of *C. pubescens*, on sunny days between 12:00 and 14:30 h in early April in both experiments. *In situ* measurements were made in HL and LL on *L. myrsinoides* ($n = 10$, except LL infected plants, $n = 8$) and *C. pubescens* ($n = 5$) 76 and 86 d after treatments had been imposed (DAT), respectively (Experiment 1); and for *U. europaeus* and *C. pubescens* ($n = 8$) at 125 DAT (Experiment 2). The PPFD ($\mu\text{mol m}^{-2}\text{s}^{-1}$) values for ETR measurements for *L. myrsinoides* and *C. pubescens* in HL were 1188 ± 4 and 933 ± 67 while for LL they were 341 ± 5 and 292 ± 4, respectively. Values for *U. europaeus* and *C. pubescens* in HL were 1033 ± 13 and 1024 ± 20 while for LL they were 307 ± 5 and 307 ± 4, respectively.

In addition, photosynthesis (A) and stomatal conductance (g_s) measurements were made on *L. myrsinoides* leaves (PLC6 U cuvette) and *U. europaeus* spine clusters (PLC5 C cuvette) using a portable Ciras-2 gas exchange system (PP Systems, Amesburg, MA). For both experiments cuvette temperature was 25 °C and the CO_2 reference supply was maintained at ~390 ppm. Cuvette leaf temperature was 24.5 ± 0.4 and 25.3 ± 0.1 °C for *L. myrsinoides* and *U. europaeus*, respectively. *In situ* measurements in HL and LL were made on uninfected and infected plants between 10:30 and 13:15 h on a sunny day in April, at 81 DAT for *L. myrsinoides* ($n = 5$) and 137 DAT for *U. europaeus* ($n = 6$, except HL uninfected plants, $n = 5$). The PPFD values ($\mu\text{mol m}^{-2}\text{s}^{-1}$) during gas exchange measurement for *L. myrsinoides* were 1464 ± 10 and 535 ± 11 and those for *U. europaeus* were 1057 ± 18 and 313 ± 5 in HL and LL, respectively.

Midday shoot water potential (Ψ) was determined on freshly cut shoots of uninfected and infected plants. Immediately after excision, shoots were placed into a Scholander-type pressure bomb with a digital gauge (PMS Instrument Company, Albany, OR, USA) and balancing pressure was recorded when xylem sap first appeared at the cut end. Measurements were made between 12:00 and 13:40 h on a sunny day in April at 83 DAT for *L. myrsinoides* ($n = 6$) and 138 DAT for *U. europaeus* ($n = 6$, except HL uninfected $n = 5$ and infected plants $n = 7$).

A destructive harvest of uninfected and infected plants and parasite was conducted at 104 and 157 DAT for Experiment 1 and Experiment 2, respectively. Stems, leaves and roots of *L. myrsinoides* (Experiment 1, $n = 5$), stems, spines (Experiment 2, very few if any leaves) and roots of *U. europaeus* ($n = 6$) and stems of *C. pubescens* from Experiment 1 ($n = 5$) and Experiment 2 ($n = 6$) were collected and oven-dried at 70 °C for 3 d prior to weighing. Leaf area for both *L. myrsinoides* and *U. europaeus* was determined using the relationships between leaf area and dry weight obtained from a subsample of foliage from each treatment (Rolston and Robertson, 1976). For these positive relationships, R was ≥ 0.95 for all treatments in both experiments. Nitrogen concentration of oven-dried *C. pubescens* stems, *L. myrsinoides* leaves and *U. europaeus* spines (replication as above) was determined using the Elementar Rapid N III Nitrogen Analyzer Version J by Waite Analytical Services (University of Adelaide).

Statistical analyses

The variances of the data were homogeneous and Experiments 1 and 2 were analysed separately. The effects of light and infection on hosts were assessed using two-way ANOVA. When significant interactions between light and infection were detected, the analyses for the four combinations were continued. If no interaction was detected, we then considered independent effects of light (uninfected and infected HL plants pooled versus uninfected and infected LL plants pooled) and independent effects of infection (uninfected HL and LL plants pooled versus infected HL and LL plants pooled). One-way ANOVA was used to determine the effect of light on *C. pubescens*. When a significant effect for a parameter was detected by the model, a Tukey–Kramer HSD was then used for *post hoc* pairwise comparisons of means. All data were analysed with the software JMP version 4.0.3 (SAS Institute, 2000) with $\alpha = 0.05$.

RESULTS

Parasite and host ETR

Our aim was to limit photosynthesis of the hemiparasite *C. pubescens* by growing plants in LL, and, as expected, midday ETR of *C. pubescens* on both *L. myrsinoides* and *U. europaeus* was significantly lower in LL than HL (Table 1). Midday ETRs of *C. pubescens* growing in HL were 51 and 43 % higher relative to those in LL when growing on *L. myrsinoides* or *U. europaeus*, respectively (Fig. 1A, B).

Midday ETR of *L. myrsinoides* was significantly affected by infection in HL but not in LL (significant interaction; Table 2,

Fig. 2A). Midday ETR was 39 % lower in HL-grown infected plants relative to uninfected plants. By contrast, there was no significant interaction between light and infection for midday ETR of *U. europaeus*, but there were independent infection and light effects (Table 2, Fig. 2B–D). On average, midday ETR of infected plants was 24 % lower than that of uninfected plants, irrespective of light conditions (Fig. 2C). Midday ETR of HL-grown *U. europaeus* was 53 % higher, on average, than that of LL plants, regardless of their infection status (Fig. 2D).

Host A, g_s and Ψ

There was no interaction between light and infection for *A* in *L. myrsinoides* (Table 2, Fig. 3A). On average, photosynthetic rates of infected plants were 43 % lower compared with those of uninfected plants, irrespective of light conditions (significant infection effect; Table 2, Fig. 3B). Similarly, there was no significant interaction between light and infection for g_s of *L. myrsinoides*, but this parameter was also independently affected by infection (Table 2, Fig. 3C, D). Stomatal conductance of infected *L. myrsinoides* was, on average, 37 % less compared with that of uninfected plants, across the light treatments (Fig. 3D).

There was also no interaction between light and infection for *A* in *U. europaeus* (Table 2, Fig. 3E). Infection had no effect on this parameter, whereas light did (Table 2). On average,

photosynthetic rates of *U. europaeus* in HL were 48 % higher than those in LL, regardless of their infection status (Fig. 3F). By contrast, there was a significant interaction between light and infection for g_s of *U. europaeus* (Table 2). Stomatal conductance was unaffected by infection regardless of light treatment; there was a trend for g_s of infected plants to be lower when grown in HL, but the opposite occurred in LL (Fig. 3G). Uninfected plants in HL had significantly higher g_s than uninfected plants in LL (Fig. 3G).

There was no interaction for midday Ψ in *L. myrsinoides* (Table 2). There was no independent infection effect on this parameter but it was independently affected by light (Table 2). Midday Ψ in HL *L. myrsinoides* was 17 % lower relative to that in LL plants (Table 3). Likewise, there was no significant interaction between light and infection for midday Ψ of *U. europaeus* (Table 2). Infection also had no significant, independent effect on this parameter in *U. europaeus*, whereas light did (Table 2). Water potentials at midday of HL *U. europaeus* were 2-fold lower than those of LL plants (Table 3).

TABLE 1. *One-way ANOVA results (P values) for the effect of light on* C. pubescens *midday electron transport rate (ETR), biomass, biomass per gram host biomass and stem nitrogen concentration (N), when infecting* L. myrsinoides *or* U. europaeus *(each host species was analysed separately)*

Source of variation	ETR	Biomass	Grams dry weight of parasite per g dry weight of host	N
L. myrsinoides				
Light	**0·002**	0·191	0·388	0·829
U. europaeus				
Light	**0·012**	**0·001**	0·073	**0·0004**

Significant effects are in bold.
F and sum of square values and d.f. are provided in Supplementary Data Table S1.

TABLE 2. *Two-way ANOVA results (P values) for the effect of* C. pubescens *and light on midday electron transport rate (ETR), photosynthetic rates (A), stomatal conductance (*g_s*), midday shoot water potentials (*Ψ*), total, shoot and root biomass, leaf or spine area (L/S A), shoot/root ratio (S/R) and leaf or spine nitrogen (N) concentration of* L. myrsinoides *and* U. europaeus *(each species was analysed separately)*

Parameter	*L. myrsinoides*			*U. europaeus*		
	I × L	I	L	I × L	I	L
ETR	**0·0009**	**0·018**	**<0·0001**	0·084	**0·012**	**<0·0001**
A	0·450	**0·011**	0·939	0·178	0·908	**<0·0001**
g_s	0·727	**0·010**	0·176	**0·010**	0·825	0·262
Ψ	0·058	0·333	**0·0009**	0·371	0·651	**<0·0001**
Total	**0·006**	0·774	**<0·0001**	0·153	**<0·0001**	**<0·0001**
Shoot	**0·016**	0·421	**<0·0001**	0·071	**<0·0001**	**<0·0001**
Root	**0·015**	0·249	**<0·0001**	0·532	**0·041**	**<0·0001**
L/S A	0·776	0·423	**0·0002**	0·261	**<0·0001**	**0·0002**
S/R	0·115	0·385	**0·003**	0·928	**0·034**	**0·003**
N	**0·040**	0·714	**0·0004**	0·745	0·123	**0·007**

I, infection; L, light.
Significant effects are in bold.
F and sum of square values and d.f. are provided in Supplementary Data Tables S2 and S3.
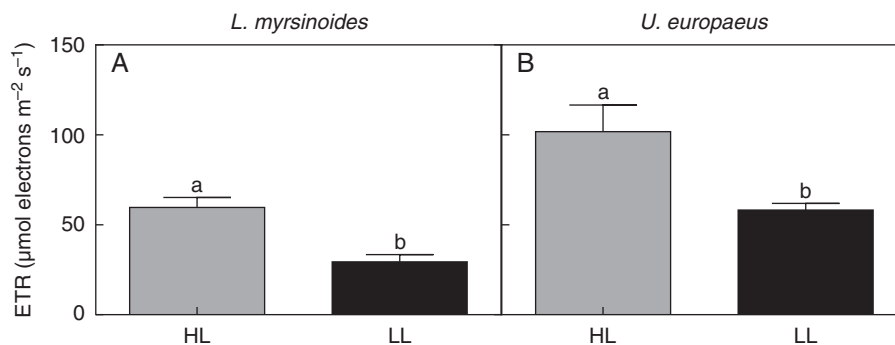

FIG. 1. *In situ* midday electron transport rates (ETRs) of *C. pubescens* growing on *L. myrsinoides* (A) or *U. europaeus* (B) in high (HL, dark grey bars) or low light (LL, black bars). Letters indicate significant differences; bars are means (± 1 s.e.) and $n = 5$ (A) and 8 (B).

Host growth

Total and shoot biomass of *L. myrsinoides* was not significantly affected by infection in HL or LL; however, biomass of uninfected HL plants was significantly higher compared with that of uninfected LL plants (significant interaction for both total and shoot biomass; Table 2, Fig. 4A). Root biomass of *L. myrsinoides* was negatively affected by infection in HL but not in LL, and again that of uninfected HL plants was significantly higher than that of uninfected LL plants (significant interaction; Table 2, Fig. 4A). There was no significant interaction or infection effect on leaf area or shoot/root ratio of *L. myrsinoides* (Table 2). Light, however, did affect these parameters, and for LL plants leaf area and shoot/root ratio were 29 and 26 % higher, respectively, relative to those of HL plants (Tables 2 and 4).

By contrast, there were no significant interactions between light and infection for any of the growth measures for

U. europaeus (Table 2, Fig. 4B). Infection had a significant, independent impact on all growth parameters for this host (Table 2, Fig. 4C). Total biomass of infected plants was 40 % lower, on average, than that of uninfected plants (Fig. 4C), regardless of light treatment. Shoot and root biomass were 40 and 28 %, respectively, lower compared with values for uninfected plants (Fig. 4C). Leaf area and shoot/root ratio of infected *U. europaeus* were 40 and 22 %, respectively, lower than those of uninfected plants (Table 4). Light also significantly affected all growth parameters of *U. europaeus* (Table 2). Total biomass of plants grown in LL was 40 % lower, on average, relative to that of the HL-grown plants, regardless of infection (Fig. 4D). Shoot and root biomass of *U. europaeus* in LL were 34 and 55 %, respectively, lower than in HL plants (Fig. 4D). Leaf area and shoot/root ratio of LL *U. europaeus* were 34 % less and 31 % higher, respectively, compared with HL-grown plants (Table 4).
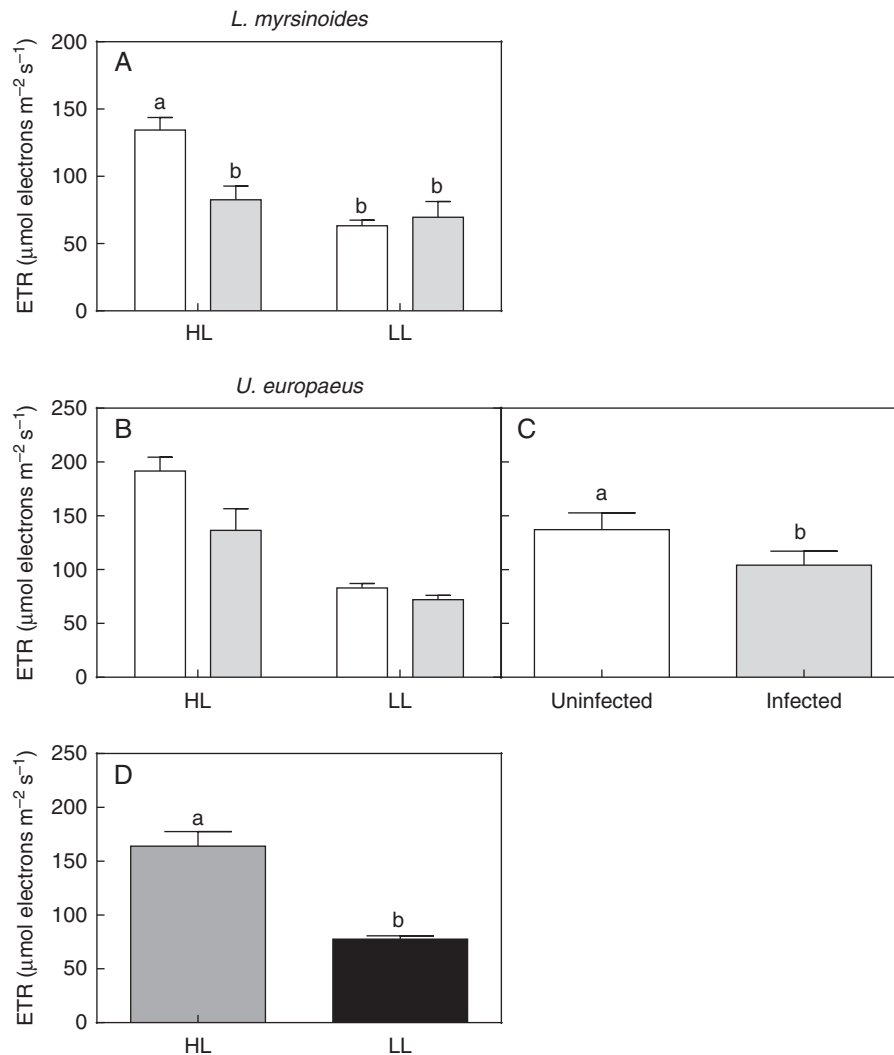

FIG. 2. *In situ* midday electron transport rates (ETRs) of *L. myrsinoides* (A) and *U. europaeus* (B) grown in high (HL) or low light (LL), and uninfected (open bars) or infected (grey bars) with *C. pubescens*. (C) Independent effect of infection on *in situ* midday ETR of *U. europaeus* (open bar, average of HL and LL uninfected plants pooled; grey bar, average of HL and LL infected plants pooled). (D) Independent effect of light on *in situ* midday ETR of *U. europaeus* in HL (dark grey bars, average of uninfected and infected HL plants pooled) versus LL (black bars, average of uninfected and infected LL plants pooled). Letters indicate significant differences; bars are means ($\pm$1 s.e.) and $n = 8$–10 (A), 8 (B) and 16 (C, D).
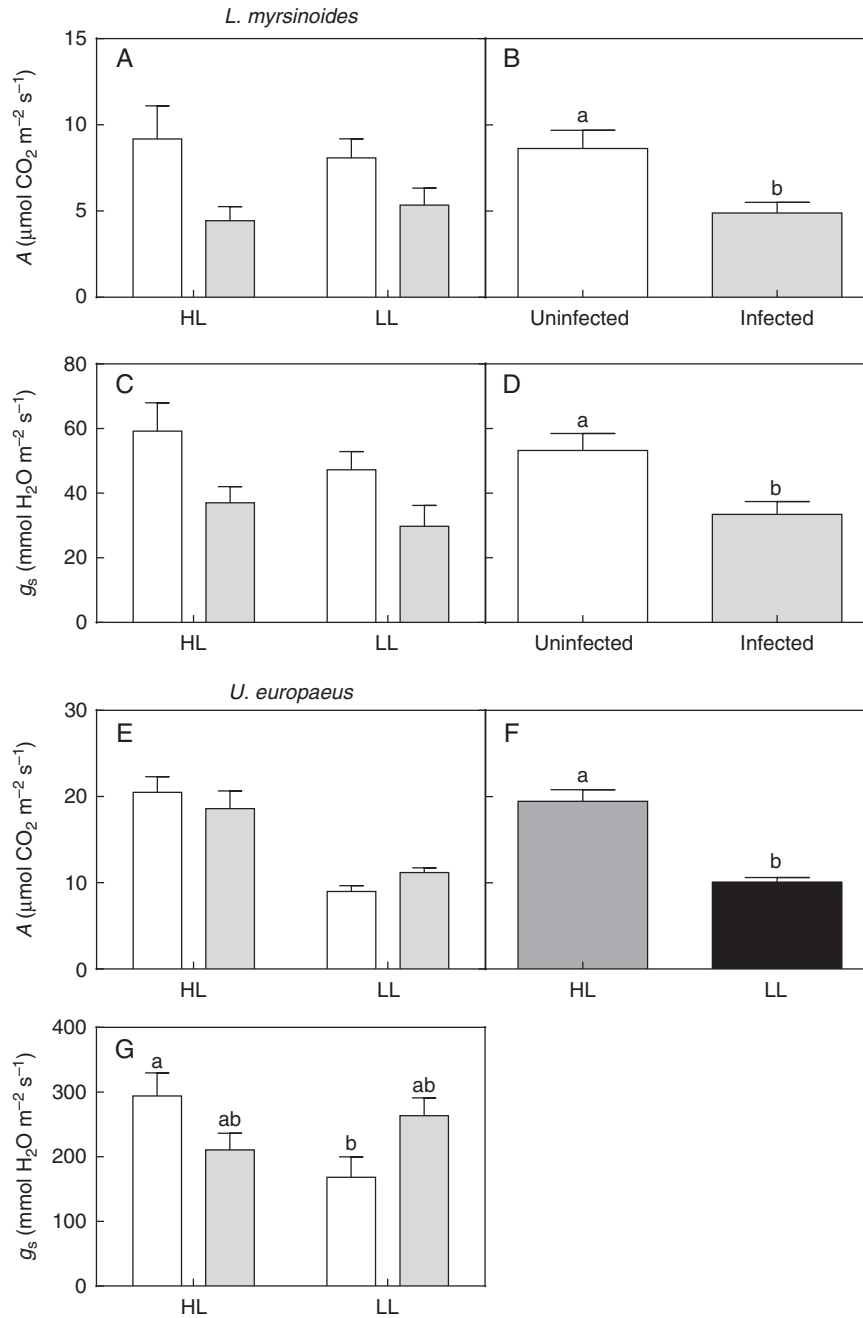
FIG. 3. *In situ* photosynthetic rates (*A*) and stomatal conductance (g_s) of *L. myrsinoides* (A, C) and *U. europaeus* (E, G) grown in high (HL) or low light (LL) and uninfected (open bars) or infected (grey bars) with *C. pubescens*. Independent effect of infection on *in situ A* (B) and g_s (D) of *L. myrsinoides* (open bars, average of HL and LL uninfected plants pooled; grey bars, average of HL and LL infected plants pooled). (F) Independent effect of light on *in situ A* of *U. europaeus* in HL (dark grey bars, average of uninfected and infected HL plants pooled) versus LL (black bars, average of uninfected and infected LL plants pooled). Letters indicate significant differences; bars are means (± 1 s.e.) and $n = 5$ (A, C), 10 (B, D), 6 (E, G, except uninfected HL plants, $n = 5$) and 11–12 (F).

Parasite growth

Final biomass of *C. pubescens* growing on *L. myrsinoides* was similar between light treatments (no significant light effect; Table 1, Fig. 5A). Likewise, there was no light effect on parasite biomass per unit dry weight of *L. myrsinoides* hosts (Table 1, Fig. 5B). By contrast, biomass of *C. pubescens* growing on *U. europaeus* in HL was 65 % higher than that in LL (significant light effect; Table 1, Fig. 5C). However, light did not

affect parasite biomass per unit dry weight of *U. europaeus* hosts (Table 1, Fig. 5D).

Parasite and host N

There was no difference in N concentration of *C. pubescens* stems when growing on *L. myrsinoides* in HL ($1 \cdot 8 \pm 0 \cdot 08$ %) or LL ($1 \cdot 8 \pm 0 \cdot 03$ %) (Table 1). By contrast, N concentration of

TABLE 3. *Midday shoot water potential (Ψ, MPa) of* L. myrsinoides *and* U. europaeus *in high (HL) or low light (LL), uninfected (−) or infected (+) with* C. pubescens. *The two species were analysed separately.* L. myrsinoides: *no interaction (n = 6), no infection but significant independent light effect (n = 12).* U. europaeus: *no interaction (n = 5−7), no infection but significant independent light effect (n = 12)*

Treatment	*L. myrsinoides*	*U. europaeus*
HL−	−1·98 ± 0·10	−2·12 ± 0·07
HL+	−1·74 ± 0·07	−2·08 ± 0·11
LL−	−1·50 ± 0·10	−0·98 ± 0·09
LL+	−1·58 ± 0·04	−1·11 ± 0·07
Infection effect		
−	−1·74 ± 0·10	−1·50 ± 0·19
+	−1·66 ± 0·05	−1·63 ± 0·15
Light effect		
HL	−1·86 ± 0·07a	−2·10 ± 0·07a
LL	−1·54 ± 0·05 b	−1·05 ± 0·06b

Data are means (±1 s.e.) and letters denote significant differences.

C. pubescens growing on *U. europaeus* in HL (1·8 ± 0·15 %) was 43 % lower compared with that in LL (3·2 ± 0·21 %) (Table 1).

With reference to *L. myrsinoides*, leaf N concentration of uninfected HL plants was not significantly different from that of infected HL plants but was significantly less than in LL uninfected and infected plants, which did not differ significantly from each other (significant interaction; Tables 2 and 4). By contrast, there was no interaction between light and infection for spine N of *U. europaeus* (Table 2). Infection had no significant independent effect on spine N of *U. europaeus*, while light did (Table 2). Nitrogen concentration of HL *U. europaeus* was 19 % less relative to that of LL plants (Table 4).

DISCUSSION

As predicted, photosynthesis (ETR) of *C. pubescens* was significantly lower in LL than HL. However, contrary to our hypothesis, this did not result in a greater relative impact of infection on biomass of either host in LL. Biomass of *U. europaeus* infected with *C. pubescens* was 40 % lower than that of uninfected plants, regardless of light treatment. In contrast, infection had no effect on total biomass of *L. myrsinoides* in either LL or HL. There was a trend for parasite biomass per unit *U. europaeus* biomass to be lower in LL compared with HL, but this was not significant.

Previous studies have also shown that photosynthesis of hemiparasites such as mistletoes is impacted by light (Strong *et al.*, 2000; Matsubara *et al.*, 2002), but to our knowledge only one study has investigated whether this also influences the parasite's effect on host growth. A recent study by Borowicz and Armstrong (2012) found that light did not influence the effect of the perennial root hemiparasite *Pedicularis canadensis* on the grass *Andropogon gerardii*. Similarly, we found that light had no impact on the relative effect of the stem hemiparasite on host growth. Hemiparasites are known to remove significant amounts of C from their hosts (Press *et al.*, 1991; Press and Whittaker, 1993; Těšitel *et al.*, 2010), but our results suggest

that, despite the lower potential for C fixation in LL, *C. pubescens* did not increase its dependency for C on either host to the point where it affected host growth.

We found no effect of light on the relative impact of *C. pubescens* on host growth; however, it is possible that the parasite's demand for host C may still have increased in LL but that this was met by an increase in host photosynthesis. Stimulatory parasite effects on host photosynthesis have been reported for associations involving the root heimparasite *S. hermonthica* (Cechin and Press, 1993) and the stem and root holoparasites *Cuscuta reflexa* and *Orobanche cernua*, respectively (Jeschke *et al.*, 1994, 1997; Jeschke and Hilpert, 1997; Hibberd *et al.*, 1998, 1999). In contrast, several studies have found that parasites, including *C. pubescens*, can have deleterious effects on host photosynthesis (Gurney *et al.*, 2002; Hwangbo *et al.*, 2003; Meinzer *et al.*, 2004; Shen *et al.*, 2007, 2010; Mauromicale *et al.*, 2008; Prider *et al.*, 2009). Increases in host photosynthesis are explained by the parasite acting as an extra sink for C, thus reducing the accumulation of carbohydrate in host foliage, which would normally act as a signal to downregulate photosynthesis (Jeschke and Hilpert, 1997; Jeschke *et al.*, 1997; Hibberd *et al.*, 1998, 1999). We did find some evidence that photosythesis of infected *U. europaeus* may have been slightly stimulated in LL, as there were small but non-significant increases in both photosynthesis and stomatal conductance relative to uninfected plants (Fig. 3E, G). Similarly, infection appeared to have a greater negative effect on ETR of both hosts in HL than in LL (Fig. 2A, B and Supplementary Data Fig. S1).

While light did not alter the relative effect of *C. pubescens* on total biomass of either host, there were differences in the absolute impact of infection on each host. In Experiment 1, *C. pubescens* had no effect on total biomass of the native *L. myrsinoides*. In contrast, in Experiment 2 total biomass of the introduced *U. europaeus* infected with *C. pubescens* was 40 % lower than that of uninfected plants, in both HL and LL. These differences may be related to the evolutionary history of each host. *Ulex europaeus* was introduced to Australia in the late 19th century, whereas *L. myrsinoides* and *C. pubescens* are both native to Australia and co-occur across eastern and southern parts of the country. Other studies have also reported that native parasites have a greater effect on growth of introduced hosts compared with native hosts (Prider *et al.*, 2009; Li *et al.*, 2012). The longer association between native hosts and parasites could have resulted in the evolution of mechanisms of resistance or tolerance to infection in the native hosts. Consistent with this, *L. myrsinoides* appears to have evolved some tolerance to infection with *C. pubescens*, as it is a common host in the wild but seems not to be significantly impacted by infection (Prider *et al.*, 2009). Mechanisms of tolerance may include preventing formation of effective haustorial connections between host and parasite, thus reducing the ability of the parasite to remove resources. For example, Tsang (2010) used ^{32}P to demonstrate that transfer of phosphorus to *C. pubescens* was more effective from the introduced host *C. scoparius* than the native host *Acacia myrtifolia*. Thus, despite the fact that *C. pubescens* affected photosynthesis of *L. myrsinoides* (likely driven by a decrease in stomatal conductance; Fig. 3D), the lack of an effect of infection on total biomass in this host may be largely explained by a poor haustorial connection. Conversely, the negative effect of *C. pubescens* on *U. europaeus* may be
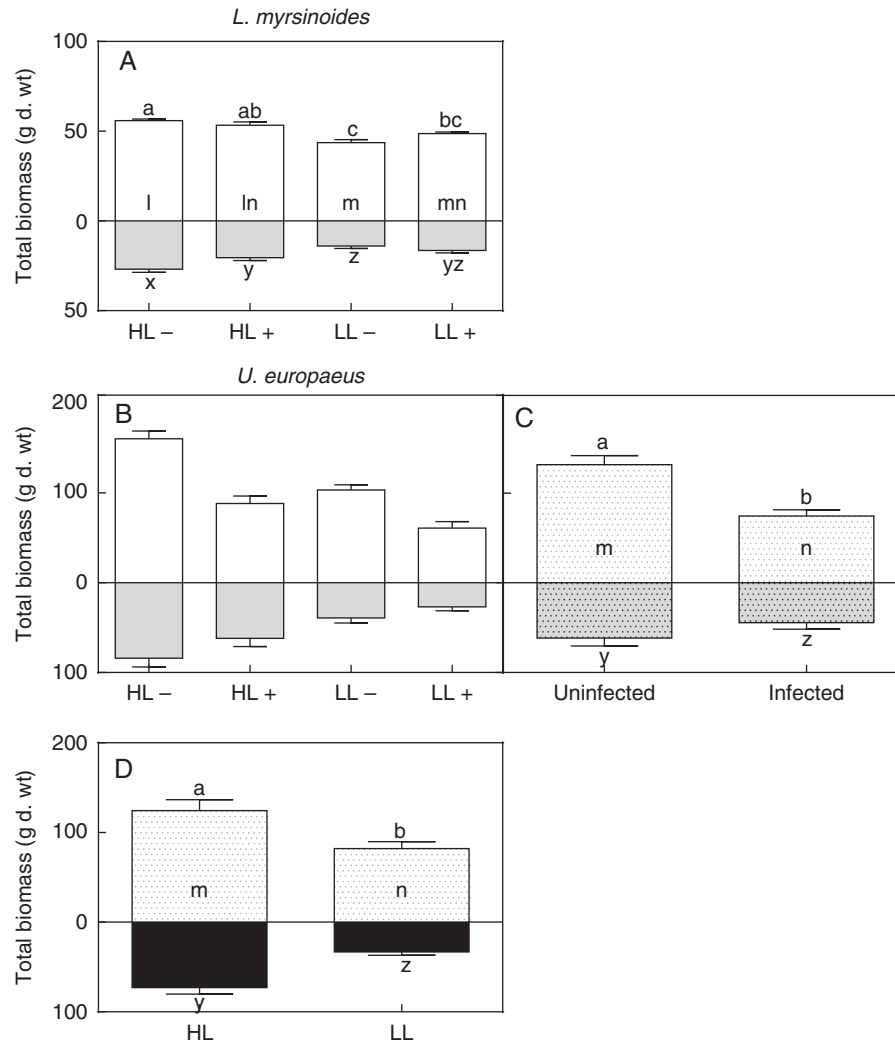
FIG. 4. Total, shoot (open bars) and root (grey bars) biomass of *L. myrsinoides* (A) and *U. europaeus* (B) grown in high (HL) or low light (LL), and uninfected (minus) or infected (plus) with *C. pubescens*. (C) Independent effect of infection on total, shoot (open dotted bar) and root biomass (dotted grey bars) of *U. europaeus* (left bar, average of uninfected HL and LL plants pooled; right bar, average of infected HL and LL plants pooled). (D) Independent effect of light on total, shoot (open dotted bar) and root biomass (black bars) of *U. europaeus* (left bar, average of uninfected and infected HL plants pooled; right bar, average of uninfected and infected LL plants pooled). Letters indicate significant differences for total (a–c), shoot (l–n) and root (x–z) biomass; bars are means (± 1 s.e.) and $n = 5$ (A), 6 (B) and 12 (C, D).

primarily due to an effective haustorial connection and removal of resources from this host (as may be inferred from the vigorous growth of the parasite), in addition to effects on host photosynthesis.

A number of studies have shown that more vigorous parasite growth is generally associated with a greater effect on the host (Gibson and Watkinson, 1991; Matthies, 1996; Keith *et al.*, 2004; Cameron *et al.*, 2008; Prider *et al.*, 2009; Li *et al.*, 2012; but see Cameron *et al.*, 2006). This is consistent with our results, where there was minimal parasite growth and effect on total biomass of *L. myrsinoides*. By contrast, *U. europaeus* supported a higher biomass of *C. pubescens* and was strongly affected by infection. Similarly, *C. pubescens* was also found to grow more vigorously and achieved significantly greater biomass on the introduced host, *C. scoparius*, compared with *L. myrsinoides* in the field (Prider *et al.*, 2009). Vigorous

growth of the parasite on *U. europaeus* might be partly due to the higher ETR of *C. pubescens* relative to that on *L. myrsinoides*. It may also be explained by a more effective haustorial connection as mentioned above. Whereas light had no effect on parasite biomass supported by *L. myrsinoides*, total parasite biomass on *U. europaeus* was much lower in LL than HL. This may be partly explained by LL significantly decreasing the ETR of the parasite and thus autotrophic contributions to its own growth. Further, *U. europaeus* hosts were smaller in LL relative to HL (Fig. 4D), and thus would have had a lower capacity for resource uptake and supply to the parasite in these conditions. There was also a trend for parasite biomass per unit *U. europaeus* biomass to be lower in LL relative to HL ($P = 0.073$). Thus, it is possible that resource uptake by *C. pubescens* was lower, per unit of host biomass, in LL versus HL on this host.

TABLE 4. *Leaf or spine area (L/S A) (cm^2), shoot/root ratio and leaf or spine nitrogen (N) concentration (%) of* L. myrsinoides *and* U. europaeus *in either HL or LL and either uninfected (−) or infected (+) with* C. pubescens. *The two species were analysed separately.* L. myrsinoides: *no interactions except for N (n = 5), no independent infection but significant light effect for leaf area and shoot/root ratio (n = 10).* U. europaeus: *no interactions (n = 6), but significant independent effect of infection on spine area and shoot/root ratio (n = 12) and significant independent effect of light on all three parameters (n = 11–12)*

Treatment	L/S area	Shoot/root	N
L. myrsinoides			
HL−	2816 ± 113	2·12 ± 0·134	1·84 ± 0·07a
HL+	2695 ± 234	2·66 ± 0·182	1·98 ± 0·07[ab]
LL−	3983 ± 252	3·22 ± 0·208	2·19 ± 0·06b
LL+	3731 ± 257	3·06 ± 0·262	2·10 ± 0·03b
Infection effect			
−	3400 ± 234	2·67 ± 0·216	−
+	3213 ± 238	2·86 ± 0·164	−
Light effect			
HL	2756 ± 124a	2·39 ± 0·139a	−
LL	3857 ± 175b	3·14 ± 0·160b	−
U. europaeus			
HL−	1267 ± 73	2·06 ± 0·291	1·50 ± 0·10
HL+	773 ± 109	1·51 ± 0·109	1·30 ± 0·07
LL−	827 ± 40	2·84 ± 0·291	1·78 ± 0·09
LL+	512 ± 78	2·33 ± 0·146	1·65 ± 0·13
Infection effect			
−	1047 ± 77a	2·45 ± 0·229a	1·66 ± 0·08
+	643 ± 75b	1·92 ± 0·152b	1·48 ± 0·09
Light effect			
HL	1020 ± 97a	1·78 ± 0·170a	1·39 ± 0·06a
LL	670 ± 63b	2·59 ± 0·173b	1·72 ± 0·08b

Data are means (± 1 s.e.) and letters denote significant differences.

Despite the lower rates of photosynthesis in the parasite in LL, our results suggest that the parasite is removing a similar amount of C per unit host biomass in both light conditions, but this needs to be confirmed. Thus, growth of the parasite seems to be tightly coupled to host growth, suggesting that parasite growth is determined by the extent to which the host supplies resources. However, it is also possible that growth of the parasite is determined by its own ability to fix C. If this were so, however, we would have expected much greater biomass of *C. pubescens* on *L. myrsinoides* than we observed, as photosynthesis of the parasite on this host was half that of the parasite on *U. europaeus*, but parasite biomass was 10-fold greater on *U. europaeus* than *L. myrsinoides*.

Conclusions

It is concluded from our experiments that, despite having lower rates of photosynthesis in LL, the parasite did not increase its dependency on host C to the point where it affected host growth or photosynthesis. With reference to *U. europaeus*, there appears to be coordination between host and parasite, with a smaller infected host in LL supporting a smaller parasite. Such coordination in responses between host and parasite growth has also been suggested for associations involving mistletoes that access resources from the host xylem and the stem holoparasites *Cuscuta campestris* and *Cuscuta reflexa* (Marshall *et al.*, 1994; Shen *et al.*, 2013). In general, our studies demonstrated that growth of the introduced host *U. europaeus*, but not the native host *L. myrsinoides*, is negatively affected by the native stem hemiparasite *C. pubescens* and is independent of light. Finally, our data indicated that *C. pubescens* will have
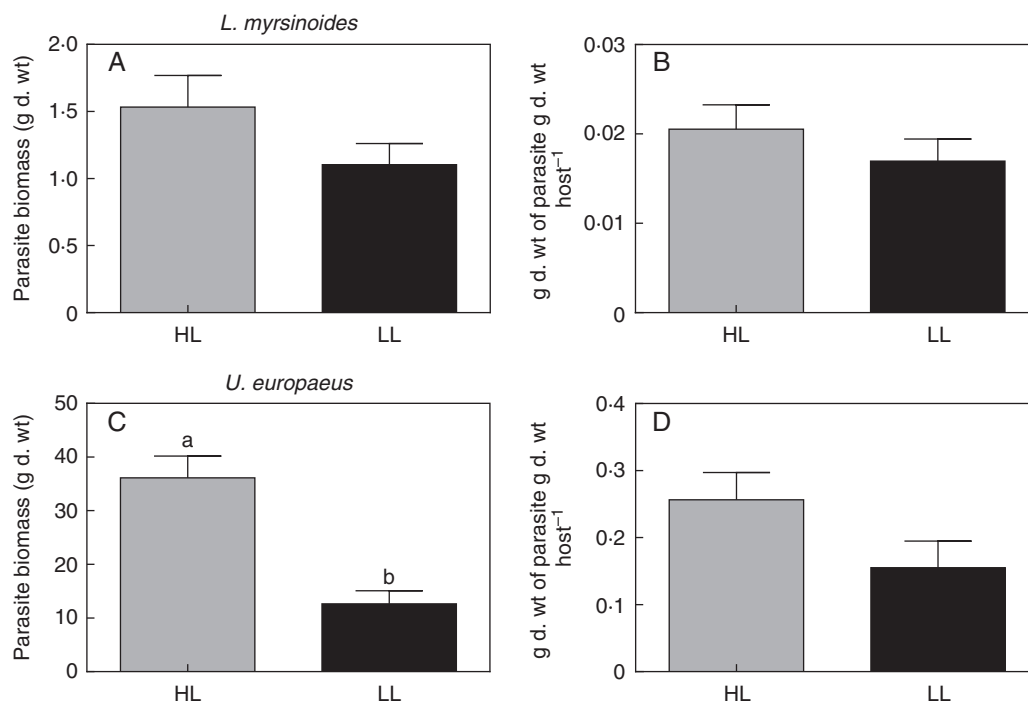

FIG. 5. Total biomass and grams of parasite dry weight per gram of host dry weight, respectively, of *C. pubescens* growing on *L. myrsinoides* (A, B) or *U. europaeus* (C, D) in high (HL, dark grey bars) or low light (LL, black bars). Letters indicate significant differences; bars are means (±1 s.e.) and n = 5 (A, B) and 6 (C, D).

a similar negative effect on the growth of *U. europaeus* in areas of both high and low light availability in the field.

SUPPLEMENTARY DATA

Supplementary data are available online at www.aob.oxfordjournals.org and comprise the following. Figure S1: rapid light response curves for both parasite and host from either experiment. Table S1: one-way ANOVA results (*F*, sum of square values and d.f.) for the effect of light on ETR, biomass, grams of parasite dry weight per gram of host dry weight and stem nitrogen concentration of parasite infecting either host. Table S2: two-way ANOVA results (*F* and sum of squares values and d.f.) for the effect of light and infection on ETR, *A*, g_s and Ψ of either host. Table S3: two-way ANOVA results (*F* and sum of squares values and d.f.) for the effect of light and infection on total, shoot and root biomass, leaf or spine area, shoot/root ratio and leaf or spine nitrogen concentration of either host.

ACKNOWLEDGEMENTS

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