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Native hemiparasite and light effects on photoprotection and photodamage in a native host

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Publication Details

Cirocco, R. M., Waterman, M. J., Robinson, S. A., Facelli, J. M. & Watling, J. R. (2015). Native hemiparasite and light effects on photoprotection and photodamage in a native host. *Functional Plant Biology: an international journal of plant function*, 42 (12), 1168-1178.

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Native hemiparasite and light effects on photoprotection and photodamage in a native host

Abstract

Plants infected with hemiparasites often have lowered rates of photosynthesis, which could make them more susceptible to photodamage. However, it is also possible that infected plants increase their photoprotective capacity by changing their pigment content and/or engagement of the xanthophyll cycle. There are no published studies investigating infection effects on host pigment dynamics and how this relates to host susceptibility to photodamage whether in high (HL) or low light (LL). A glasshouse experiment was conducted where *Leptospermum myrsinoides* Schltdl. either uninfected or infected with *Cassytha pubescens* R.Br. was grown in HL or LL and pigment content of both host and parasite were assessed. Infection with *C. pubescens* significantly decreased all foliar pigment concentrations (except chlorophyll b) in *L. myrsinoides* in both HL and LL. Xanthophyll cycle (violaxanthin, antheraxanthin, zeaxanthin; VAZ) and chlorophyll (Chl) pigments decreased in parallel in response to infection, hence, VAZ/Chl of the host was unaffected by *C. pubescens* in either HL or LL. Pre-dawn and midday de-epoxidation state $[(A + Z)/(V + A + Z)]$ of *L. myrsinoides* was also unaffected by infection in both HL and LL. Thus, *L. myrsinoides* infected with *C. pubescens* maintained similar photoprotective capacity per unit chlorophyll and engagement of the xanthophyll cycle as uninfected plants. Even though midday quantum yield (Φ_{PSII}) of HL plants was affected by infection, pre-dawn maximum quantum yields (F_v/F_m) of hosts were the same as uninfected plants whether in HL or LL. This ability of *L. myrsinoides* to maintain photoprotective capacity/engagement when infected by *C. pubescens* thereby preventing photodamage could explain this host's tolerance to hemiparasite infection.

Disciplines

Medicine and Health Sciences | Social and Behavioral Sciences

Publication Details

Cirotto, R. M., Waterman, M. J., Robinson, S. A., Facelli, J. M. & Watling, J. R. (2015). Native hemiparasite and light effects on photoprotection and photodamage in a native host. *Functional Plant Biology: an international journal of plant function*, 42 (12), 1168-1178.

Native hemiparasite and light effects on photoprotection and photodamage in a native host

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Abstract

Plants infected with hemiparasites often have lowered rates of photosynthesis which could make them more susceptible to photodamage. It is also however possible that infected plants may increase their photoprotective capacity by changing their pigment content and/or engagement of the xanthophyll cycle. There are no studies investigating infection effects on host pigment dynamics and how this relates to host susceptibility to photodamage whether in high (HL) or low light (LL). A glasshouse experiment was conducted where *Leptospermum myrsinoides* Schltdl. was grown either uninfected or infected with *Cassytha pubescens* R. Br. and pigment content of both host and parasite assessed under both HL and LL conditions. Infection with *C. pubescens* significantly decreased all foliar pigment concentrations in *L. myrsinoides* in both HL and LL. Xanthophyll cycle (violaxanthin, antheraxanthin, zeaxanthin; VAZ) and chlorophyll (Chl) pigments decreased in parallel in response to infection, hence, VAZ/Chl of the host was unaffected by *C. pubescens* in either HL or LL. Pre-dawn and midday de-epoxidation state $[(A+Z)/(V+A+Z)]$ of *L. myrsinoides* was also unaffected by infection in both HL and LL. Thus, *L. myrsinoides* infected with *C. pubescens* maintained similar photoprotective capacity per unit chlorophyll and engagement of the xanthophyll cycle as uninfected plants. Even though midday quantum yield (Φ_{PSII}) of HL plants was affected by infection, pre-dawn maximum quantum yields (F_v/F_m) of hosts were the same as uninfected plants whether in HL or LL. This ability of *L. myrsinoides* to maintain photoprotective capacity/engagement when infected by *C. pubescens* thereby preventing photodamage could explain this host's tolerance to hemiparasite infection.

Additional keywords: carotenoid pigments, chlorophyll fluorescence, lutein epoxide, shading, xanthophylls.

Introduction

Parasitic plants are a diverse group that vary greatly in physiology and morphology but all have haustoria (Kuijt 1969). Haustoria are typically 'disk' like organs that fuse to and penetrate host tissue forming a bridge between their vasculature and that of the host (Kuijt 1969). Hemiparasites typically tap the host xylem and remove water, nutrients and other solutes while holoparasites along with removal of these resources extract carbohydrate from the host phloem (see Press and Graves 1995). A relatively lower water potential in the parasitic plant drives the transfer of resources from host to parasite (Ehleringer and Marshall 1995). How effectively haustoria connect to a particular host also varies and can explain why some parasitic plant species affect some hosts more severely than others (Gurney, Grimanelli *et al.* 2003; Cameron and Seel 2007). These impacts on the host can range from negligible to host death (Press and Graves 1995). For example, growth of the forb *Plantago lanceolata* was unaffected by the root hemiparasite *Rhinanthus minor* (Cameron, Geniez *et al.* 2008), whereas Shen, Ye *et al.* (2005) found that nearly all aboveground biomass of the vine *Mikania micrantha* died as a result of infection by the stem holoparasite *Cuscuta campestris*.

Parasite effects on host photosynthesis also vary but are generally deleterious (Jeschke, Bäuml *et al.* 1994; Watling and Press 1998; Hwangbo, Seel *et al.* 2003; Meinzer, Woodruff *et al.* 2004). For example, photosynthesis of *Sorghum bicolor* cultivar CSH-1 was more severely affected by the root hemiparasite *Striga hermonthica* than the more tolerant variety Ochuti (Frost, Gurney *et al.* 1997). The decline in photosynthesis is often caused by hosts closing their stomata (Frost, Gurney *et al.* 1997). This response may be due to increases in host ABA levels resulting from localized water removal by the parasite, and/or a wounding response to infection (Frost, Gurney *et al.* 1997; Chen, Shen *et al.* 2011). Declines in host photosynthesis may also be due to infection effects on Rubisco and/or chlorophyll content (Johnson and Choinski 1993; Shen, Hong *et al.* 2011).

Parasitic plants can also affect host photosystem II (PSII) efficiency, and thus light use (Gurney, Taylor *et al.* 2002; Cameron, Geniez *et al.* 2008; Rodenburg, Bastiaans *et al.* 2008). PSII efficiency declines when plants are exposed to excess photosynthetically active light, and photodamage can occur if exposure to excess absorbed light is prolonged. Excess photosynthetic light occurs when the ratio of photosynthetic photon flux density (PPFD) to photosynthesis is high, which can occur when PPFD increases or when photosynthesis decreases at a constant PPFD (e.g. as a consequence of infection by hemiparasites) (Demmig-

Adams and Adams III 1992). Thus, even in low light if photosynthesis decreases absorbed light energy may become excessive. However, plants can harmlessly dissipate excess excitation energy as heat via engagement of photoprotective xanthophyll cycles involving either violaxanthin (V), antheraxanthin (A) and zeaxanthin (Z; the VAZ cycle) (Demmig-Adams and Adams III 1992) or lutein (L) and lutein epoxide (Lx; the lutein epoxide cycle) (Bungard, Ruban *et al.* 1999; García-Plazaola, Hernández *et al.* 2003; Matsubara, Morosinotto *et al.* 2003; García-Plazaola, Matsubara *et al.* 2007). While the VAZ cycle is ubiquitous the Lx cycle is found in many, but not all, plant species and plants growing in low light tend to have more Lx cycle activity than those in growing in high light (see García-Plazaola, Matsubara *et al.* 2007; Matsubara, Krause *et al.* 2009; Matsubara, Förster *et al.* 2012; Nichol, Pieruschka *et al.* 2012). Both these cycles allow the light harvesting complexes (LHCs) to harvest light efficiently when light levels are low (using V and Lx) but quench excess energy (using Z and L) if absorbed light becomes excessive (Matsubara, Naumann *et al.* 2005; Pascal, Liu *et al.* 2005; Nilkens, Kress *et al.* 2010; Horton 2012). If for some reason the photoprotective capacity of a plant is insufficient to cope with excess absorbed light, then chlorophyll may become over-excited, enter its triplet state and promote formation of oxygen radicals (Logan 2008). These radicals can damage DNA, lipids and proteins (Lambeth 2004) such as the D1 protein of PSII and or inhibit its repair (Horton, Ruban *et al.* 1996; Takahashi and Badger 2011). Such photodamage resulting from infection may result in significant reductions in plant growth in the field (Gurney, Taylor *et al.* 2002).

Sustained photoprotection due to constitutive engagement of the xanthophyll cycle and/or photodamage can be detected as chronic suppression of PSII efficiency, often measured by chlorophyll fluorescence as decreases in pre-dawn maximum quantum yields (F_v/F_m) (Maxwell and Johnson 2000; Demmig-Adams and Adams III 2006). The ability of the host to provide sufficient photoprotection via the xanthophyll cycle could be critical for preventing photodamage resulting from parasite effects on photosynthesis. However, there have been no studies evaluating infection effects on these pigment dynamics of hosts (Watling and Press 2001). Further, there have been no investigations of the above in differing light conditions which would be frequently encountered by plants in the field. It is important to quantify these mechanisms and processes as they may help explain why some native hosts display tolerance to infection with native parasites.

Here, our study explored the effects of infection by the stem hemiparasite *Cassytha pubescens* on *Leptospermum myrsinoides* when grown in either high (HL) or low light (LL). Previous work by Cirocco, Facelli *et al.* (submitted) found that midday electron transport rates of *L. myrsinoides* were affected by *C. pubescens* in HL but not LL. Thus, it was expected that infected *L. myrsinoides* grown in HL would have the highest xanthophyll cycle capacity and engagement in order to avoid photodamage as a consequence of exposure to excess absorbed light. Pigment composition (including, xanthophyll cycle capacity and engagement) and susceptibility to photodamage of *L. myrsinoides* were assessed. They were also measured for the parasite as a means of investigating its performance in HL and LL. This is of interest because many parasitic plants have an active Lx cycle in the shade (Matsubara, Förster *et al.* 2012) and in general, have low photosynthetic capacities and tend to have lower quantum yields than non-parasitic plants (Strong, Bannister *et al.* 2000; Matsubara, Gilmore *et al.* 2002).

Materials and methods

Study species

Leptospermum myrsinoides Schltdl. (Myrtaceae) is a native Australian perennial shrub that reaches 1–2 m in height (Harden 1991). Also native to Australia *Cassytha pubescens* R. Br. (Lauraceae) is a coiling, perennial hemiparasitic vine 0.5–1.5 mm in diameter that has no true leaves but does have photosynthetic stems that attach to host stems and leaves via multiple haustoria (McLuckie 1924). Both species are widespread in the Mount Lofty Ranges (South Australia) where *C. pubescens* is frequently found infecting this host (Prider, Watling *et al.* 2009).

Plant material and growth conditions

Ten month old nursery tubed *L. myrsinoides* were transplanted into 140 mm pots (1 plant per pot) containing sandy/loam (60/40 v/v) in early May 2010. They were provided with liquid fertiliser (Nitrosol; Rural Research Ltd, Auckland, New Zealand; NPK 8:3:6) in accordance with manufacturer's directions. Four months later they were re-potted (1 plant per pot) into 200 mm pots of sandy loam (60/40 v/v) and supplied with slow release fertiliser (Osmocote; Scotts-Sierra Horticultural Products, Marysville, OH, USA) at the recommended dosage for the remainder of the experiment. Synchronous infection of randomly selected *L. myrsinoides* with *C. pubescens* was achieved following the technique of Shen, Prider *et al.* (2010).

Briefly, *C. pubescens* already established on *Ulex europaeus* L. (gorse) was allowed to attach

to and infect stems of experimental hosts. Three months later, stems of *C. pubescens* attached to the newly infected study species were severed from the gorse donor plant. Plants were monitored for a further week to ensure that *C. pubescens* had successfully established on the new hosts.

Infected and uninfected *L. myrsinoides* were randomly allocated to two light treatments, HL or LL, and two blocks. Each block was on a separate bench in the same glasshouse and contained 4–5 uninfected and 4–5 infected HL or LL plants. HL plants were grown in ambient light conditions. Adjacent, LL plants were housed in a 2 (H) x 1.45 (D) x 1.2 (W) m frame completely covered by black neutral density shade cloth (~35% light penetration, which is similar to understorey light conditions within the host's and parasite's natural range). Plants were re-randomised fortnightly to account for small light differences within the glasshouse. Treatments ran from mid-January 2011 to April 2011. Plants were well watered throughout the experiment and grown in an evaporatively cooled glasshouse (Thermostat: 26 °C) at the University of Adelaide. *In situ* midday summer and autumn mean PPFDs ($\mu\text{mol quanta m}^{-2} \text{s}^{-1}$) in HL were 1670 ± 127 and 1182 ± 66 , respectively. In LL they were 591 ± 8 and 351 ± 22 , respectively (LI-190SA quantum sensor; LI-1400 datalogger, LI-COR, Lincoln NEB.).

Pigment content

Three green *L. myrsinoides* leaves per plant (including one used for chlorophyll fluorescence measurements) and 6 cm of *C. pubescens* (taken 15 cm from the growing tip) were collected 76 and 86 days after treatments had been imposed (DAT), respectively. Plant material was collected at pre-dawn and midday on a sunny day in early April 2011, placed in foil and immediately frozen in liquid nitrogen. Samples were then stored at -80°C . Five weeks after collection they were transported to the University of Wollongong on dry ice which took less than 24 hrs. On arrival at Wollongong they were again stored at -80°C until used for pigment analysis.

Photosynthetic and photoprotective pigments were extracted according to the method of Förster, Osmond *et al.* (2009). Pigments in extracts were separated and quantified using high pressure liquid chromatography according to Miller, Watling *et al.* (2009) for *L. myrsinoides* leaves, and Förster, Osmond *et al.* (2009) for *C. pubescens* stem. Xanthophyll cycle (VAZ)

activity is expressed as de-epoxidation state $[(A+Z)/(V+A+Z)]$, and Lx cycle activity as L, and Lx per unit of total chlorophyll (Lx/Chl). Total carotenoids (Car) represent: VAZ, L, Lx (if present), neoxanthin and β -carotene (no α -carotene detected in either species).

Chlorophyll fluorescence

Chlorophyll *a* fluorescence was measured with a portable pulse-modulated chlorophyll fluorometer (Mini-PAM, Walz, Effeltrich, Germany) fitted with a leaf-clip (2030-B, Walz, Effeltrich, Germany). Maximum quantum yield (F_v/F_m) was recorded after dark-recovery overnight. F_v (variable fluorescence) is the difference between maximal (F_m : all PSII reaction centres closed) and minimal (F_o : all PSII reaction centres open) fluorescence of a dark adapted sample. The quantum yield in the light (Φ_{PSII}) is calculated as $\Delta F/F_m'$, where ΔF is the increase in fluorescence yield due to a saturating pulse, and measures the efficiency of PSII photochemistry (Genty, Briantais *et al.* 1989; Maxwell and Johnson 2000; Klughammer and Schreiber 2008). Pre-dawn (F_v/F_m) and midday quantum yields (Φ_{PSII}) (Maxwell and Johnson 2000) were measured on a single leaf per plant of *L. myrsinoides* and 15 cm from the growing tip of *C. pubescens*. Measurements were made on *L. myrsinoides* and *C. pubescens* 76 and 86 DAT, respectively. Mean midday PPFD values in HL and LL for *L. myrsinoides* at the time of measurement were 1188 ± 4 and 341 ± 5 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$, respectively ($n = 18-20$). For *C. pubescens* in HL and LL they were 933 ± 67 and 292 ± 4 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$, respectively ($n = 5$).

Data analysis

The variances of the data were homogeneous and a standard least squares model was implemented to detect treatment differences for all parameters. A Tukey-Kramer HSD *post hoc* analysis was used for pairwise comparisons where interactions between light x infection were significant. Where this was not the case, significant additive infection effects (HL and LL plants pooled) and significant additive light effects (uninfected and infected plants pooled) were considered. All data were analysed with the software JMP Ver. 4.0.3 (SAS Institute Inc., 2000) and $\alpha = 0.05$.

Results

Leptospermum myrsinoides

Pigment composition

There were no light x infection interactions for pigment concentrations of *L. myrsinoides* (Table 1). On average, infection had a significant impact on total xanthophyll cycle pool (VAZ), chlorophyll (Chl), carotenoids (Car), lutein (L) and on Chl *a*, regardless of light treatment (Table 1). As a result of infection, VAZ and Chl decreased by 17% and 14%, respectively (Table 1). Car and L concentrations in infected plants (HL and LL plants pooled) were 12% and 10% less than for uninfected plants (HL and LL plants pooled), respectively (Table 1). Chl *a* decreased by 14% in response to infection (Table 1). Chl *b* was the only pigment affected by light (Table 1). On average, Chl *b* of HL plants (uninfected and infected plants pooled) was 16% less compared with that of LL plants (uninfected and infected plants pooled). In contrast to pigment concentrations, there was a significant interaction between light x infection for Chl *a/b* ratio (Table 1). In HL, Chl *a/b* was unaffected by *C. pubescens* whereas in LL, it significantly decreased in response to infection (Table 1). This decrease was driven by a strong decline in Chl *a* relative to Chl *b* in response to infection (Table 1).

Photoprotective capacity and xanthophyll cycle engagement

There was no light x infection interaction or independent effect of infection on VAZ/Chl of *L. myrsinoides*, but this parameter was affected by light (Fig. 1a, b). On average, VAZ/Chl of HL plants (uninfected and infected plants pooled) was 8% higher than that of LL plants (uninfected and infected plants pooled) (Fig. 1b). By contrast, light did interact with infection for Car/Chl (Fig. 1c). In HL, Car/Chl was unaffected by *C. pubescens* whereas in LL it significantly increased in response to infection (Fig. 1c).

There was no interactive effect of light x infection or independent infection effect on de-epoxidation state $[(A+Z)/(V+A+Z)]$ but this parameter was significantly affected by light at both pre-dawn and midday (Fig. 2). Pre-dawn de-epoxidation state of HL plants (uninfected and infected plants pooled) was more than an order of magnitude higher than that of LL plants (uninfected and infected plants pooled) (Fig. 2c). Midday de-epoxidation state of plants in HL was 71% higher relative to that of LL plants, regardless of infection status (Fig. 2d).

PSII efficiency

There was no significant light x infection effect on pre-dawn quantum yield (F_v/F_m) of *L. myrsinoides*. There was also no infection effect on F_v/F_m however, there was a significant, but small light effect (Fig. 3a, c). On average, F_v/F_m of HL plants was 3% lower than that of

LL plants, regardless of their infection status (Fig. 3c). By contrast, there was a significant light x infection interaction for midday quantum yield (Φ_{PSII}) (Fig. 3b). Φ_{PSII} of HL infected plants was 38% less than that of uninfected plants, whereas in LL it was 12% higher for infected compared with uninfected plants; although the difference in LL plants was not significant (Fig. 3b).

Cassytha pubescens

Pigments and chlorophyll fluorescence

There were no significant light effects on pigment composition of *C. pubescens* except for VAZ which was only just significant (Table 2). VAZ of the parasite in HL was 38% higher compared with that in LL (Table 2). Light had a significant effect on VAZ/Chl but not on Car/Chl or Lx/Chl (Fig. 4). VAZ/Chl of *C. pubescens* in HL was 42% higher than that in LL (Fig. 4a).

Light had no effect on the pre-dawn de-epoxidation state of *C. pubescens* but did significantly affect it at midday (Fig. 5a). At midday, de-epoxidation state of HL was 34% higher than it was in LL *C. pubescens* (Fig. 5a). Lx/Chl at both pre-dawn and midday was unaffected by light (Fig. 5b). Light also had no significant influence on either F_v/F_m or Φ_{PSII} of the parasite (Fig. 6).

Discussion

Our study investigated pigment composition and susceptibility to photodamage in *L. myrsinoides* in response to infection with *C. pubescens* in both HL and LL. The data clearly demonstrated that while foliar pigment content of *L. myrsinoides* strongly decreased in response to infection, there was no significant impact on photoprotective capacity/engagement or susceptibility to photodamage in this host.

Impacts of infection and light on L. myrsinoides pigment composition

Previous studies have found that host pigment concentrations can increase (Frost, Gurney *et al.* 1997), remain unchanged (Watling and Press 1997; Gurney, Taylor *et al.* 2002; Logan, Huhn *et al.* 2002) or decrease (Johnson and Choiniski 1993; Cameron, Geniez *et al.* 2008; Mauromicale, Lo Monaco *et al.* 2008; Shen, Xu *et al.* 2013) in response to infection. Our study found that *C. pubescens* had a strong effect on foliar content of all pigments in *L.*

myrsinoides except Chl *b* (Table 1). By contrast, Shen, Prider *et al.* (2010) found that total chlorophyll of *Cytisus scoparius* stems was unaffected by *C. pubescens*. In a study by Logan, Huhn *et al.* (2002) there was also no effect of infection by *Arceuthobium pusillum* on pigment content of *Picea glauca* needles. This may be due to a strong decrease in needle size resulting from infection, which could have concentrated pigments to similar values as those for uninfected plants with larger needles. Similarly, in another study, leaf area of *L. myrsinoides* did not change in response to infection by *C. pubescens* (Cirocco *et al.* submitted), and thus changes in pigment content in the current study are unlikely to be due to changes in leaf area. As nitrogen is critical for their synthesis, the strong decrease in pigment content of *L. myrsinoides* observed here may be due to removal of this resource by the parasite. In a preliminary study, foliar nitrogen concentration of this host was found to be significantly affected by *C. pubescens* (data not shown). Similar examples of host nitrogen levels strongly decreasing in response to infection by parasitic plants are well represented in the literature (Watling and Press 2000; Hwangbo, Seel *et al.* 2003; Meinzer, Woodruff *et al.* 2004; Shen, Xu *et al.* 2013).

Interactively, Chl *a/b* ratio of HL plants was unaffected by *C. pubescens* whereas that of LL plants decreased in response to infection (Table 1). In contrast, Shen, Prider *et al.* (2010) found that Chl *a/b* ratio of *C. scoparius* stems increased in response to infection with *C. pubescens* under ambient light. Most other studies have reported no effect of parasitism on host Chl *a/b* ratio (Cechin and Press 1994; Hibberd, Quick *et al.* 1996; Jeschke, Baig *et al.* 1997; Logan, Huhn *et al.* 2002; Reblin, Logan *et al.* 2006; Cameron, Geniez *et al.* 2008; Shen, Hong *et al.* 2011; Shen, Xu *et al.* 2013). In our study, both Chl *a* and Chl *b* declined to a similar degree in the infected plants in HL, whereas in LL, there was a strong decrease in Chl *a* but not Chl *b* as a result of infection, causing the significant decline in Chl *a/b* for these plants. This enhanced shade response to infection in LL plants might possibly be due to additional shading by the parasite. *Cassytha pubescens* is a stem hemiparasitic vine that can grow over the host canopy and, if that growth is extensive it can limit light penetration to the host; although this doesn't seem to have occurred for the HL plants. The Chl *a/b* ratio data indicate that infected plants in LL favoured production of LHCs over reaction centres which would improve light energy capture (Lichtenthaler 2007).

Photoprotection in L. myrsinoides

The xanthophyll cycle protects plants from excess light by dissipating that light safely as heat before it reaches PSII reaction centres (Horton 2012). As light has to pass through chlorophyll pigments to be used in photochemistry, it is more physiologically meaningful to consider the amount of xanthophyll pigment relative to chlorophyll (VAZ/Chl) than to use the absolute amount of VAZ as an indicator of photoprotective capacity. Here, VAZ decreased in parallel with Chl in response to infection (Table 1). Thus, infection had no effect on the photoprotective capacity of the xanthophyll cycle in *L. myrsinoides* (Fig. 1). Although there are no other reports for parasite effects on host xanthophyll cycle capacity, similar concurrent decreases in VAZ and Chl in response to low relative to high nitrogen supply have been reported for *Spinacia oleracea* and *Clematis vitalba* (Bungard, McNeil *et al.* 1997; Logan, Demmig-Adams *et al.* 1999). We also found no interactive or infection effect on de-epoxidation state of *L. myrsinoides* in HL or LL at either pre-dawn or midday (Fig. 2). Interestingly, de-epoxidation state of *C. vitalba* was unaffected by nitrogen whereas that of *S. oleracea* strongly increased in response to low versus high nitrogen supply (Bungard, McNeil *et al.* 1997; Logan, Demmig-Adams *et al.* 1999). Effectively, our VAZ/Chl and de-epoxidation state results indicate that both uninfected and infected plants had the same potential for xanthophyll mediated photoprotection against excess excitation energy that could excitate triplet state chlorophyll and or singlet oxygen (Faria, Silvério *et al.* 1998; see Logan, Demmig-Adams *et al.* 1999).

Given that infection can have a strong effect on host photosynthesis, it might still be expected that infected plants would be more susceptible to photodamage despite the lack of any impact of infection on VAZ/Chl or de-epoxidation state. Infection having an effect on Φ_{PSII} at midday in HL infected plants (Fig. 3b) is consistent with them having lower rates of photosynthesis than uninfected plants. Despite this however, there was no effect of infection on pre-dawn F_v/F_m for either HL or LL plants. A previous field study also found no infection effect on F_v/F_m for *L. myrsinoides* and the introduced host *C. scoparius* (Prider, Watling *et al.* 2009). However, Shen, Prider *et al.* (2010) found that F_v/F_m of *C. scoparius* in the glasshouse was severely affected by infection with *C. pubescens*. They suggested that this host may not have adequate photoprotective capacity to cope with excess absorbed light resulting from the stress of infection. Our results suggest that *L. myrsinoides* whether in HL or LL was not becoming photodamaged (F_v/F_m data) as a result of infection. Thus, this native host appears to have adequate photoprotection (VAZ/Chl and de-epoxidation state data) to prevent damage from excess absorbed light regardless of infection. This may partly explain the lack of any

infection effect on growth of this host in both low and high light (Cirocco, Facelli *et al.* submitted).

There was a small, but significant effect of light on VAZ/Chl with LL plants having somewhat lower values than HL plants, although this was more evident in uninfected *L. myrsinoides* (Fig. 1a, b). In contrast to VAZ/Chl, there was an interactive effect of light x infection on Car/Chl for *L. myrsinoides*, with a significant increase in response to infection but only in LL plants (largely driven by increases in L/Chl, data not shown). Lutein made up the largest proportion of the carotenoid pool in *L. myrsinoides* followed by VAZ with the remainder comprising neoxanthin and β -carotene. An increase in Car/Chl could improve light energy capture which is consistent with the strategy of these plants decreasing their Chl *a/b* ratio. Also, these carotenoid increases, particularly L and neoxanthin would help quench triplet state chlorophylls while not compromising yield (Pascal, Liu *et al.* 2005; Ruban, Berera *et al.* 2007). β -carotene is proposed to quench singlet oxygen (see Telfer 2005) and may also afford more protection against excitation energy and photodamage. It is interesting that infection by aphids (Phylloxera) also elicited an increase in Car/Chl in two grape vine species in the field (Blanchfield, Robinson *et al.* 2006), presumably due to host water stress. Studies have also found that increases in Car/Chl can occur in response to nitrogen and other nutrient deficiencies (e.g. iron, potassium, sulphur and magnesium) (Kumar Tewari, Kumar *et al.* 2004; Morales, Abadía *et al.* 2006). For example, Logan, Demmig-Adams *et al.* (1999) found that *S. oleracea* significantly increased lutein, neoxanthin and had slightly elevated VAZ on a chlorophyll basis, in response to nitrogen limitation. Hence, the increase in Car/Chl in *L. myrsinoides* in response to infection in LL might also be due to increased parasite removal of nutrients in these conditions. The maintenance of host yield in these conditions versus HL may be evidence that the parasite acts as an additional sink for carbohydrate and possibly other resources in LL on account of its own photosynthesis being limited.

Parasite (C. pubescens) pigments

VAZ of *C. pubescens* was higher (38%) in HL compared with LL. There was also more Chl *a* and Chl *b*, but a lower Chl *a/b* ratio for *C. pubescens* in LL versus HL. Although not significant these findings are consistent with other studies on various mistletoes (Strong, Bannister *et al.* 2000; Matsubara, Gilmore *et al.* 2001; Matsubara, Gilmore *et al.* 2002) and if

the experiment ran for longer a stronger decrease in the Chl *a/b* ratio of the parasite in response to LL might have been observed.

Parasite photoprotection and PSII efficiency

VAZ/Chl of *C. pubescens* in HL was significantly higher than that in LL, which is consistent with findings for the mistletoe *A. miquelii* (Matsubara, Gilmore *et al.* 2001; Matsubara, Gilmore *et al.* 2002). As expected, the VAZ/Chl data clearly demonstrated that *C. pubescens* in HL had a greater photoprotective capacity than in LL. Further, the midday de-epoxidation state of *C. pubescens* was much greater in HL versus LL. Similarly, de-epoxidation state of *A. miquelii* was also found to be higher in sun compared with shade leaves at 0800 hrs and from June through to September (Matsubara, Gilmore *et al.* 2001; Matsubara, Gilmore *et al.* 2002). The midday de-epoxidation state data indicate that *C. pubescens* in HL had greater engagement of the xanthophyll cycle relative to LL and may explain why they had a marginally lower Φ_{PSII} as similarly found for *A. miquelii* (Matsubara, Gilmore *et al.* 2002). However, the pre-dawn de-epoxidation state of *C. pubescens* in HL versus LL was not statistically different. This suggests there was no sustained overnight retention of zeaxanthin in HL relative to LL and probably explains why F_v/F_m did not differ between light treatments. Matsubara, Gilmore *et al.* (2001) also found that light had no effect on predawn F_v/F_m of *A. miquelii*. Thus, like other plants, *C. pubescens* is able to respond to different light conditions by modifying its pigment composition to reflect the need for photoprotection.

Lutein epoxide cycle operation in C. pubescens

Notably, the Lx cycle was detected in *C. pubescens* as previously found by Close, Davidson *et al.* (2006) but was not evident in the host *L. myrsinoides*. Pre-dawn Lx/Chl in *C. pubescens* was similar in HL and LL. By contrast, Matsubara, Gilmore *et al.* (2001) found that Lx/Chl of *A. miquelii* at pre-dawn in shade leaves was approximately 75% higher than it was in sun leaves. There was a trend for Lx/Chl levels to decline from pre-dawn to midday in LL *C. pubescens* but this was not significant (data not shown). Matsubara, Gilmore *et al.* (2001) found that Lx/Chl in sun and shade leaves of *A. miquelii* from pre-dawn to 0800 hrs declined by around 60% and 40% respectively. Our data indicate that *C. pubescens* whether in HL or LL had similar capacity and engagement of the Lx cycle and potential for excess light dissipation by its operation.

Conclusion

We conclude that *C. pubescens* had a significant effect on foliar pigment concentrations of *L. myrsinoides*. However, this did not result in diminished photoprotective capacity (VAZ/Chl) of the host, as both VAZ and Chl were similarly affected by *C. pubescens* in HL and LL. Further, infection had no effect on engagement of the xanthophyll cycle (de-epoxidation state) whether in HL or LL. Thus, *C. pubescens* had no effect on the ability of *L. myrsinoides* to dissipate excess excitation energy in HL or LL. As a result, even though Φ_{PSII} was affected by infection in HL, *C. pubescens* had no effect on F_v/F_m of the host. Thus, our pigment data can help explain why *L. myrsinoides* did not become photodamaged and shows tolerance to *C. pubescens* in terms of its overall growth in both the glasshouse and the field (Prider, Watling *et al.* 2009; Cirocco, Facelli *et al.* submitted). Similar investigations of pigment dynamics and PSII efficiency of introduced hosts may help explain why they are more severely affected by *C. pubescens* than native hosts such as *L. myrsinoides* (Prider, Watling *et al.* 2009; Shen, Prider *et al.* 2010). The effects of light treatment on both *L. myrsinoides* and *C. pubescens* were similar to those reported by others for a range of plants. In contrast to other plant species, including parasites, we found no evidence of Lx cycle activity for *C. pubescens* in HL or LL or accumulation of Lx in the latter. We also found that the parasite tended to have lower pigment concentrations but similar ratios of VAZ/Chl and Car/Chl to its host.

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Funding

Part funding for this experiment was provided by Nature Foundation SA Inc (60106911).

Acknowledgements

Special thanks to Dr. Jane N. Prider, Hong T. Tsang, Dr. Rebecca E. Miller and A/Prof. Robert J. Reid, the University of Wollongong and the University of Adelaide for all their assistance and support.

Table 1. Foliar content ($\mu\text{mol m}^{-2}$) of xanthophyll pigments (VAZ), total chlorophyll (Chl), total carotenoids (Car), lutein, chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*) and the chlorophyll *a/b* ratio (Chl *a/b*) of *Leptospermum myrsinoides* growing in either high (HL) or low light (LL) and either uninfected (minus) or infected (plus) with *Cassytha pubescens* ($n = 15\text{--}16$). Data are means ($\pm 1\text{SE}$), $df = 1, 58$ for all parameters, *P* values from two-way ANOVA are in bold and letters denote significant differences for significant interactive infection (I) x light (L) effect for Chl *a/b* ratio, independent significant effect ($n = 31\text{--}32$) of infection (I) on VAZ, Chl, Car, lutein and Chl *a* and light (L) effect on Chl *b*.

	VAZ	Chl	Car	Lutein	Chl <i>a</i>	Chl <i>b</i>	Chl <i>a/b</i>
HL-	17 $\pm$ 1	511 $\pm$ 30	76 $\pm$ 4	39 $\pm$ 2	354 $\pm$ 21	157 $\pm$ 10	2.26 $\pm$ 0.06a
HL+	14 $\pm$ 1	448 $\pm$ 26	64 $\pm$ 3	34 $\pm$ 2	309 $\pm$ 18	139 $\pm$ 8	2.23 $\pm$ 0.05ab
LL-	16 $\pm$ 1	558 $\pm$ 35	76 $\pm$ 4	40 $\pm$ 2	375 $\pm$ 26	183 $\pm$ 10	2.03 $\pm$ 0.05b
LL+	14 $\pm$ 1	472 $\pm$ 28	69 $\pm$ 3	37 $\pm$ 2	303 $\pm$ 21	170 $\pm$ 9	1.79 $\pm$ 0.07c
(I x L)	0.492	0.665	0.643	0.533	0.500	0.831	0.032
-	16 $\pm$ 1a	535 $\pm$ 23a	76 $\pm$ 3a	39 $\pm$ 2a	364 $\pm$ 16a	170 $\pm$ 7	-
+	14 $\pm$ 1b	461 $\pm$ 19b	67 $\pm$ 2b	35 $\pm$ 1b	306 $\pm$ 14b	155 $\pm$ 7	-
(I)	0.004	0.015	0.016	0.054	0.009	0.063	
HL	15 $\pm$ 1	481 $\pm$ 21	70 $\pm$ 3	36 $\pm$ 1	332 $\pm$ 14	148 $\pm$ 7a	-
LL	15 $\pm$ 1	515 $\pm$ 23	73 $\pm$ 3	38 $\pm$ 1	339 $\pm$ 17	176 $\pm$ 7b	-
(L)	0.529	0.244	0.508	0.264	0.751	0.001	

Table 2. Stem concentrations ($\mu\text{mol m}^{-2}$) of xanthophyll pigments (VAZ), total chlorophyll (Chl), total carotenoids (Car), lutein, lutein epoxide (Lx), chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), and chlorophyll *a/b* ratio (Chl *a/b*) of *Cassytha pubescens* stems when infecting *Leptospermum myrsinoides* in either high (HL) or low light (LL). Data are means ($\pm 1\text{SE}$), $n = 9$, $df = 1, 15$ for all parameters, *P* values from one-way ANOVA are in bold and letters denote significant light (L) effect for VAZ. Area for the parasite was determined according to the equation for the surface area of a cylinder (not including cylinder ends).

	VAZ	Chl	Car	Lutein	Lx	Chl <i>a</i>	Chl <i>b</i>	Chl <i>a/b</i>
HL	8.46 $\pm$ 1.42a	230 $\pm$ 24	43 $\pm$ 5	23 $\pm$ 3	2.83 $\pm$ 0.49	163 $\pm$ 17	67 $\pm$ 7	2.45 $\pm$ 0.07
LL	5.24 $\pm$ 0.89b	269 $\pm$ 41	42 $\pm$ 6	25 $\pm$ 4	3.19 $\pm$ 0.56	188 $\pm$ 30	81 $\pm$ 12	2.31 $\pm$ 0.06
(L)	0.051	0.507	0.773	0.673	0.785	0.555	0.400	0.146

Fig. 1. Xanthophyll cycle pool per unit chlorophyll (VAZ/Chl) (a) and the total carotenoid pool (Car/Chl) (c) of *Leptospermum myrsinoides* either in high (HL) or low light (LL) and uninfected (open bars) or infected (light grey bars) with *Cassytha pubescens*. Additive light effect on VAZ/Chl (b) of *L. myrsinoides* (dark grey bars are average of uninfected and infected HL plants; black bars are average of uninfected and infected LL plants). Data are means (± 1 SE), $n = 15-16$ (a, c), $n = 31-32$ (b), $df = 1, 58$, letters denote significant differences and P values (two-way ANOVA) for infection (I) $\times$ light (L) interaction, additive I or L effect are included in panels.

Fig. 2. Pre-dawn (a) and midday (b) de-epoxidation state $[(A+Z)/(V+A+Z)]$ of *Leptospermum myrsinoides* grown in either high (HL) or low light (LL) and uninfected (open bars) or infected (light grey bars) with *Cassytha pubescens*. Additive light effect on pre-dawn (c) and midday de-epoxidation state (d) of *L. myrsinoides* (dark grey bars are average of uninfected and infected HL plants, black bars are average of uninfected and infected LL plants). Data are means ($\pm 1SE$), $df = 1, 27$ and $1, 26$ for pre-dawn and midday de-epoxidation state, respectively, $n = 7-8$ (a, b), $n = 16$ (c), $n = 15-16$ (d). Letters denote significant differences and P values (two-way ANOVA) for infection (I) x light (L) interaction, additive I or L effect are included in panels.

Fig. 3. Quantum yield measured at pre-dawn (F_v/F_m) (a) and midday (Φ_{PSII}) (b) for *Leptospermum myrsinoides* grown in either high (HL) or low light (LL) and uninfected (open bars) or infected (light grey bars) with *Cassytha pubescens*. Additive light effect on F_v/F_m (c) of *L. myrsinoides* (dark grey bars are average of uninfected and infected HL plants, black bars are average of uninfected and infected LL plants). Data are means (± 1 SE), $n = 8-10$ (a, b), $n = 18-20$ (c) and $df = 1, 33$. Letters denote significant differences and P values (two-way ANOVA) for infection (I) $\times$ light (L) interaction, additive I or L effect are included in panels.

Fig. 4. VAZ/Chl (a), Car/Chl (b) and Lx/Chl (c) of *Cassytha pubescens* when infecting *Leptospermum myrsinoides* in high (HL) or low light (LL). Data are means (± 1 SE), $n = 9$, $df = 1, 15$ and P values (one-way ANOVA) for light effect are included in panels with letters denoting significant effect

Fig. 5. De-epoxidation state of the xanthophyll (a) and lutein epoxide cycles (b) of *Cassythia pubescens* when infecting *Leptospermum myrsinoides* in high (HL) or low light (LL), at pre-dawn (hatched bars) or at midday (dotted bars). Data are means (± 1 SE) and $n = 4-5$, $df = 1, 6$ and P values (one-way ANOVA) for light effect are included in panels. Different letters denote significant effects for pre dawn (PD, a, b) and midday (MD, m, n), which were analysed separately.

Fig. 6. Quantum yield measured at pre-dawn (F_v/F_m) (a) and midday (Φ_{PSII}) (b) of *Cassytha pubescens* infecting *Leptospermum myrsinoides* in high (HL) or low light (LL). Data are means ($\pm 1SE$), $n = 5$, $df = 1, 7$ and P values (one-way ANOVA) for light effect are included in panels with no significant differences detected.