The influence of abiotic factors on the impact of a native stem hemiparasite on introduced versus native hosts.

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Thesis submitted for the degree of a Doctor of Philosophy

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April 2016

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Abstract

Over 100 years, the impact of parasitic plants on their hosts has been a major and fascinating field of research. Recently, there is evidence for native parasites having a greater effect on growth of introduced compared with native hosts. However, there is little known about the mechanisms behind these differential impacts. Further, there have been surprisingly very few studies in the field in general, that have incorporated the influence of abiotic factors on parasite effects on their hosts. A series of glasshouse studies were conducted to explore these gaps in the literature.

Light experiment (Ch. 2): The influence of high (HL) or low light (LL) on the effects of the Australian native stem hemiparasite *Cassytha pubescens* on the native and introduced perennial evergreen shrubs *Leptospermum myrsinoides* and *Ulex europaeus*, respectively. It was hypothesised that as a result of decreased parasite photosynthesis in LL, *C. pubescens* would become more dependent on host carbon and have a greater effect on host performance (particularly, *U. europaeus*) in these conditions. Parasite photosynthesis was significantly lower in LL relative to HL when infecting either host. However, contrary to my prediction, light was not found to influence the effect of *C. pubescens* on overall growth of these two hosts. Independent of light, the parasite did have a significant negative impact on overall growth of *U. europaeus* but not *L. myrsinoides* and also grew much more vigorously on the introduced host.

Pigments (Ch. 3): The influence of high (HL) or low light (LL) and *C. pubescens* on pigment dynamics and photo-damage of *L. myrsinoides*. It was hypothesised that excess light would occur as a result of infection effects on host photosynthesis in HL and in response; the native host would increase its photo-protective capacity (VAZ/Chl) and engagement (de-epoxidation state) in these conditions. As total xanthophyll (VAZ) and chlorophyll content (Chl) significantly decreased in parallel in response to infection, regardless of light, VAZ/Chl of *L. myrsinoides* was unaffected by *C. pubescens* in either HL or LL. The de-epoxidation state of the host was also unaffected by infection in both HL and LL. Consequently, infected *L. myrsinoides* had the same photo-protective capacity/engagement as uninfected plants and thus, showed no signs of photo-damage.

These findings may explain why this native host shows tolerance to *C. pubescens* both in the light experiment (Ch. 2), and in the field.

Nitrogen experiment (Ch. 4): The influence of nitrogen (N) when supplied (HN) or not (LN) on the effect of *C. pubescens* on two leguminous hosts, (native: *Acacia paradoxa*; introduced: *U. europaeus*). It was hypothesised that the combination of infection along with the added carbon burden of rhizobia at LN would result in *C. pubescens* having a greater effect on hosts in these conditions. Contrary to this prediction, N was not found to influence the effect of the parasite on overall growth of hosts. Similarly, as with the light experiment (Ch. 2), *C. pubescens* had a significant negative effect on total biomass of *U. europaeus* but not that of *A. paradoxa*, regardless of N and also grew significantly greater on the introduced host, irrespective of N. Maximum electron transport rates (ETR_{max}) of *U. europaeus*, but not *A. paradoxa* were also found to be affected by *C. pubescens* which may explain the parasite's negative effect on growth of *U. europaeus*.

Water experiment (Ch. 5): The influence of water on the effect of *C. pubescens* on *U. europaeus*. It was hypothesised that the parasite would grow better and have a greater effect on the host in well-watered (HW) compared with low water (LW) conditions. Again, as with the experiments above, the parasite negatively affected growth of this introduced host, but in contrast, water did influence the effect of the parasite. Supporting this hypothesis, total biomass of *U. europaeus* was affected by *C. pubescens* in both treatments, but more severely in the HW treatment. This greater effect may be explained by the significantly higher photosynthetic performance (F_v/F_m) and increased growth of the parasite in the HW compared with LW. Thus, it seems a more hydrated healthy *C. pubescens* in HW was capable of removing more resources and therefore had a greater effect on growth of *U. europaeus* in these conditions.

These studies have revealed that light and N (specifically when hosts are legumes) may not be important in modulating the effects of stem hemiparasites on their hosts. By contrast, water was an important factor, with the parasite having a more severe effect when the host was well hydrated. It seems that from these experiments, parasite performance is controlled by host supply rather than parasite demand. Such 'fine tuning' between parasite and host has also been reported for the stem holoparasitic vine *Cuscuta*. Nevertheless, studies

looking at the effects of the parasite when these abiotic factors are combined will further clarify potential outcomes of these associations. Results from these experiments also consolidate the idea that native parasites more negatively affect introduced compared with native hosts. Consequently, my data continues to support the potential-use of *C. pubescens* as a native bio-control agent against major introduced weeds in Australia. At the same time, my information adds to the discussion on pre-existing ecological theory; are introduced species successful invaders because their newly encountered enemies lack the effective arsenal. Or are they naïve invaders in the sense that new enemies do have an effective arsenal, my findings support the latter hypothesis.

Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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Acknowledgements

I greatly thank my supervisors Jenny and José who provided me with a sound theoretical and practical scientific framework which I took with both hands. When needed, they questioned my scientific thinking, as I did theirs. Their brilliant guidance will not be forgotten.

Special thanks to Jane Prider who was also my honours supervisor and integral to my development as a scientist. Her previous work on *Cassytha* was critical to the success of my own. The technique she developed for infecting plants with *Cassytha* using 'mother plants' was fundamental to executing my experiments. Indeed, some of her plants already infected with *Cassytha* were used to infect my own, which saved me much time and potential heartache.

I'm very thankful to Steven Tsang and Elizabeth Maciunas who also shared a passion for working on *Cassytha*. They stimulated and shared many discussions with me on scientific thinking and life in general. I couldn't have asked for better compadres.

I'm extremely grateful to Sharon Robinson and Melinda Waterman who provided time and space to teach me the technique of using HPLC. They were a pleasure to work with and our time together showed me how collaboration is the key to success in the scientific world.

I would like to thank Rob Reid for his expert advice on plant physiology. He always had time to clarify and expand my thinking on the subject.

I would also like to thank Steven Delean for his expert advice on statistics. He too always had time for me and my research is much better for it.

Many thanks to Ron Smernik who headed up writing group which I attended for two years. My scientific writing is much improved from his expertise and tuition.

Many thanks also to John Stanley and David Ladd for their help with infrastructural support; they shaped me into a much handier man.

I’m very grateful to Nenah Mackenzie, Mark Rollog, Teresa Fowles (and Waite Analytical) for their expert elemental analysis of my samples.

Many thanks to The Field Naturalists Society of South Australia Inc, Nature Foundation SA and the Native Vegetation Council for seeing merit in and helping fund my research.

I’m so very grateful to Maria Johns, Russ Sinclair (and the National Trust) and Milton Hearn for being so hospitable, allowing and trusting me to work on their sites in the field. This was paramount for the advancement of my research and development as a scientist. They shared my enthusiasm on *Cassytha,* always keen to hear about what I discovered.

I would like to thank my angels, Matthew Pearson and Sonia Croft for assisting me in the field. It takes a special person who will arise at 2 am for someone else’s research so we can arrive in the field to conduct pre-dawn measurements. They too were a pleasure to work with, being there when I needed them the most.

What can I say to my friends and the Terrestrial Plant Ecology Lab group; thanks so much for the great times and many laughs shared.

Finally, thank you to my family for providing me with a life of strong values, traditions, beliefs and support that have help lead me to this path of science that I have chosen.

Chapter 1

The direct impacts of parasitic plants on host performance under various environmental conditions.

Robert M. Cirocco

Introduction

Parasitic plants are herbs, shrubs, trees or even vines (Fig. 1) that, partially or completely, depend on other plants for water, nutrients, and other solutes. They are found in all areas inhabited by higher plants (Press *et al.*, 1999). The parasitic mode of life has evolved independently 12-13 times, and the ca. 4,500 parasitic species are currently known to occur in about 28 families and 280 genera (Westwood *et al.*, 2010; Rubiales and Heide-Jørgensen, 2011; Heide-Jørgensen, 2013). They all connect to their hosts via haustoria, the organs by which they access host resources (Kuijt, 1969) (Fig. 2). Parasitic plants which primarily access resources from the host phloem and have little or no chlorophyll are called holoparasites (Shen *et al.*, 2006). Those which typically access resources from the host xylem and are capable of photosynthesis are termed hemiparasites (Press and Whittaker, 1993). Parasitic plants are also categorised by whether they attach to the roots or stems of their hosts (Press and Graves, 1995). In addition, those that require a host at some stage of their life-cycle such as *Striga* (Orobanchaceae) are termed obligate parasites (Westwood *et al.*, 2010). Those that do not require a host, but will generally infect accessible hosts, which typically increases their fitness are referred to as facultative e.g. *Rhinanthus* (Orobanchaceae) (Seel and Press, 1993).

The total number of potential host species that a specific parasitic plant can infect is termed host range (Musselman and Press, 1995). Most parasitic plants have a broad host range (Pennings and Callaway, 2002) and at the extreme lies the mistletoe *Lysiana exocarpi* (Behr.) Tieghem ssp. *exocarpi* (Loranthaceae) which infects over 100 species from 25 families including other parasitic plants (Downey, 1998). Although less common, some have a very specific host range e.g. *Arceuthobium apachecum* Hawksw. & Wiens (Viscaceae) only infects *Pinus strobiformis* Engelm. (Pinaceae) (Hawksworth and Weins,

1996). This review will focus on the direct impacts parasitic plants have on their potential host(s). In particular, how their effects on host performance are modulated by variables, especially abiotic factors (light, nitrogen and water).

Fig. 1. The stem hemiparasitic vine *Cassytha pubescens* (native to Australia) "prospecting" (arrow) for a potential host. Photo by Robert Michael Cirocco.

Fig. 2. Close up of the haustorium (arrow; which also acts as 2-3 mm scale bar) of *C. pubescens*, attached to the stem of the introduced shrub *Ulex europaeus*. Photo by Casey Lauren O'Brien.

Factors influencing impacts of parasitic plants on their hosts

There have been a multitude of studies investigating the impacts of parasitic plants on host physiology and growth (see reviews by Ameloot *et al.*, 2005; Press and Phoenix, 2005; Shen *et al.*, 2006; Irving and Cameron, 2009; Bell and Adams, 2011). Depending on parasite-host species involved, deleterious infection effects on hosts may be due to resource removal (Jeschke *et al.*, 1994; Hibberd *et al.*, 1998; Mathiasen *et al.*, 2008), decreases in photosynthesis (Watling and Press, 2001; Shen *et al.*, 2007; Mauromicale *et al.*, 2008), as well as perturbations to hormonal balances (Taylor *et al.*, 1996; Frost *et al.*, 1997; Chen *et al.*, 2011).

The impacts of parasitic plants on their hosts vary from negligible (Bowers and Turner, 2001; Ward, 2005; MacRaild *et al.*, 2009) to lethal (Dobbertin and Rigling, 2006; Ejeta, 2006; Mathiasen *et al.*, 2008; Carnegie *et al.*, 2009; Yu *et al.*, 2009), and a number of factors may alter the severity of effect. One of these factors is host species, with the same parasite negatively affecting one host but not another. For example, *Rhinanthus minor* L. negatively affects grasses (Seel and Press, 1996; Cameron *et al.*, 2006; Cameron *et al.*, 2008) but not forbs (Cameron *et al.*, 2006; Cameron *et al.*, 2008). Moreover, *Striga hermonthica* (Del.) Benth. strongly affects growth of some members of the Poaceae e.g. *Sorghum bicolor* (L.) Moench., *S. arundinaceum* (Desv.) Stapf., *Zea mays* L., *Z. mays*-*Tripsacum dactyloides* hybrid, but not *T. dactyloides* (L.) L. (Gurney *et al.*, 2002a; Gurney *et al.*, 2003). One of the reasons for this difference in response between hosts is the effectiveness of the haustorial connection formed by the parasite (Gurney *et al.*, 2003; Cameron *et al.*, 2006; Cameron and Seel, 2007).

When an effective haustorial connection is made, the effect of a single parasite on a specific host species can also be altered by a range of factors. These include proximity of the parasite to the host (Keith *et al.*, 2004), host defoliation (Puustinen and Salonen, 1999a; Van Hoveln *et al.*, 2011), intensity and timing of infection (Gurney *et al.*, 1999; Puustinen and Salonen, 1999b) as well as biotic factors. For example, inoculation of hosts with mycorrhizae has eliminated (Gworgwor and Weber, 2003), enhanced (Stein *et al.*, 2009) or not influenced the negative impact of parasitic plants on host biomass (Davies and Graves, 1998; Salonen *et al.*, 2001). Although there are some studies on the influence of

mycorrhizae on parasitic plant effects on their hosts, to my knowledge, there are none on the influence of rhizobia (i.e. low versus high colonisation) on parasite impacts. This gap needs to be addressed considering many hosts of parasitic plants are leguminous and it is unknown how increasing rhizobial abundance and accompanying carbon cost may alter the outcomes of the association.

The effect of abiotic factors on host/parasite associations

Factors such as CO_2 or phosphorous can interact with impacts of parasitic plants on host performance. For instance, elevated CO_2 may cancel (Dale and Press, 1998), mitigate (Watling and Press, 2000) or have no influence on the impact of parasitic plants on host growth (Watling and Press, 1997, 1998; Hwangbo *et al.*, 2003). One of the few studies on phosphorous found that high supply helped alleviate the effect of *R. minor* on *Lolium perenne* L. (Poaceae) by enhancing host growth (and consequently its sink strength relative to the parasite) and its root thickness which hindered haustial attachment (Davies and Graves, 2000). Light, nitrogen and water availability can also influence the effect of parasitic plants on their hosts.

Light availability

Light is essential for photosynthesis and changes in its availability may be particularly pertinent for associations involving hemiparasites as they are capable of photosynthesis but also known to remove large amounts of carbon from their hosts (Těšitel *et al.*, 2010). Hypothetically, decreases in hemiparasite photosynthesis as a result of low light may result in greater dependency on the host for carbon. This is plausible, considering a pioneering study by Press *et al.* (1987) that compared carbon isotope ratios between the C_3 parasites *S. hermonthica* and *S. asiatica* (L.) Kuntze. and C_4 host *S. bicolor* suggests that when these parasites are immature and less photosynthetically active they have increased demand for host carbohydrate. However, there is very little information on the influence of light on parasite/host associations. In one study, Borowicz and Armstrong (2012) found that light had no influence on the effect of the root hemiparasite *Pedicularis canadensis* L. (Orobanchaceae) on growth of the grass *Andropogon gerardii* Vitman (Poaceae). However, in this study light was not completely controlled for as the host was allowed to grow into full sun through slits made in the shade cloth. This was done to recreate natural

growth conditions as this grass can grow above shaded areas resulting from the presence of other plants in the community. Also, in this study, no physiological measurements were made on either host or parasite under the different light environments. Apart from this very recent study, to my knowledge, there is nothing else in the literature about how light influences the effect of parasitic plants on host growth and physiology including photosynthesis.

Any decreases in host photosynthesis resulting from infection would increase the ratio of photon flux density (PFD) to photosynthesis (even in low light at a constant PFD) which creates conditions of excess absorbed light (Demmig-Adams and Adams III, 1992). Plants can harmlessly dissipate this excess excitation energy as heat via engagement of the xanthophyll cycle which comprises the pigments violaxanthin (V), antheraxanthin (A) and zeaxanthin (Z) (Demmig-Adams and Adams III, 1992). In light, V is converted via A to Z which is the proposed quencher of excess absorbed light (Demmig-Adams and Adams III, 1996). However, if for some reason the xanthophyll capacity (VAZ per unit chlorophyll) and engagement (A+Z/V+A+Z) of a host is insufficient to cope with excess excitation energy (e.g. parasite removes nitrogen which is required for pigment construction), they may become chronically photoinhibited (Horton *et al.*, 1996) (please refer to the introduction of Ch. 3 for a more detailed account). Studies have found that some less tolerant hosts are susceptible to photoinhibition as a consequence of infection with parasites (Gurney *et al.*, 2002b; Mauromicale *et al.*, 2008; Shen *et al.*, 2010). However, I am unaware of any investigations into the effects of parasitic plants on hosts' xanthophyll capacity/engagement (Watling and Press, 2001) let alone how these parameters are impacted by infection under high versus low light availability. Such quantification of host pigment dynamics would be a powerful tool in explaining why some species show no signs of photoinhibition and thus, have the ability to tolerate infection across varying light conditions.

Nitrogen

Nitrogen is critical for plant growth, especially as it is needed to synthesise the pigments and enzymes involved in photosynthesis (Evans, 1989). Thus, its removal by parasites may have devastating consequences for host health. There are numerous reports on nitrogen

relations between parasites and their hosts (e.g. Küppers, 1992; Pate, 2001; Meinzer *et al.*, 2004; Irving and Cameron, 2009; Yu *et al.*, 2009; Bell and Adams, 2011). There have been several studies on the influence of nitrogen on parasite effects on hosts, but to my knowledge, they are limited to only two parasitic plant genera; *Striga* (e.g. Cechin and Press, 1993a, 1994) and *Cuscuta* (Convolvulaceae) (Shen *et al.*, 2013). The influence of nitrogen on associations involving *S. hermonthica* appears more related to its effects on parasite incidence rather than resource-relations between host and parasite *per se* (Farina *et al.*, 1985). In some associations but not others, nitrogen fertilization has been found to suppress infection with *Striga* (Bebawi, 1981; Farina *et al.*, 1985). For example, high nitrogen supply has been found to eliminate the severe effect of *S. hermonthica* on growth of *Sorghum bicolor* cv. CSHI (Cechin and Press, 1993a). This may be attributed to high nitrogen strongly suppressing growth of the parasite, likely due to these conditions inhibiting synthesis or release of germination cues required for successful development of *S. hermonthica* (Cechin and Press, 1993b). Cechin and Press (1994) also found that *S. hermonthica* negatively affected growth of *Oryza sativa* L. (Poaceae), but this affect was less severe at high versus low nitrogen supply. It is not clear to why effects were less severe at high nitrogen for this host as they do not seem related to inhibition of *S. hermonthica* development (but see Jamil *et al.*, 2011). This is because at high nitrogen, parasite biomass per unit biomass of *O. sativa* was double that at low nitrogen (Cechin and Press, 1994). Some field studies have found no influence of nitrogen on *S. hermonthica* impacts on a range of sorghum and maize varieties (Gurney *et al.*, 1995; Aflakpui *et al.*, 1998, 2002, 2005; Sinebo and Drennan, 2001). These authors suggested that the supply of nitrogen may not have been high enough to effectively inhibit emergence and thus, impacts of *S. hermonthica* on its hosts.

Studies on associations involving *Cuscuta campestris* Yuncker-*Mikania micrantha* H.B.K. (Asteraceae), *C. reflexa* Roxb.-*Ricinus communis* L. (Euphorbiaceae) and *C. reflexa*-*Coleus blumei* Benth. (Lamiaceae) have found that both parasite and host growth decline with decreasing nitrogen supply (Jeschke and Hilpert, 1997; Jeschke *et al.*, 1997; Shen *et al.*, 2013). In reference to the first two associations, the parasite was found to have a greater impact on host growth at low relative to high nitrogen treatments (Jeschke and Hilpert, 1997; Shen *et al.*, 2013). This greater impact was attributed to increased resource

removal by the parasite in low versus high nitrogen conditions. By contrast, Jeschke *et al.* (1997) found that *C. reflexa* affected growth of *Coleus blumei* similarly in low and high nitrogen treatments.

For the most part, (albeit for seemingly different reasons) there appears to be a pattern that high nitrogen supply weakens infection effects on host performance irrespective of whether the parasite is root hemiparasitic or stem holoparasitic. To the best of my knowledge, as there have been no studies manipulating nitrogen for root holo- or stem hemiparasitic plants, no generalities can be made with the effects of parasite types studied above. In addition, I am unaware of any field studies where different soils have been used as proxy for the manipulation of nitrogen to assess the influence of this resource on stem hemiparasite effects on their hosts. Furthermore, from my reading, I gathered no information in relation to manipulation of nitrogen supply where the host is a legume (Bell and Adams, 2011), which highlights another major gap in the literature. Legumes are commonly infected by many parasitic plant species (see Matthies, 1996; Ameloot *et al.*, 2005; Mathiasen *et al.*, 2008; Rubiales and Fernández-Aparicio, 2012; Lu *et al.*, 2014) and it is unknown how the added carbohydrate burden associated with rhizobia at low versus high nitrogen supply could influence the effect of the parasite on the host.

Water

Water is vital for plant growth and its removal by parasitic plants may be a key driver of their effects with some infected hosts showing signs of water stress (Taylor *et al.*, 1996; Lei, 1999; Sala *et al.*, 2001; Mathiasen *et al.*, 2008). Moreover, understanding the influence of water on parasite effects on host performance is paramount, being especially pertinent now with climate change where frequency of drought and precipitation is predicted to increase in dry and wet areas of the World, respectively (Dore, 2005). Yet, there have been surprisingly very few studies investigating parasite impacts on their hosts in high versus low water conditions. A study by Inoue *et al.* (2013) found that relative water content, stomatal conductance and photosynthesis of *S. bicolor* were unaffected by *S. hermonthica* regardless of whether the host was subjected to well-watered or droughted conditions. However, water treatments in this study only lasted 1-2 days so it is difficult to make any conclusions regarding their findings. Le *et al.* (2015) found that photosynthesis

of *M. micrantha* was affected by *Cuscuta australis* R. Br. independent of well-watered and droughted conditions (water withheld from plants for one week and measurements made the following week). In contrast, they found that stomatal conductance of the host was more severely affected in the low water treatment. Unfortunately in both these studies no information on host (or parasite) growth in response to water/infection was provided. Conversely, Evans and Borowicz (2013) did not provide any physiological evidence such as water and nutrient relations or photosynthesis for the association between *Cuscuta gronovii* Willd. ex Schult.-*Verbesina alternifolia* (L.) Britton ex Kearney (Asteraceae) but did report the effects of infection and water on host growth. In this study where treatments ran for 32 days they found that *Cuscuta gronovii* grew much better (Evans and Borowicz, 2015) and had a greater negative effect on growth of *Verbesina alternifolia* in well-watered than pulse or continuous drought conditions.

There is a significant gap in the literature with regard to water and parasitic plants for at least four reasons along with the fact that so few species have been studied; 1) it is important to determine if there are general patterns in host responses to infection with various parasites and water. But from the studies above, comparisons cannot confidently be made among hemi and holoparasite effects on host growth or physiology with regard to water availability; 2) as hemiparasites primarily access resources from the xylem of the host, water may be the most important driver that modulates their effects. But again we have no information on how host growth may be affected in response to both hemiparasites and water availability; 3) the absence of this information becomes a fundamental problem when we consider that hemiparasites make up around 90% of the approximately 4,500 species of parasitic plants (Heide-Jørgensen, 2013); 4) to my knowledge in all the years of research on the field of parasitic plants, there are no studies comparing the influence of high versus low water conditions on root holo or stem hemiparasite effects on their hosts. Moreover, I am aware of only one study that used a salinity gradient as a way of investigating the effects of water and infection with a stem hemiparasite. Miller *et al.* (2003) found that pre-dawn water potentials and carbon isotope composition of *Eucalyptus largiflorens* F. Muell. (Myrtaceae) were unaffected by the mistletoe *Amyema miquelii* (Lehm. ex Miq.) Tiegh. (Loranthaceae) across a range of soil salinities.

General knowledge gap for stem hemiparasites

It is understandable why studies on the influence of abiotic factors such as light, nitrogen and water on stem hemiparasite effects are lacking from the literature as the majority of stem hemiparasite species are mistletoes. As mistletoes infect woody perennial hosts (in many instances large shrubs/trees), this makes it very difficult to conduct field manipulations and near impossible to conduct glasshouse experiments where abiotic factors are controlled. Thus, to the best of my knowledge, it is unknown how abiotic factors may modulate the effects of stem hemiparasites on their hosts. Further, as the majority of stem hemiparasite species are mistletoes, there is no information on how stem hemiparasites affect host root growth, because of the difficulties in accessing the root systems of shrubs and trees in the field. These gaps in knowledge need to be filled considering that stem hemiparasites constitute approximately 30% of all known parasitic plant species (Watson, 2001; Heide-Jørgensen, 2013). If we can find a stem hemiparasite that infects smaller shrubs which would be suitable for glasshouse experiments (also where abiotic conditions can be manipulated) this system may offer a gateway to information on the influence of abiotic factors on stem hemiparasites and hemiparasites in general with regard to their impacts on host performance. I accomplished this with my PhD by using the stem hemiparasite *Cassytha pubescens* to fill in the gaps highlighted above.

Cassytha pubescens R. Br. (Lauraceae) is a perennial, stem hemiparasitic coiling vine that accesses resources from the host xylem via multiple haustoria, has indeterminate growth and can infect more than one host at any one time (Fig. 3, McLuckie, 1924). It is native only to Australia (Kokubugata *et al.*, 2012) being found in all states (except Western Australia and the Northern Territory) and in New Zealand (Weber, 1981). In Australia, it infects both native and introduced host species but has been found to have a much greater impact on the introduced host *Cytisus scoparius* L. Link (Fabaceae) compared with the native host *Leptospermum myrsinoides* Schltdl. (Myrtaceae) (Prider *et al.*, 2009). The mechanism(s) and processes behind this differential impact remain unclear, but there is evidence that the haustoria of *C. pubescens* connect more effectively to introduced than native host species (Tsang, 2010). To elucidate other physiological mechanisms, and place findings into a more real world context and fill in the gaps highlighted above my project investigated the impacts of the Australian native stem hemiparasite *Cassytha pubescens* on

native and introduced hosts, and how these effects are modulated by availability of light, nitrogen or water.

Fig. 3. *Cassytha pubescens* (arrow; which also acts as a 2-3 mm scale bar) coiling around the stem of the native host *Leptospermum myrsinoides*. (Note the multiple haustoria). Photo by David Hollingworth.

Overarching aim and objective: By conducting glasshouse experiments I will determine whether light, nitrogen or water influence the effect of *C. pubescens* on its hosts by quantifying a range of physiological and growth measurements of both uninfected and infected hosts and parasite.

Overarching hypothesis: *Cassytha pubescens* would have a negative effect on introduced but not native hosts and abiotic factors would influence these impacts.

Significance

Thus, my project appeals on multiple levels. It will provide information lacking on the influence of abiotic factors on parasitic plant effects on their hosts. This will importantly allow for an appraisal of the fundamental principles that accompany such manipulation and my choice of hosts (e.g. carbon relations between host and parasite in response to limiting light or nitrogen in terms of rhizobial demand for this resource, or hydrologic strategies

employed by the host and parasite under different watering regimes). Also, the results of these experiments (mechanisms and processes) could be used to explain patterns of survival, abundance and distribution of native versus introduced hosts infected with *C. pubescens* in varying environmental field settings. Consequently, my project will generate evidence that could be used to make informed decisions about the potential use of a native parasite as an effective management tool in helping eradicate major introduced weeds in Australia and thus, helping restore native biodiversity and preserve endangered species. Additionally, in terms of ecological invasion theory which has not previously included parasitic plant-host associations, my project will contribute mechanistical knowledge of how the impacts of a native parasitic plant on native versus introduced hosts, fit into the naïve invader, biotic resistance/enemy-release hypotheses which are already lacking in terms of how these outcomes are shaped by abiotic factors (Mack *et al.*, 2000; Maron and Vilà, 2001; Shea and Chesson, 2002; Verhoeven *et al.*, 2009).

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Prologue

Dear Examiner,

Three experimental chapters in this thesis have been published in international journals and the fourth will soon be resubmitted to New Phytologist. Consequently, I have presented these manuscripts (along with supplementary data at the end of each chapter) in the corresponding journal style. Chapter 3 was published without supplementary data so sum of square and *F* values for this experiment are shown in Appendix 1. Tables and graphs are presented at the end of each experimental chapter. I also conducted a field study during my PhD candidature and have included the methods and results sections as Appendix 2. It is closely related to the theme of the thesis, provides external validation for some of my findings in the glasshouse, and also shows that I extended myself and have the ability to perform quality research in the field as well as the glasshouse.

* In reference to Chapter 2 (light experiment) I will say that all my *Cytisus scoparius* (including the 100 or so spares) died from an unidentified pathogen, so the experiment could only be run at that time with the native host. The experiment was repeated the following year using *Ulex europaeus* as this introduced host was not susceptible to the disease. The window for host and parasite pigment analysis which will be described in Chapter 3 was only open for the first experiment (native host-parasite relationship) which is why the same analysis was not carried out for the introduced host-parasite association.

* I will also say that with regard to Chapter 5 (water experiment) another novel native host (*Leptospermum continentale*) was used but did not successfully become infected (resistance?) in the time allocated for this process, so I only have information for *U. europaeus*.

Enjoy!

Chapter 2: Light

FIG. 1a. Light experiments for *Cassytha pubescens* in association with *Leptospermum myrsinoides* (above; Experiment 1, 2011) and *Ulex europaeus* (below; Experiment 2, 2012).

Statement of Authorship

Title of Paper	Does light influence the relationship between a native stem hemiparasite and a native or introduced host?
Publication Status	☑ Published ☐ Accepted for Publication ☐ Submitted for Publication ☐ Unpublished and Unsubmitted work written in manuscript style
Publication Details	Cirocco, R. M., Facelli, J. M., & Watling, J. R. (2016). Does light influence the relationship between a native stem hemiparasite and a native or introduced host? Annals of Botany, mcv193

Principal Author

Name of Principal Author (Candidate)	Robert Cirocco
Contribution to the Paper	Co-conceived and designed the experiment, performed the experiment, analysed and interpreted the data, wrote manuscript and acted as corresponding author.
Overall percentage (%)	80
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.
Signature	Date 26/2/2016

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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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* ($P = 0{\cdot}073$). 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 x 1·5 m deep x 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 ± 66 μmol m^{-2} s^{-1} (±1 s.e.) in Experiment 1 and 1159 ± 11 μmol m^{-2} s^{-1} in Experiment 2. For the LL treatment blocks they were 351 ± 22 μmol m^{-2} s^{-1} in Experiment 1 and 300 ± 65 μmol m^{-2} 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:

ETR = yield x PAR x 0·5 x 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 μmol quanta m^{-2} 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 (μmol m^{-2} s^{-1}) values for ETR measurements for *L. myrsinoides* and *C. pubescens* in HL were 1188 ± 64 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 (μmol m^{-2} 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 $\alpha = 0{\cdot}05$.

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).

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).

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 (1·8 ± 0·08 %) or LL (1·8 ± 0·03 %) (Table 1). By contrast, N concentration of *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 photosynthesis 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 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.

Light and native hemiparasite effects on native and introduced hosts

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.

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 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

Special thanks to Dr Jane N. Prider, Hong T. Tsang, Elizabeth C. Maciunas, Associate Professor Robert J. Reid, Angela Cirocco and Michele Cirocco for all their assistance. This work was supported by the Nature Foundation SA Inc. (60106911) and the Field Naturalists Society of South Australia Lirabenda Endowment Fund (75108097).

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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.

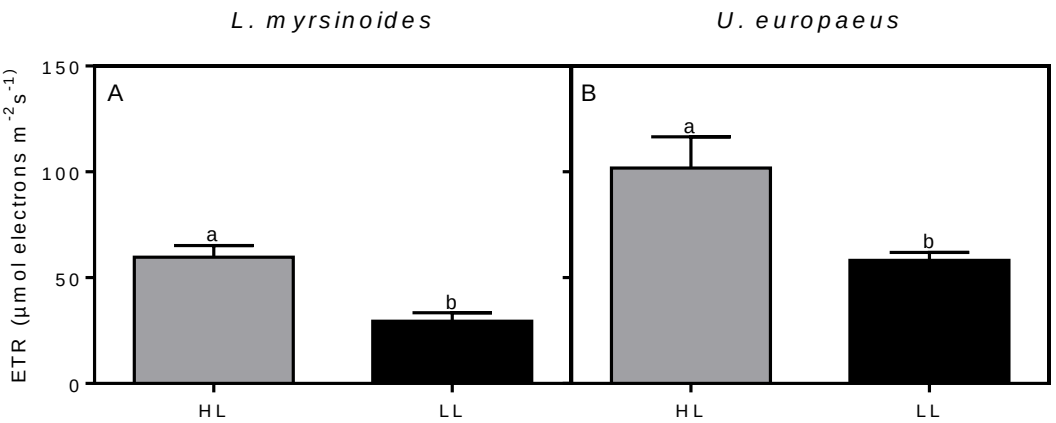

FIG. 1. *In situ* midday electron transport rates (ETRs) of *C. pubescens* growing on *L. myrsinoides* (A) or *U. europaeus* (B) in high (HL, dark grey bars) or low light (LL, black bars). Letters indicate significant differences; bars are means (±1 s.e.) and $n = 5$ (A) and 8 (B).

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 x L	I	L	I x 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.

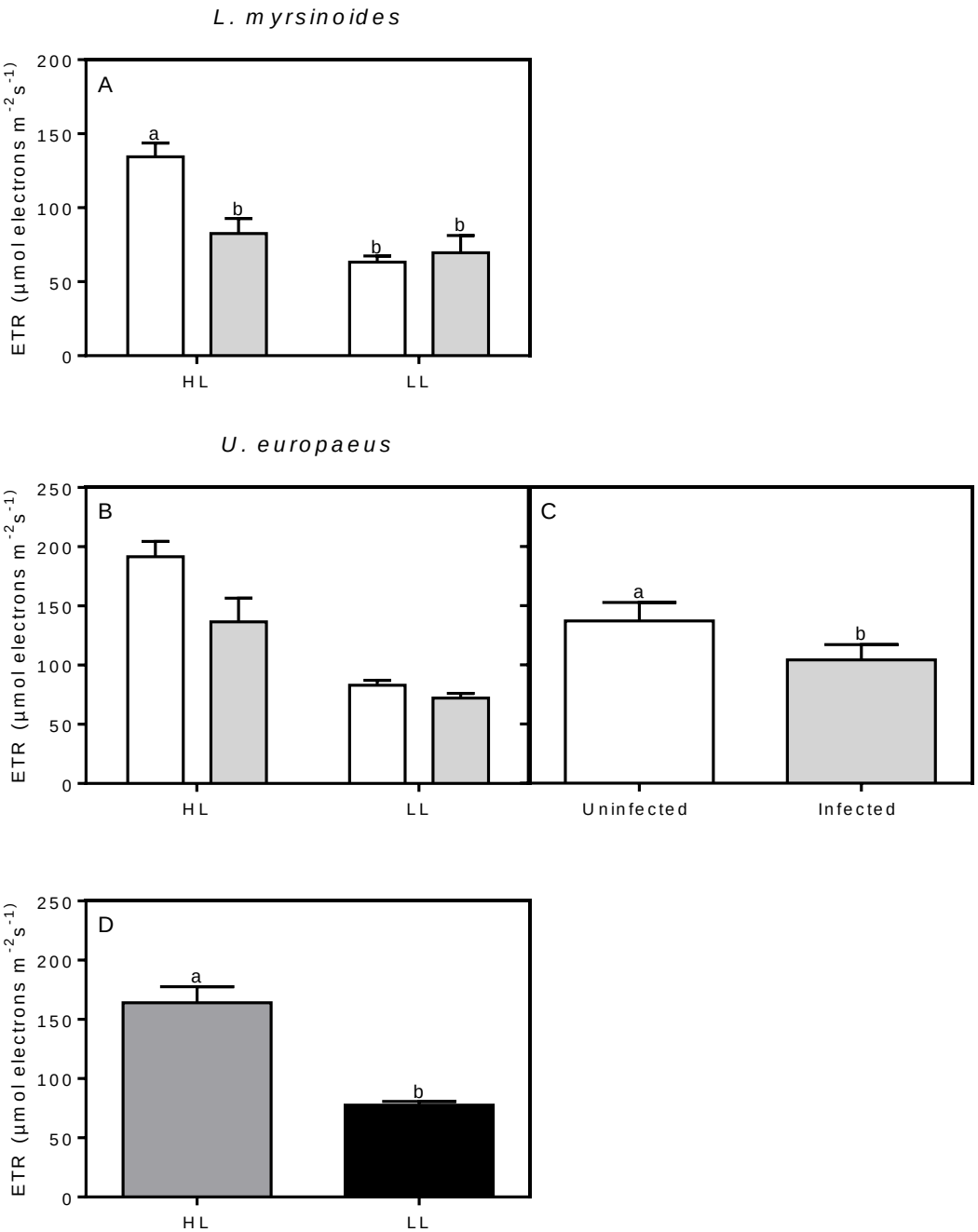

FIG. 2. *In situ* midday electron transport rates (ETRs) of *L. myrsinoides* (A) and *U. europaeus* (B) grown in high (HL) or low light (LL), and uninfected (open bars) or infected (grey bars) with *C. pubescens*. (C) Independent effect of infection on *in situ* midday ETR of *U. europaeus* (open bar, 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 (±1 s.e.) and n = 8–10 (A), 8 (B) and 16 (C, D).

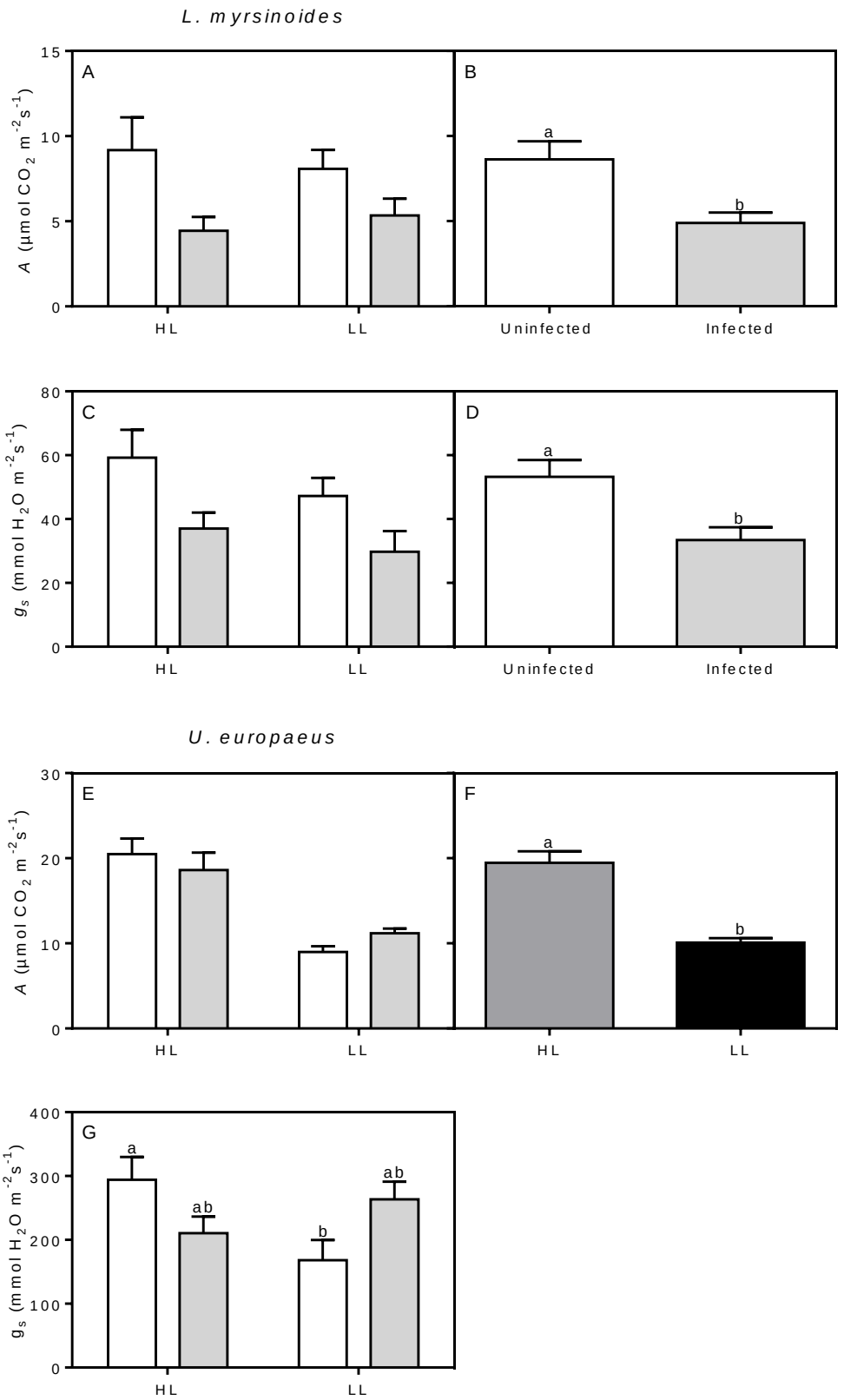
L. myrsinoides
A
B
C
D
E
F
G
A (µmol CO2 m-2s-1)
gs (mmol H2O m-2s-1)
HL
LL
Uninfected
Infected
a
b
ab

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).

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*
	Ψ	Ψ
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 ± 0.05b	−1.05 ± 0.06b

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

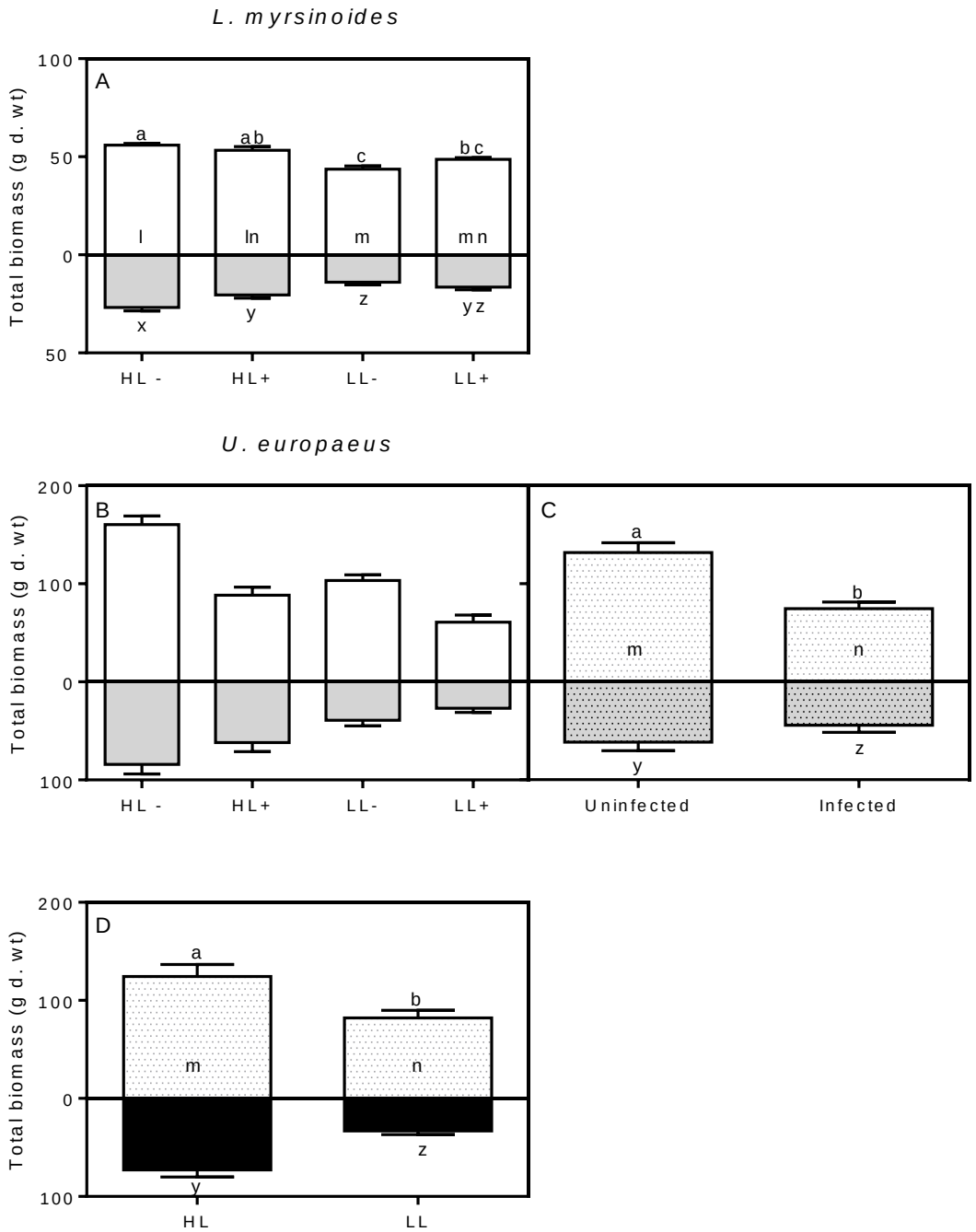

FIG. 4. Total, shoot (open bars) and root (grey bars) biomass of *L. myrsinoides* (A) and *U. europaeus* (B) grown in high (HL) or low light (LL), and uninfected (minus) or infected (plus) with *C. pubescens*. (C) Independent effect of infection on total, shoot (open dotted bar) and root biomass (dotted 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)

Light and native hemiparasite effects on native and introduced hosts

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).

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 ± 0.07ab
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.

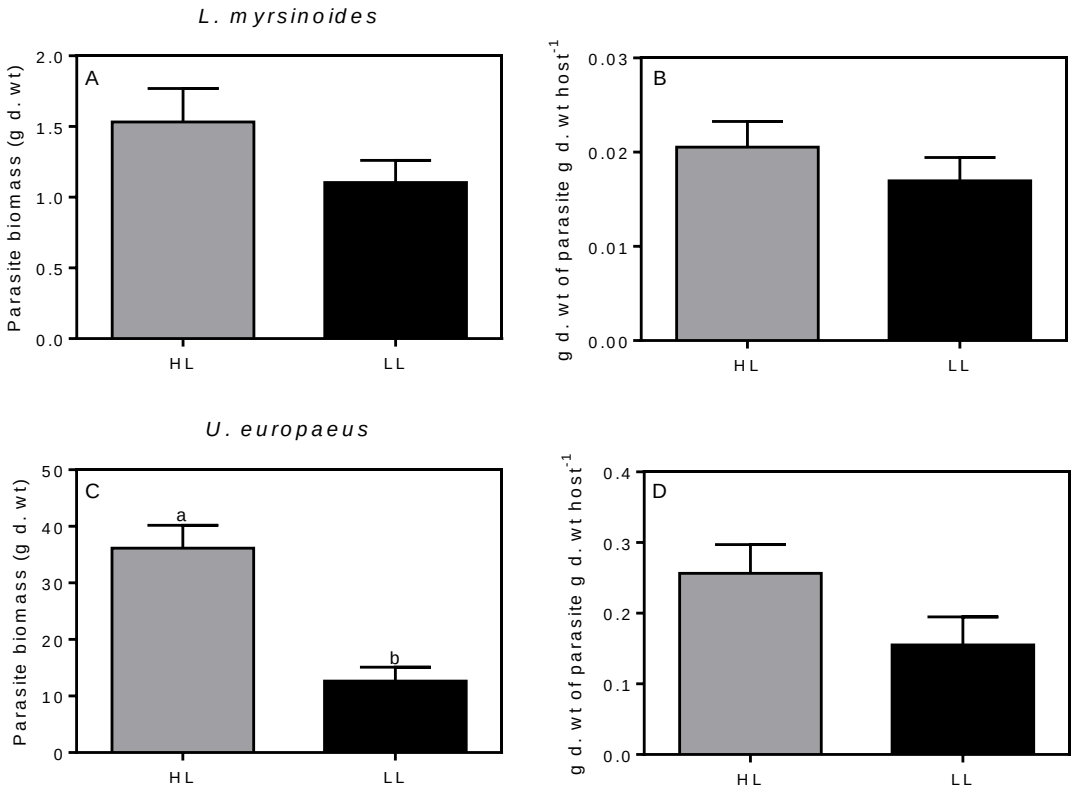

FIG. 5. Total biomass and grams of parasite dry weight per gram of host dry weight, respectively, of *C. pubescens* growing on *L. myrsinoides* (A, B) or *U. europaeus* (C, D) in high (HL, dark grey bars) or low light (LL, black bars). Letters indicate significant differences; bars are means (±1 s.e.)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

Supplementary Data

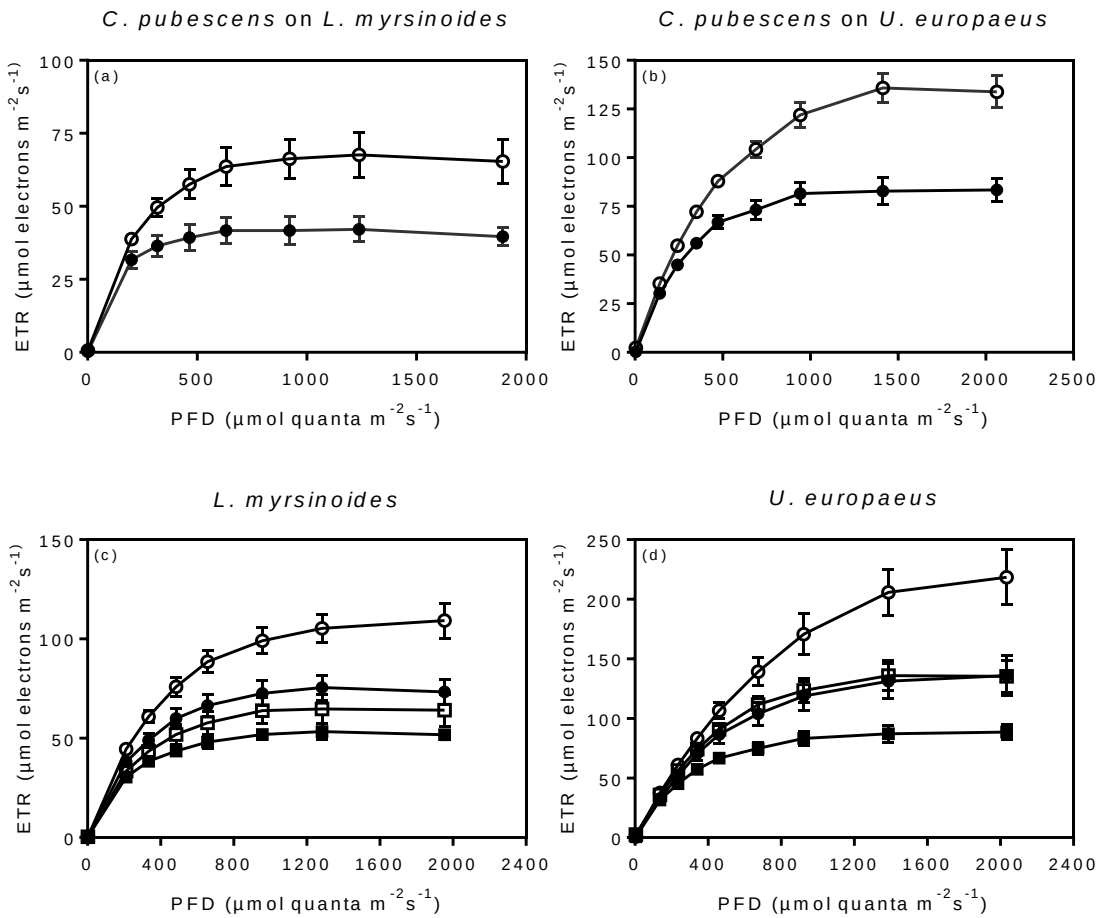

Figure S1. The response to light of ETR (Rapid light response curves) for *Cassytha pubescens* growing on (a) *Leptospermum myrsinoides*, or (b) *Ulex europaeus* in high (HL, open symbols) or low light (LL, closed symbols), and for the hosts (c) *L. myrsinoides* and (d) *U. europaeus* (uninfected are circles, and infected squares), also grown in HL or LL. Data points are means (± 1 SE), and *n*=4–5 (a), *n*=8 (b), *n*=5 (c) and *n*=8 (except HL uninfected *n*=6) (d). Measurements were performed using a MINI-PAM chlorophyll fluorometer on sunny days between 10:00 am–13:00 pm.

Table S1. One-way ANOVA results (*F*, sum of squares (SS) and degrees of freedom (df)), for the effect of light on *C. pubescens* midday electron transport rates (ETR), biomass, biomass per gram host biomass and stem nitrogen concentration (N), when infecting either *L. myrsinoides* or *U. europaeus* (each host species was analysed separately).

Source of variation	**ETR**	**Biomass**	**g dwt of parasite g dwt host^{-1}**	**N**
L. myrsinoides				
F	21.6	2.10	0.846	0.050
SS	2277	0.458	0.099	0.001
Block	1.77	0.407	0.521	0.209
	187	0.089	0.061	0.004
Error (SS)	739	1.53	0.816	0.140
df	1, 7	1, 7	1, 7	1, 7
U. europaeus				
F	8.51	22.5	4.11	29.3
SS	0.0002	1657	1.15	5.47
Block	0.196	0.178	1.41	1.29
	0.000004	13.1	0.394	0.241
Error (SS)	0.0002	664	2.52	1.68
df	1, 13	1, 9	1, 9	1, 9

Table S2. Two-way ANOVA results (*F*: above, and sum of square (SS) values: below, SS only provided for Error) for the effect of *C. pubescens* and light on midday electron transport rates (ETR), photosynthetic rates (*A*), stomatal conductance (g_s) and midday shoot water potentials (Ψ) of *L. myrsinoides* and *U. europaeus* (each species was analysed separately). Infection=I and Light=L; interactive effects=I x L.

Source of variation	**ETR**	***A***	g_s	**Ψ**
L. myrsinoides				
I	6.25	8.43	8.60	0.986
	0.067	69.9	1960	0.039
L	25.3	0.006	2.02	15.4
	0.270	0.050	461	0.611
I x L	13.5	0.603	0.126	4.07
	0.144	5.00	28.8	0.162
Block	0.871	0.898	0.459	1.48
	0.009	7.45	105	0.059
Error	0.352	124	3420	0.755
df	1, 33	1, 15	1, 15	1, 19
U. europaeus				
I	7.17	0.014	0.051	0.212
	8689	0.163	270	0.011
L	49.4	42.7	1.34	126
	59815	511	7166	6.58
I x L	3.23	1.97	8.35	0.840
	3910	23.5	44600	0.044
Block	0.773	0.0002	0.480	0.243
	936	0.002	2564	0.013
Error	32707	215	96124	0.995
df	1, 27	1, 18	1, 18	1, 19

Table S3. Two-way ANOVA results (*F*: above, and sum of square (SS) values: below, SS only provided for Error) for the effect of *C. pubescens* and light on 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). Infection=I and Light=L; interactive effects=I x L.

Source of variation	**Total**	**Shoot**	**Root**	**L/S A**	**S/R**	**N**
L. myrsinoides						
I	0.085	0.684	1.44	0.677	0.800	0.140
	2.87	6.82	18.6	173832	0.173	0.002
L	42.5	35.9	27.7	23.6	12.8	20.1
	1432	358	358	6067946	2.78	0.288
I x L	10.1	7.37	7.56	0.084	2.81	5.03
	341	73.5	97.6	21571	0.608	0.072
Block	0.088	0.005	0.175	0.330	0.004	4.57
	2.96	0.048	2.25	84582	0.001	0.065
Error	506	150	194	3850278	3.25	0.215
df	1, 15	1, 15	1, 15	1, 15	1, 15	1, 15
U. europaeus						
I	31.7	55.4	4.80	27.2	5.23	2.62
	33274	19667	1778	979619	1.67	0.160
L	38.5	30.2	25.7	20.4	12.1	9.29
	40481	10730	9528	736877	3.86	0.568
I x L	2.22	3.66	0.406	1.34	0.009	0.109
	2335	1300	150	48345	0.003	0.007
Block	0.156	0.159	0.076	1.522	0.015	0.029
	164	56.3	28.0	54856	0.005	0.002
Error	19967	6752	7046	684930	6.08	1.10
df	1, 19	1, 19	1, 19	1, 19	1, 19	1, 18

Chapter 3: Pigments

Fig. 1a. Light experiment (2011) for the *Cassytha pubescens-Leptospermum myrsinoides* association. Shade (above) and sun (below) treatments for a single block.

Statement of Authorship

Title of Paper	Native hemiparasite and light effects on photoprotection and photodamage in a native host.
Publication Status	☑ Published ☐ Accepted for Publication ☐ Submitted for Publication ☐ Unpublished and Unsubmitted work written in manuscript style
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, 42(12), 1168-1178.

Principal Author

Name of Principal Author (Candidate)	Robert Cirocco
Contribution to the Paper	Co-conceived and designed the experiment, performed the experiment, analysed and interpreted the data, wrote manuscript and acted as corresponding author.
Overall percentage (%)	75
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.
Signature	Date 26/2/2016

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
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iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Melinda Waterman
Contribution to the Paper	Provided training and expertise to principal author in using high performance liquid chromatography for pigment analysis, and helped with manuscript evaluation
Signature	Date 2/3/2016

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Contribution to the Paper	Supervised development of work, helped in data interpretation and manuscript evaluation
Signature	Date 26/02/2016

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Signature	Date 26/02/2016

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. 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.

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 (Kuijt1969). 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, whereas holoparasites remove these resources and also extract carbohydrate from the host phloem (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 *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* L. was unaffected by the root hemiparasite *Rhinanthus minor* L. (Cameron *et al.* 2008), whereas Shen *et al.* (2005) found that nearly all aboveground biomass of the vine *Mikania micrantha* Kunth died as a result of infection by the stem holoparasite *Cuscuta campestris* Yuncker.

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

Parasitic plants can also affect host PSII efficiency, and thus light use (Gurney *et al.* 2002; Cameron *et al.* 2008; Rodenburg *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 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 1992) or lutein (L) and lutein epoxide (Lx; the lutein epoxide cycle) (Bungard *et al*. 1999; García-Plazaola *et al*. 2003, 2007; Matsubara *et al*. 2003). Although 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 *et al*. 2007; Matsubara *et al*. 2009, 2012; Nichol *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 *et al*. 2005; Pascal *et al*. 2005; Nilkens *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 overexcited, 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 *et al*. 1996; Takahashi and Badger 2011). Such photodamage resulting from infection may result in significant reductions in plant growth in the field (Gurney *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 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 published studies evaluating infection effects on these pigment dynamics of

hosts (Watling and Press 2001). Further, there have been no reported investigations of the above in differing light conditions that 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* R.Br. on *Leptospermum myrsinoides* Schltdl. when grown in either high (HL) or low light (LL). Previous work by R. M. Cirocco, J. M. Facelli, J. R. Watling (unpubl. data) 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 *et al*. 2012) and in general, have low photosynthetic capacities and tend to have lower quantum yields than non-parasitic plants (Strong *et al*. 2000; Matsubara *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 *et al*. 2009).

Plant material and growth conditions

Ten month old nursery tubed *L. myrsinoides* were transplanted into 140 mm pots (one 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, NZ; N:P:K 8:3:6) in accordance with manufacturer's directions. Four months later they were re-potted (one 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 reported by Shen *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 (height) x 1.45 (depth) x 1.2 (width) 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 evapouratively cooled glasshouse (thermostat: 26°C) at the University of Adelaide. *In situ* midday summer and autumn mean PPFDs (μmol quanta m^{-2} 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, NE, USA).

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 (DAT) had been imposed 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 h. 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 by Förster *et al*. (2009). Pigments in extracts were separated and quantified using high pressure liquid chromatography according to Miller *et al*. (2009) for *L. myrsinoides* leaves, and Förster *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). 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_0, 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 *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 μmol quanta m^{-2} s^{-1} respectively (n = 18–20). For *C. pubescens* in HL and LL they were 933 ± 67 and 292 ± 4 μmol quanta m^{-2} 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., Cary, NC, USA) 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 with 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. 1*a*, *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. 1*b*). By contrast, light did interact with infection for Car/Chl (Fig. 1*c*). In HL, Car/Chl was unaffected by *C. pubescens* whereas in LL it significantly increased in response to infection (Fig. 1*c*).

Light and native hemiparasite effects on host pigments

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. 2*c*). Midday de-epoxidation state of plants in HL was 71% higher relative to that of LL plants, regardless of infection status (Fig. 2*d*).

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. 3*a*, *c*). On average, F_v/F_m of HL plants was 3% lower than that of LL plants, regardless of their infection status (Fig. 3*c*). By contrast, there was a significant light x infection interaction for midday quantum yield (Φ_{PSII}) (Fig. 3*b*). 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. 3*b*).

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. 4*a*).

Light had no effect on the pre-dawn de-epoxidation state of *C. pubescens* but did significantly affect it at midday (Fig. 5*a*). At midday, de-epoxidation state of HL was 34% higher than it was in LL *C. pubescens* (Fig. 5*a*). Lx/Chl at both pre-dawn and midday was unaffected by light (Fig. 5*b*). 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 *et al*. 1997), remain unchanged (Watling and Press 1997; Gurney *et al*. 2002; Logan *et al*. 2002) or decrease (Johnson and Choinski 1993; Cameron *et al*. 2008; Mauromicale *et al*. 2008; Shen *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). In contrast, Shen *et al*. (2010) found that total chlorophyll of *Cytisus scoparius* stems was unaffected by *C. pubescens*. In a study by Logan *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* (R. M. Cirocco, J. M. Facelli, J. R. Watling, unpubl. data), 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 *et al*. 2003; Meinzer *et al*. 2004; Shen *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 *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 *et al*. 1996; Jeschke *et al*. 1997; Logan *et al*. 2002; Reblin *et al*. 2006; Cameron *et al*. 2008; Shen *et al*. 2011, 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. *C. 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 *et al*. 1997; Logan *et al*. 1999). Further, we 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). We noted that the 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 *et al*. 1997; Logan *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 promote formation of triplet state chlorophyll and or singlet oxygen (Faria *et al*. 1998; Logan *et al*. 1999).

Light and native hemiparasite effects on host pigments

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. 3*b*) 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 *et al*. 2009). However, Shen *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 (R. M. Cirocco, J. M. Facelli, J. R. Watling, unpubl. data).

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. 1*a*, *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 *et al*. 2005; Ruban *et al*. 2007). β-carotene is proposed to quench singlet oxygen (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 *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 *et al*. 2004; Morales *et al*. 2006). For example, Logan *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 vs HL. Although not significant these findings are consistent with other studies on various mistletoes (Strong *et al*. 2000; Matsubara *et al*. 2001, 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 *et al*. 2001, 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 vs LL. Similarly, de-epoxidation state of *A. miquelii* was also found to be higher in sun compared with shade leaves at 0800 hours and from June through to September (Matsubara *et al*. 2001, 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 *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 *et al*. (2001) also found that light had no effect on pre-dawn 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 *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 *et al*. (2001) found that Lx/Chl of *A. miquelii* at pre-dawn in shade leaves was ~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 *et al*. (2001) found that Lx/Chl in sun and shade leaves of *A. miquelii* from pre-dawn to 0800 h 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 *et al*. 2009; R. M. Cirocco, J. M. Facelli, J. R. Watling, unpubl. data). 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 *et al*. 2009; Shen *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.

Acknowledgements

Special thanks to Dr Jane N Prider, Hong T Tsang, Dr Rebecca E Miller and Associate Professor Robert J Reid, the University of Wollongong and the University of Adelaide for all their assistance and support. Part funding for this experiment was provided by Nature Foundation SA Inc. (60106911).

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Table 1. Foliar content (μmol 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* = 15–16)

Data are means (± s.e.), d.f. = 1, 58 for all parameters, different letters denote significant ($P \leq 0.05$) differences for significant interactive infection (I) x light (L) effect for Chl *a*/*b* ratio, independent significant effect (*n* = 31–32) of infection (I) on VAZ, Chl, Car, lutein and Chl *a* and light (L) effect on Chl *b*

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

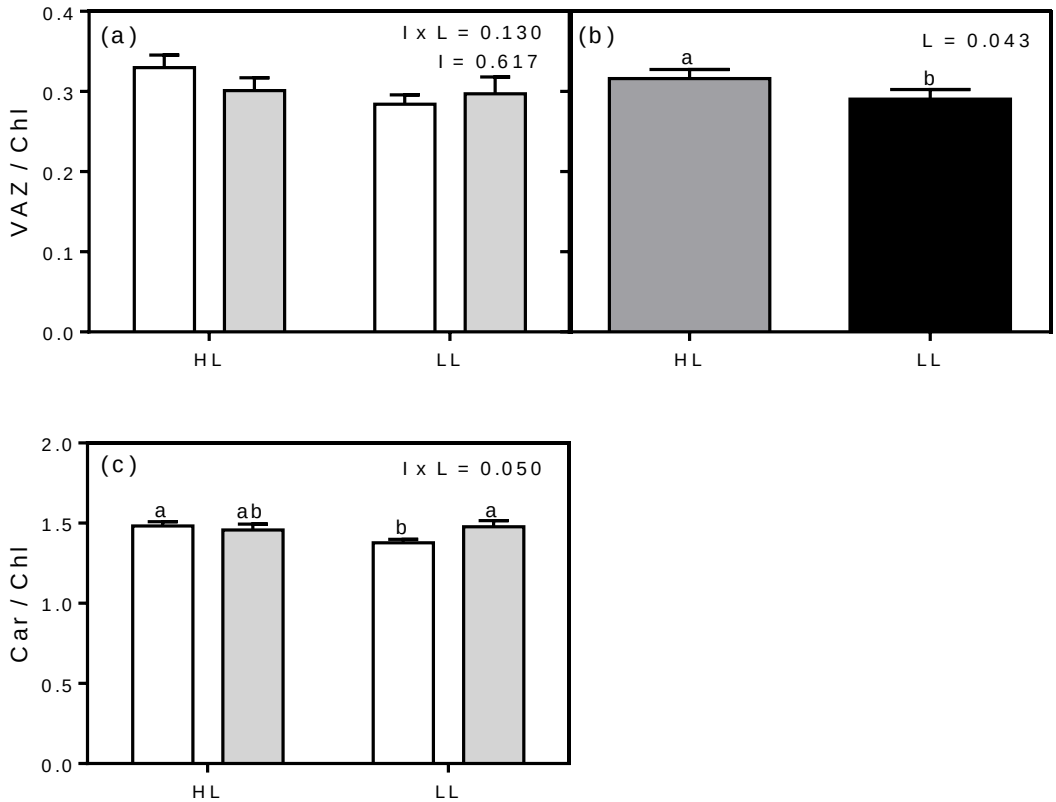

Fig. 1. Xanthophyll cycle pool per unit chlorophyll (VAZ/Chl) (*a*) and the total carotenoid pool (Car/Chl) (*c*) of *Leptospermum myrsinoides* either in high (HL) or low light (LL) and uninfected (white bars) or infected (light grey bars) with *Cassytha pubescens*. Additive light effect on VAZ/Chl (*b*) 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 (± s.e.), n = 15–16 (*a*, *c*), n = 31–32 (*b*), d.f. = 1, 58. Different letters denote significant ($P < 0.05$) differences and P-values (two-way ANOVA) for infection (I) x light (L) interaction, additive I or L effect are included in panels.

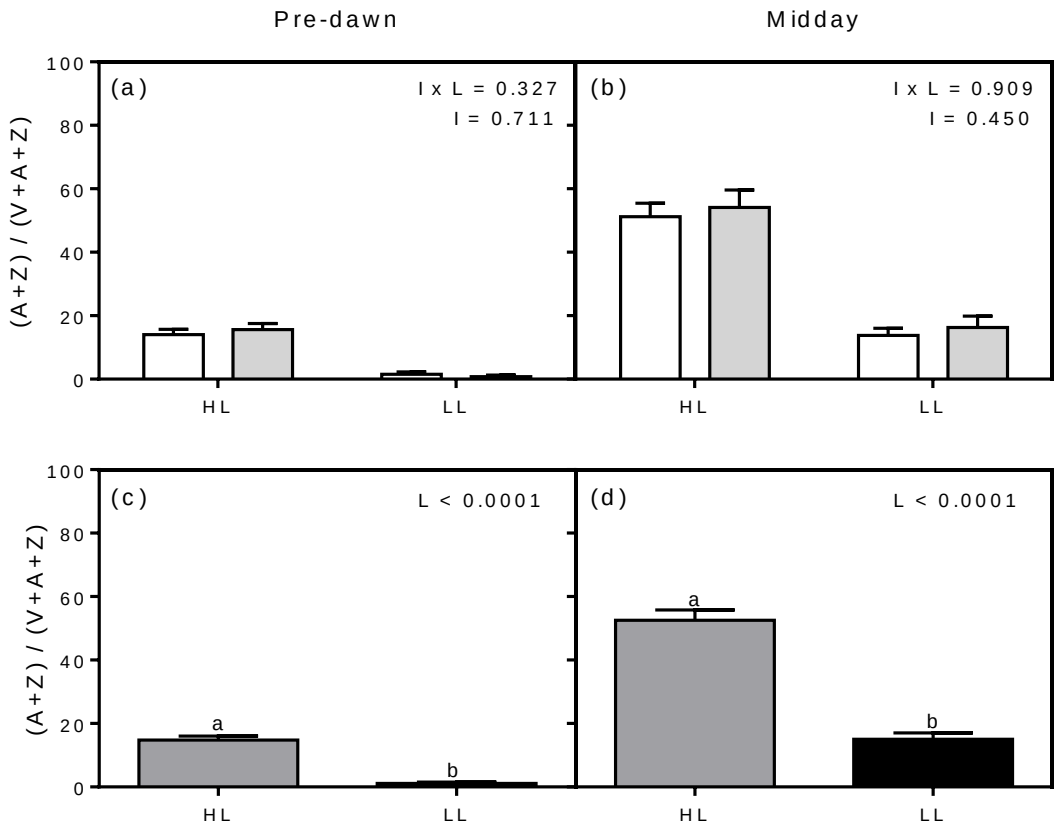

Fig. 2. Pre-dawn (*a*) and midday (*b*) de-epoxidation state [(A+Z)/(V+A+Z)] of *Leptospermum myrsinoides* grown in either high (HL) or low light (LL) and uninfected (white bars) or infected (light grey bars) with *Cassytha pubescens*. Additive light effect on pre-dawn (*c*) and midday de-epoxidation 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 (± s.e.), d.f. = 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*). Different letters denote significant ($P < 0.05$) differences and P-values (two-way ANOVA) for infection (I) x light (L) interaction, additive I or L effect are included in panels.

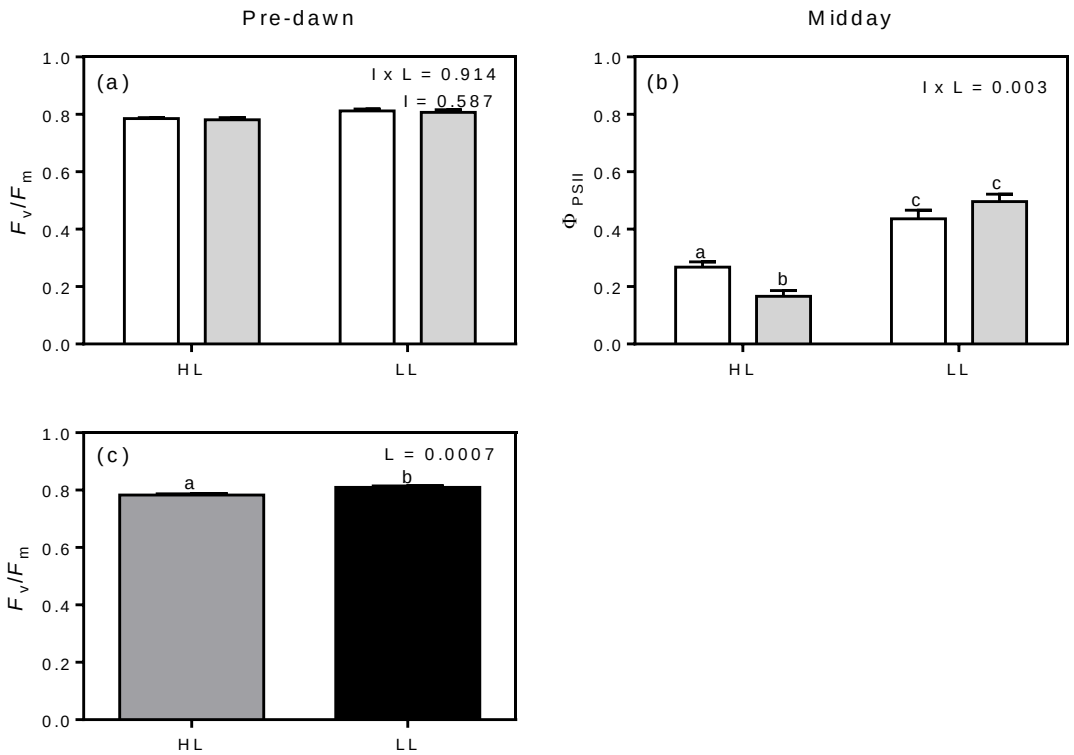

Fig. 3. Quantum yield measured at pre-dawn (F_v/F_m) (*a*) and midday (Φ_{PSII}) (*b*) for *Leptospermum myrsinoides* grown in either high (HL) or low light (LL) and uninfected (white bars) or infected (light grey bars) with *Cassytha pubescens*. Additive light effect on F_v/F_m (*c*) of *L. myrsinoides* (dark grey bars are average of uninfected and infected HL plants, black bars are average of uninfected and infected LL plants). Data are means (± s.e.), n = 8–10 (*a*, *b*), n = 18–20 (*c*) and d.f. = 1, 33. Different letters denote significant (P < 0.05) differences and P-values (two-way ANOVA) for infection (I) x light (L) interaction, additive I or L effect are included in panels.

Table 2. Stem concentrations (µmol 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 (± s.e.), $n = 9$, d.f. = 1, 15 for all parameters, different letters denote significant ($P \leq 0.05$) 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 *a*	Chl *b*	Chl *a*/*b*
HL	8.46 ± 1.42a	230 ± 24	43 ± 5	23 ± 3	2.83 ± 0.49	163 ± 17	67 ± 7	2.45 ± 0.07
LL	5.24 ± 0.89b	269 ± 41	42 ± 6	25 ± 4	3.19 ± 0.56	188 ± 30	81 ± 12	2.31 ± 0.06
(L)	**0.051**	0.507	0.773	0.673	0.785	0.555	0.400	0.146

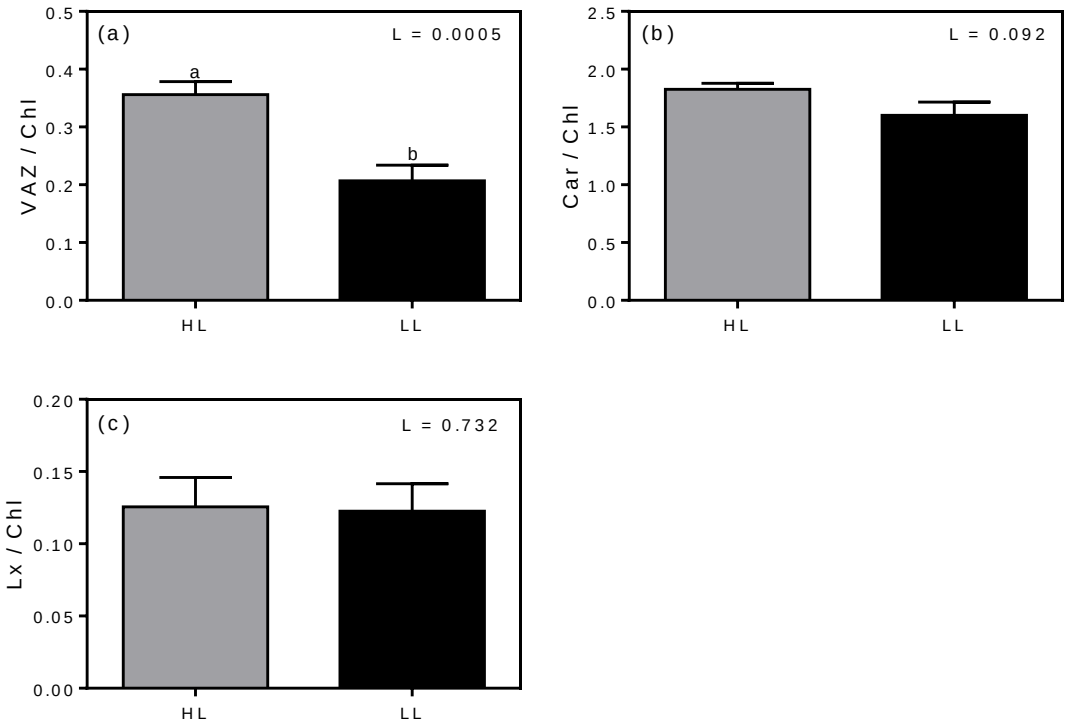

Fig. 4. VAZ/Chl (*a*), Car/Chl (*b*) and Lx/Chl (*c*) of *Cassytha pubescens* when infecting *Leptospermum myrsinoides* in high (HL) or low light (LL). Data are means (± s.e.), $n = 9$, d.f. = 1, 15 and *P*-values (one-way ANOVA) for light effect are included in panels with different letters denoting significant ($P < 0.05$) effects.

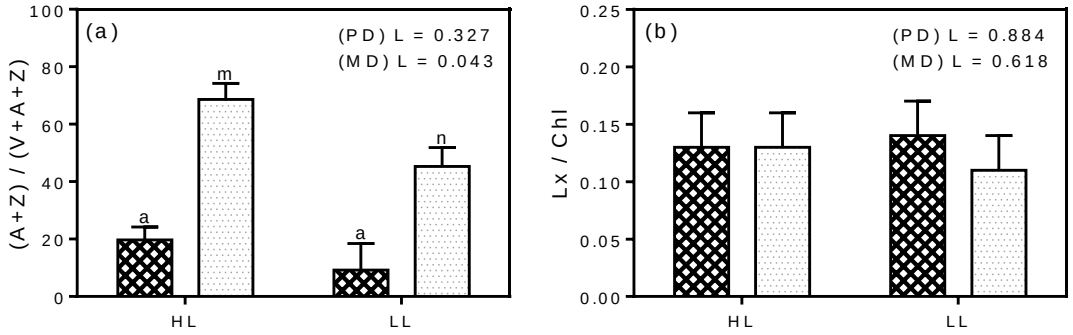

Fig. 5. De-epoxidation state of the xanthophyll (*a*) and lutein epoxide cycles (*b*) of *Cassytha pubescens* when infecting *Leptospermum myrsinoides* in high (HL) or low light (LL), at pre-dawn (hatched bars) or at midday (dotted bars). Data are means (± s.e.) and n = 4–5, d.f. = 1, 6 and P-values (one-way ANOVA) for light effect are included in panels. Different letters denote significant ($P < 0.05$) effects for pre-dawn (PD, a, b) and midday (MD, m, n), which were analysed separately.

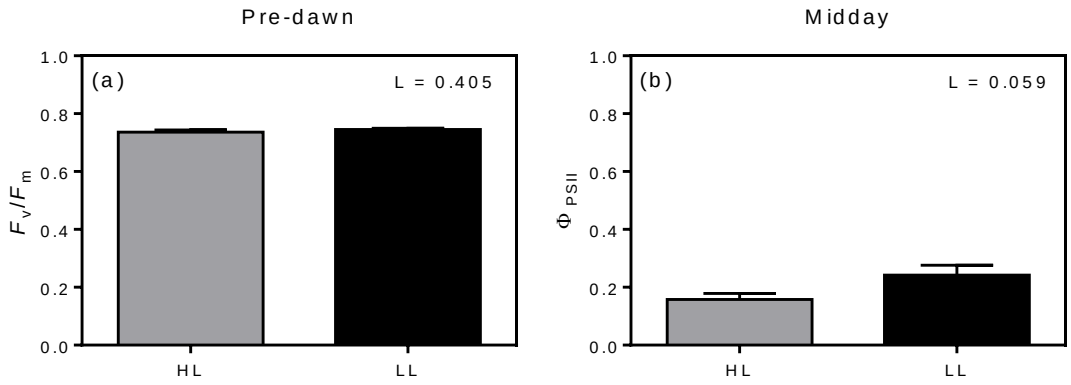

Fig. 6. Quantum yield measured at pre-dawn (F_v/F_m) (*a*) and midday (Φ_{PSII}) (*b*) of *Cassytha pubescens* infecting *Leptospermum myrsinoides* in high (HL) or low light (LL). Data are means (± s.e.), n = 5, d.f. = 1, 7 and P-values (one-way ANOVA) for light effect are included in panels with no significant ($P < 0.05$) differences detected.

Chapter 4: Nitrogen

Fig. 1a Photos of the nitrogen experiment taken from two opposite angles. Foreground of top photo: *Acacia paradoxa* and *Ulex europaeus* infected with *Cassytha pubescens*, left and right of arrow respectively, which also acts as a scale bar for approximately 15-16 cm.

Statement of Authorship

Title of Paper	Does nitrogen affect the interaction between a native hemiparasite and its native or introduced leguminous hosts?
Publication Status	☐ Published ☐ Accepted for Publication ☑ Submitted for Publication ☐ Unpublished and Unsubmitted work written in manuscript style
Publication Details	Cirocco, R. M., Facelli, J. M., & Watling, J. R. (submitted). Does nitrogen affect the interaction between a native hemiparasite and its native or introduced leguminous hosts? New Phytologist (Official Journal response: Resubmission encouraged)

Principal Author

Name of Principal Author (Candidate)	Robert Cirocco
Contribution to the Paper	Co-conceived and designed the experiment, performed the experiment, analysed and interpreted the data, wrote manuscript and acted as corresponding author.
Overall percentage (%)	80
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.
Signature	Date 26/2/2016

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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Contribution to the Paper	Supervised development of work, helped in data interpretation and manuscript evaluation
Signature	Date 26/02/2016

Please cut and paste additional co-author panels here as required.

Does nitrogen affect the interaction between a native hemiparasite and its native or introduced leguminous hosts?

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Summary

- Associations between plants and N-fixing rhizobia intensify with decreasing nitrogen (N) supply, and come at a carbon cost to the host. However, what the additional impact parasitic plants will have on their leguminous hosts' carbon budget in terms of effects on host physiology and growth is unknown.
- Under glasshouse conditions, *Ulex europaeus* and *Acacia paradoxa* either uninfected or infected with the hemiparasite *Cassytha pubescens* were supplied (HN) or not (LN) with extra N. Photosynthetic performance and growth measures of the association were measured.
- *Cassytha pubescens* had a significant negative impact on maximum electron transport rates and total biomass of *U. europaeus* but not *A. paradoxa*, regardless of N supply. Root growth but not nodule biomass of *A. paradoxa* was affected by infection at only LN. Infection had a significant negative impact on host nodule biomass. Parasite biomass (also per unit host biomass) was significantly greater when infecting *U. europaeus* than *A. paradoxa*, regardless of N treatment.
- We concluded that rhizobia do not influence the effect of a native parasite on overall growth of leguminous hosts. Our results suggest that *C. pubescens* will have a strong impact on *U. europaeus* but not *A. paradoxa*, regardless of N conditions in the field.

Key words: Biomass, gas exchange, hemiparasite, legume, nitrogen, nodulation, photosynthesis, rhizobia.

Introduction

Parasitic plants are globally important as they are found in a wide range of ecosystems and have profound effects on processes at the population, community and ecosystem levels (Press & Phoenix, 2005). They vary greatly in taxonomy, form and function, but all attach to either host stems or roots via haustoria (Press *et al.*, 1999). This structure joins the parasite to the host from which it extracts resources (Kuijt, 1969). Holoparasites access resources from the phloem and xylem of their hosts removing carbohydrate, water and nutrients but generally have very low photosynthetic ability (Stewart & Press, 1990). Conversely, hemiparasites typically access resources from the host xylem, and while being capable of photosynthesis they depend on their hosts for water, nutrients and other solutes (Press & Graves, 1995). Parasite effects on their hosts can range from negligible to host death and such outcomes can depend on a number of factors.

One such factor is nutrient supply. For example, in some host species, high nitrogen (N) supply reduces the effect of the hemiparsite, *Striga hermonthica*, on host photosynthesis and growth, even to the point of eliminating it for *Sorghum bicolor* cv. CSH1 (Cechin & Press, 1993; Cechin & Press, 1994), while in other cultivars or host species N does not influence the effect of this root hemiparasite (Gurney *et al.*, 1995; Aflakpui *et al.*, 1998; Sinebo & Drennan, 2001; Aflakpui *et al.*, 2002; Aflakpui *et al.*, 2005). These authors suggested that in their studies, insufficient amounts of N may have been added to influence the effects of *S. hermonthica* on its hosts. High N supply has also been found to dampen the effect of the stem holoparasites *Cuscuta campestris* and *Cuscuta reflexa* on growth of *Mikania micrantha* and *Ricinus communis*, respectively, but not for the *C. reflexa-Coleus blumei* association (Jeschke & Hilpert, 1997; Jeschke *et al.*, 1997; Shen *et al.*, 2013). At least for the *C. campestris-M. micrantha* association, the greater effect on host growth at low N supply was attributed to increased resource removal by the parasite in these conditions (Shen *et al.*, 2013).

The influence of N on host-parasite associations become more complex when the host plants are N-fixers, such as legumes which form associations with rhizobia to obtain N at a cost of carbohydrate (Pennings & Callaway, 2002). When supplied with sufficient N, plants have low affinity for partnerships with rhizobia, while at low N, they have a greater engagement with these bacteria and this comes at a greater cost of carbohydrate (Lambers *et al.*, 2008). This may be compounded when legumes are also infected by a parasite as carbohydrate may already be in short supply due to infection effects on host photosynthesis

as well as direct removal of host carbon by the parasite (Gurney *et al.*, 2002; Meinzer *et al.*, 2004; Shen *et al.*, 2007; Těšitel *et al.*, 2010). Thus, at low N supply, the combination of infection by a parasite and rhizobia, which may be the main N source of the plant, may result in greater pressure on host carbon and ultimately growth.

Importantly, plants that form associations with N-fixing bacteria are common hosts of parasitic plants (Matthies, 1996). One study investigating the effects of the stem holoparasite *Cuscuta reflexa* on the legume *Lupinus albus* found that nitrogen fixation, host growth and fruit setting was strongly suppressed by infection (Jeschke *et al.*, 1994). They attributed these decreases to carbon and nitrogen removal by the parasite from the host phloem, however, in this study plants were only supplied with nitrogen-free solution. Hence, although there have been a number of studies investigating the influence of mycorrhizae (inoculated versus not inoculated) (Davies & Graves, 1998; Salonen *et al.*, 2001; Gworgwor & Weber, 2003; Stein *et al.*, 2009) on parasite effects on hosts, to our knowledge, there are none on the influence of rhizobia (high versus low colonisation) via manipulation of N supply to the host. Thus, it is clear that any knowledge on the topic will advance the field of parasitic plant-host interactions. As below-ground process such as rhizobial interactions and root growth are very difficult to quantify in the field, experimentation offers a practical and strict evaluation of these variables in isolation from numerous other factors found in nature.

Here we report results of an experiment investigating how N availability affected the association between the Australian native stem hemiparasite, *Cassytha pubescens* and two N-fixing hosts, a native (*Acacia paradoxa*) and an introduced weed (*Ulex europaeus*) (Supporting Information Fig. S1). *Cassytha pubescens* has been found to negatively affect introduced hosts more than native hosts (Prider *et al.*, 2009). We hypothesised that *C. pubescens* would have a greater effect on host performance at low N supply. This is because of carbohydrate limitations resulting from infection effects on host photosynthesis coupled with the additional C demand from rhizobia in these conditions. However, we also expected the impact of infection with *C. pubescens* would be greater in the introduced host, *U. europaeus*, than the native host, *A. paradoxa*.

Materials and Methods

Study species

Cassytha pubescens R. Br. (Lauraceae) is a perennial, stem hemiparasitic vine native to Australia (Kokubugata *et al.*, 2012) and abundant in the southern part of the continent. It has much reduced scale-like leaves on a coiling stem (0.5−1.5 mm in diameter) and attaches to host stems and leaves via multiple haustoria (McLuckie, 1924; Harden, 1990; Prider *et al.*, 2009). *Acacia paradoxa* DC. (Fabaceae) is a perennial, evergreen, leguminous shrub native to southern Australia that grows on a range of soils and is often found in eucalypt-dominated woodlands (Cunningham *et al.*, 2011). *Acacia paradoxa* grows to *c.* 2.5–4 m in height and has dark green 0.8–3 cm long phyllodes (Harden, 1991).

Ulex europaeus L. (Fabaceae) is a perennial, evergreen, leguminous shrub *c.* 1.5–2 m in height that is native to Europe and Northern Africa (Clements *et al.*, 2001; Tarayre *et al.*, 2007). It is a serious, introduced weed in more than 15 countries worldwide, including Australia (Lowe *et al.*, 2000; Clements *et al.*, 2001; Tarayre *et al.*, 2007). Its leaves, spines and stems are photosynthetic (Hill *et al.*, 1991; Clements *et al.*, 2001; Tarayre *et al.*, 2007). *Ulex europaeus* thrives in disturbed areas and grows well in nutrient poor sandy soils. Both *U. europaeus* and *A. paradoxa* are N-fixing and form associations with rhizobium bacteria to obtain biologically reduced atmospheric N_2 in exchange for carbohydrate (Lawrie, 1983; Weir *et al.*, 2004).

Experimental design

Acacia paradoxa plants (~20 cm in height) were obtained from a commercial nursery and individually transplanted into 1.65 litre pots containing organic sandy loam in late April 2011. *Ulex europaeus* plants (~15cm in height) were obtained from the field (Crafers, Mt. Lofty Ranges of South Australia: 35°27’41”S, 138°43’91”E), and were individually transplanted into 1.65 litre pots containing organic sandy loam in late January 2011. Throughout the experiment, plants were grown in the commercial soil mentioned. This soil was not inoculated with field soil in case of introducing any pathogens into the system. Further, although the commercial soil was not inoculated with any rhizobial strain this may be inconsequential as nodules were present on experimental plants (total biomass of uninfected plants of both species at HN were similar with those at LN even though they received no extra N, Fig. 3; and as expected, nodule biomass per unit root biomass was significantly higher at LN versus HN (independently affected by N, Tables 3 & 4)). All plants were provided with liquid fertiliser (Nitrosol; Rural Research Ltd, Auckland, New Zealand; NPK 8:3:6) in accordance with the manufacturer’s directions.

Synchronous infection with *C. pubescens* of randomly selected individuals of both species was achieved in mid-June 2011 using the method described in Shen *et al.* (2010). Large *U. europaeus* plants already infected by *C. pubescens* were used as the source of infection, and the parasite was allowed to coil and attach to stems of experimental plants. Stems of *C. pubescens* attached to the newly parasitised plants were severed from the *U. europaeus* donor plant in early November 2011. The process of attachment took *c.* 4–5 months. Experimental plants were monitored for a further week to ensure that *C. pubescens* had successfully established on the hosts. All plants were then individually re-potted into 5 litre pots containing the soil mentioned in early December 2011.

Uninfected and infected plants of both species were randomly allocated into two N treatments. Plants in the high N treatment (HN) were provided with standard Hoagland's solution. Plants in the treatment without additional N (LN) were provided standard Hoagland's solution with KCl and $CaCl_2$ substituted for KNO_3 and $Ca(NO_3)_2.4H_2O$, respectively. All plants were randomly allocated into six blocks, each block containing all combinations of treatments, and were re-randomised fortnightly to account for small light differences in the glasshouse. Plants were provided with 400 ml of standard (HN) or modified Hoagland's solution (LN) fortnightly. Nitrogen treatments ran from early February 2012 to mid-June 2012, lasting for 164 days. The experiment consisted of a full three-way factorial design with host species, infection and N at two levels each with six replicates for each combination of factors.

Photosynthesis measurements

Rapid light response curves for hosts and parasite were determined using a portable, pulse-modulated chlorophyll fluorometer (MINI-PAM, Walz, Effeltrich, Germany) fitted with a leaf-clip (2030–B, Walz, Effeltrich, Germany) (Supporting Information Fig. S2). Electron transport rate was calculated as:

ETR = Yield x PAR x 0.5 x 0.84

Where Yield is how efficiently photosystem II is contributing to photochemistry in the light, PAR is photosynthetically active radiation, 0.5 signifies that two photons are required to transport a single electron and 0.84 is the absorptance factor for a standard leaf of an angiosperm (White & Critchley, 1999; Strong *et al.*, 2000). Actinic light levels were automatically increased in eight steps at 10 s intervals and included an initial measurement in darkness. Rates of electron transport were considered to be at their maximum (ETR_{max})

at the same actinic light level within species where highest rates where consistently reached and most representative of replicates. ETR_{max} occurred at photon flux densities (PFD) of 1904 ± 23.31 μmol m^{-2} s^{-1} for *U. europaeus*, 1308 ± 20.41 μmol m^{-2} s^{-1} for *A. paradoxa*, and 1439 ± 12.85 μmol m^{-2} s^{-1} for *C. pubescens* on both hosts. Measurements were made between 11:00 and 13:00 on the youngest fully expanded spine or phyllode, depending on species, on a sunny day in mid-May 2012, 103 days after N treatments were imposed (DAT); and on *C. pubescens* 15 cm from the growing tip on a sunny day in mid-May 2012 (107 DAT).

Measurements of photosynthesis (*A*) and stomatal conductance (g_s) were obtained using a portable Ciras–2 gas-exchange system fitted with a PLC (5) conifer cuvette (PP Systems, Amesburg, MA). This cuvette enabled gas exchange measurements on the different photosynthetic organs (stems with spines or phyllodes) of *U. europaeus* and *A. paradoxa*. Measurements were made between 10:30 and 13:00 in early June 2012 (when days where sunny between 117-129 DAT), at mean PFD=1278 ± 4 μmol m^{-2} s^{-1}, n=32.

Biomass and N concentration

A destructive harvest was conducted at the end of the experiment in mid-June 2012, 164 DAT. Nodules, roots, stems and spines (very few if any leaves present) of *U. europaeus*; nodules, roots, stems and phyllodes of *A. paradoxa*, and stems of *C. pubescens* were collected and oven dried at 70 °C for three days. Nitrogen concentration of *U. europaeus* spines, *A. paradoxa* phyllodes and *C. pubescens* stems was determined by complete combustion gas chromatography at Waite Analytical Services (University of Adelaide), on final harvest oven-dried material.

Statistical analyses

The variances of the data were homogeneous and the effects of infection with *C. pubescens,* N supply and host species were assessed using a three-way ANOVA. Where a three-way interaction was not detected, two-way interactions were considered e.g. Infection x Host species (uninfected plants at HN and LN pooled versus infected plants at HN and LN pooled for *A. paradoxa* compared with those of *U. europaeus*). A two-way ANOVA was implemented to detect the effect on N and host species on parasite parameters. Where interactions were not significant, independent effects were then considered e.g. infection effect with *C. pubescens* (uninfected plants from both host species at HN and LN pooled versus infected plants from both host species at HN and LN

pooled). Where effects were significant, a Tukey-Kramer HSD was used for pairwise comparisons of means. All data were analysed with the software JMP Ver. 4.0.3 (SAS institute Inc., 2000) and α=0.05.

Results

Photosynthetic performance

Nitrogen did not have any interactive or independent effects on ETR_{max} of either *U. europaeus* or *A. paradoxa* (Table 1, Fig. 1a). There was however, a species x infection interaction for ETR_{max} (Table 1). Infection decreased ETR_{max} of *U. europaeus* by 46% while having no effect on that of *A. paradoxa*, regardless of N treatment (Fig. 1b). There was no interactive effect of N x species or any independent effects of these factors on ETR_{max} of *C. pubescens* (Table 2, Fig. 1c).

Nitrogen had no interactive or independent effects on photosynthesis of *U. europaeus* or *A. paradoxa* (*A*; Table 1, Fig. 2a). The species x infection interaction for this parameter was also not significant (Table 1), nevertheless, photosynthetic rates of infected *U. europaeus* were close to half those of uninfected plants (Fig. 2b). No significant differences were detected for g_s, although there was a trend for them to be lower as a result of infection in *U. europaeus*, but not *A. paradoxa* (Table 1, Fig. 2c).

Growth, nodulation and N concentration

As with photosynthetic performance, N had no interactive or independent effect on total or shoot biomass of either *U. europaeus* or *A. paradoxa* (Table 3, Fig. 3a, c). There was however, a species x infection interaction for total and shoot biomass (Table 3). Total and shoot biomass of infected *U. europaeus* was *c.* 60% less than that of uninfected plants (Fig. 3b, d). Infection had no effect on total or shoot biomass of *A. paradoxa* (Fig. 3b, d). In contrast to total and shoot biomass, there was a three-way interaction for root biomass (Table 3, Fig. 3e). Root biomass of infected *U. europaeus* in HN and LN treatments was 56% and 36% lower compared with that of the respective uninfected plants (Fig. 3e). Root biomass of infected *A. paradoxa* in the LN treatment was 39% less relative to that of respective uninfected plants (Fig. 3e). Infection had no effect on root biomass of *A. paradoxa* in the HN treatment (Fig. 3e).

There were no treatment interactions for host leaf area, shoot/root ratio, nodule biomass or nodule biomass per g root biomass (Table 3). There was however, an independent effect of

infection on leaf area (Table 3). Phyllode/spine area of infected plants on the whole was 42% less than that of uninfected plants (Table 4). There was also an independent effect of infection on nodule biomass (Table 3). Nodule biomass on roots of infected plants was 41% lower compared with that of uninfected plants on the whole (Table 4). There was an independent effect of species on all four parameters. Spine area of *U. europaeus* was 70% lower relative to phyllode area of *A. paradoxa* (Table 4). Shoot/root ratio of *U. europaeus* was 48% lower than that of *A. paradoxa* (Table 4). Nodule biomass of *U. europaeus* was 43% lower compared with that of *A. paradoxa* (Table 4). Nodule biomass per g root biomass of *U. europaeus* was 58% lower relative to that of *A. paradoxa* (Table 4). This parameter was also independently affected by N treatment (Table 3). Nodule biomass per g root biomass of plants in LN (0.127 ± 0.017) was 20% higher than that of plants in HN treatment (0.102 ± 0.014). Parasite biomass, both total and on a per g host biomass basis, was independently affected by species but not by N treatment (Table 2, Fig. 4a, b). Total parasite biomass on *A. paradoxa* was 63% less than it was on *U. europaeus* (Fig. 4a), and was nearly an order of magnitude lower per g of host on *A. paradoxa* than on *U. europaeus* (Fig. 4b).

There was no three-way interaction for host foliar N concentration (Table 3, Fig. 5a). There was however, an N x infection interaction for this parameter (Table 3). Host foliar N concentration of infected plants was not significantly different from that of uninfected plants in either HN or LN (Fig. 5b). However, foliar N of infected plants in HN was significantly higher compared with that of infected plants in LN treatment (Fig. 5b). There was also an independent species effect on N concentration of spines or phyllodes (Table 3). 'Foliar' N concentration of *U. europaeus* was 32% lower than that of *A. paradoxa* (Fig. 5c). There was no N x species interaction or independent effects on N concentration of *C. pubescens* stems (Table 2, Fig. 5d).

Discussion

Our hypothesis that *C. pubescens* would have a greater effect on host performance under LN was supported by the root biomass data, although for the native not introduced host as expected. *Acacia paradoxa* root growth was negatively affected by infection at only LN. This might be due to the 44% reduction in phyllode area resulting from infection in these conditions. This would result in lower C gain on a whole plant basis, of which was evidently allocated to maintaining similar nodulation relative to that of respective uninfected plants at LN than root growth. This is in line with Resource Allocation Theory;

in order to help recover N losses to the parasite, more C may have been allocated to nodules than roots of *A. paradoxa* to help maintain sufficient N acquisition as rhizobia are likely the host's primary source of N at LN. In contrast to *A. paradoxa*, although *C. pubescens* had a negative impact on root growth of *U. europaeus*, it was less severe at LN. The effect of *C. pubescens* on root growth of *U. europaeus* in either N treatment may be due to infection effects on spine area and photosynthesis of this host which would negatively affect its C budget. But in contrast to *A. paradoxa* at LN, of that less available C it seems that *U. europaeus* allocated more toward root growth rather than nodule biomass which was 56% less than that of respective uninfected plants. Presumed increased allocation of C by *U. europaeus* to roots relative to nodules possibly to increase N uptake may be how this host responds to LN, especially as *U. europaeus* generally had much lower nodulation than *A. paradoxa*. Root biomass of uninfected plants was unaffected by N treatment, but nodulation increased in response to LN in *U. europaeus* likely to obtain sufficient N which enabled similar growth compared with that of uninfected HN *U. europaeus*. However, as total biomass of infected *U. europaeus* at LN was much less than that of respective uninfected plants, this much smaller plant would require relatively less N mitigating the need to expend energy for greater nodulation and instead this species responded to LN by increasing root biomass. The opposite was the case for *A. paradoxa* which increased nodulation, at the expense of root biomass to presumably obtain levels of N that could sustain normal overall growth relative to that of respective uninfected plants. These responses (increasing root biomass coupled with much less total biomass for *U. europaeus* or nodulation for *A. paradoxa*) may help explain why infected plants at LN were able to maintain similar concentrations of foliar N than respective uninfected plants. Moreover within host species, LN plants were able to maintain similar foliar N concentrations than HN plants likely because they had significantly higher nodule biomass per gram root biomass. This should afford hosts sufficient access to N from rhizobia in these conditions. Therefore from the above, it makes sense that N treatment had no influence on photosynthesis nor total biomass of either host species and in turn no interactive effect with *C. pubescens* infection on these parameters. On the other hand, Shen *et al.* (2013) found that the negative effect of stem holoparasite *Cuscuta campestris* on total biomass of *Mikania micrantha* was more severe at low N supply. Parasites can affect host growth due to effects on host photosynthesis and/or resource removal (Shen *et al.*, 2006). As Shen *et al.* (2013) found no significant N x infection interaction on host photosynthesis; they attributed the greater effect on host growth at low N to increased

resource removal by *Cuscuta campestris* in these conditions. This discrepancy between findings may be in part related to *Cuscuta campestris* and *C. pubescens* being holo and hemiparasites and or being associated with non-leguminous and leguminous hosts in these studies, respectively.

Cassytha pubescens had negative effect on nodule biomass of both species, regardless of N supply. By contrast, Tennakoon *et al.* (1997) found that nodule biomass and number on roots of *Acacia littorea* were unaffected by the root hemiparasite *Olax phyllanthi*. This difference may be due to infection having a significant effect on photosynthesis of *U. europaeus* and foliar area of both hosts in our study, whereas *O. phyllanthi* had no effect on either host photosynthesis or leaf area of its host (Tennakoon *et al.*, 1997). As a result, infected plants in our study may have had less carbohydrate available for rhizobia, which would explain why infection had a negative effect on nodulation.

Another important finding of our study is that total biomass of the introduced host *U. europaeus* but not that of the native host, *A. paradoxa*, was affected by *C. pubescens*, regardless of N conditions. This is similar to other studies that have reported greater negative effects of native parasites on growth of introduced rather than native hosts (Prider *et al.*, 2009; Li *et al.*, 2012). Our results may be explained by the negative effect of infection on photosynthetic performance of *U. europaeus*, but not that of *A. paradoxa* (Figs. 1b, 2b). It may also in part be due to more effective resource removal by the parasite from *U. europaeus* compared with *A. paradoxa*, resulting from a more effective haustorial connection to the introduced host (see Gurney *et al.*, 2003; Cameron *et al.*, 2006; Gurney *et al.*, 2006; Cameron & Seel, 2007; Rümer *et al.*, 2007). This is plausible considering that an earlier study with *C. pubescens* using ^{32}P labelling, demonstrated that haustoria formed on the introduced host *Cytisus scoparius* (broom) were more effective at removing phosphorus than those on the native host *Acacia myrtifolia* (Tsang, 2010).

This idea is further supported by the fact that in our study, photosynthesis of the parasite was similar on both hosts, while the parasite grew significantly larger both in absolute and per unit host biomass terms on *U. europaeus* than *A. paradoxa* (Figs. 1c, 4 a, b). Again, our finding builds on consistent reports that native parasites with indeterminate growth such as *C. pubescens*, grow much more vigorously on introduced versus native hosts (Prider *et al.*, 2009; Yu *et al.*, 2011; Li *et al.*, 2012). Nitrogen was not found to influence parasite biomass in absolute terms nor on a per g host biomass basis. By contrast, Shen *et al.* (2013) found that biomass of *Cuscuta campestris* infecting *M. micrantha* was significantly greater

at high than low N supply. It appears that in their study, hosts grew larger in response to high N and so too did the parasite (Shen *et al.*, 2013). Here, infected plants did not grow larger in the HN than in LN (likely due to hosts in our study being legumes with access to nitrogen from rhizobia under LN) which may explain why *C. pubescens* did not grow more in the HN treatment.

Nitrogen had no influence on the effect of *C. pubescens* on photosynthetic performance (ETR_{max}, *A* and g_s) of hosts as similarly found for the *Cuscuta campestris-M. micrantha* association (Shen *et al.*, 2013). The negative effect of *C. pubescens* on photosynthetic performance of *U. europaeus* does not seem related to nitrogen stress as infected plants did not have a significantly lower foliar N concentration than uninfected plants. Although not significant, decreases in g_s of *U. europaeus* as a result of infection may explain the negative impact of *C. pubescens* on photosynthetic performance of this host. Negative effects of *C. pubescens* on photosynthesis of the introduced *Cytisus scoparius* and native *Leptospermum myrsinoides* have been ascribed to decreases in stomatal conductance/transpiration rate resulting from infection (Prider *et al.*, 2009; Shen *et al.*, 2010). Importantly, our study revealed that *A. paradoxa* is the first native host studied whose photosynthesis was not affected by the native *C. pubescens*.

Acknowledgements

Special thanks to Dr. Jane N. Prider, Hong T. Tsang, Elizabeth C. Maciunas, A/Prof. Robert J. Reid, Angela Cirocco and Michele Cirocco for all their assistance. Part funding for this experiment was provided by the Native Vegetation Council (56109204).

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Nitrogen and native hemiparasite effects on native and introduced hosts

Supporting Information

Fig. S1 Photos of hosts uninfected or infected with the parasite from the experiment.

Fig. S2 Rapid light response curves of hosts and parasite.

Table S1 Three-way ANOVA results for host photosynthesis and stomatal conductance.

Table S2 Two-way ANOVA results for parasite photosynthesis, biomass and nitrogen.

Table S3 Three-way ANOVA results for host growth measures, nodulation and nitrogen.

Table 1 *P*-values from three-way ANOVA for the effects of host species (Sp), infection with *Cassytha pubescens* (I) and nitrogen supply (N) on maximum electron transport rates (ETR_{max}), photosynthetic rates (*A*) and stomatal conductance (g_s) of *Ulex europaeus* and *Acacia paradoxa*

	ETR_{max}	***A***	**g_s**
Sp	0.944	**0.035**	0.368
I	**0.0005**	0.205	0.497
Sp x I	**0.003**	0.085	0.152
N	0.954	0.489	0.915
Sp x N	0.219	0.431	0.555
I x N	0.546	0.359	0.613
Sp x I x N	0.080	0.394	0.277
Block	0.744	0.462	0.519

Significant effects are in bold; *F* and sum of square values are presented in Supporting Information Table S1.

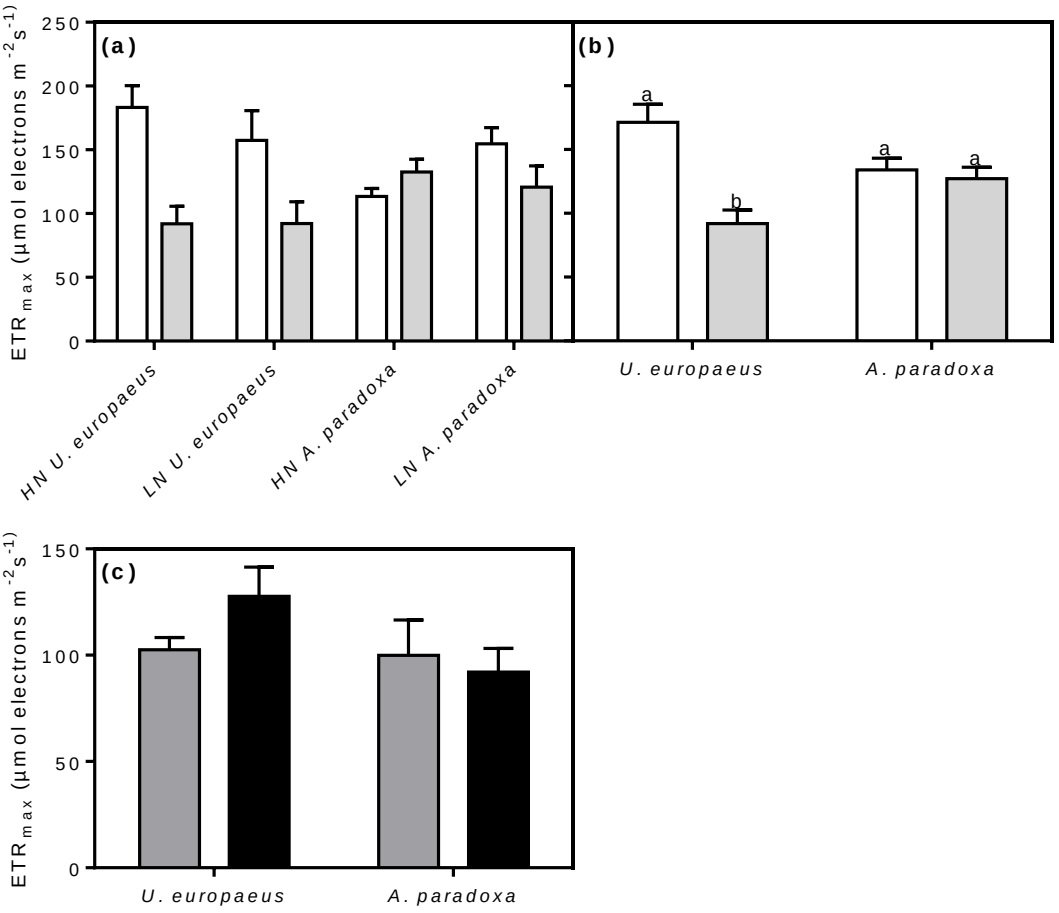

Fig. 1 (a) Maximum electron transport rates (ETR_{max}) of *Ulex europaeus* and *Acacia paradoxa* either uninfected (open bars) or infected (grey bars) with *Cassytha pubescens*, and supplied (HN) or not supplied with nitrogen (LN). (b) Species x infection interaction for host ETR_{max}. (c) ETR_{max} of *C. pubescens* when infecting either host species supplied (dark grey bars) or not supplied (black bars) with nitrogen. Different letters denote significant differences, data are means ± 1SE, *n*=5–6 (a); *n*=11–12 (b) and *n*=4–6 (c).

Table 2 *P*-values from two-way ANOVA for effects of host species (Sp) and nitrogen treatments (N) on maximum electron transport rates (ETR_{max}), parasite biomass, parasite biomass g^{-1} host biomass, and stem nitrogen concentration [N] of *Cassytha pubescens* infecting either *Ulex europaeus* or *Acacia paradoxa*

	ETR_{max}	**Parasite biomass**	**Parasite biomass g^{-1} host**	**[N]**
Sp	0.069	**<0.0001**	**0.0008**	0.395
N	0.844	0.628	0.599	0.566
Sp x N	0.078	0.733	0.746	0.860
Block	0.121	0.646	0.553	0.457

Significant effects are in bold; *F* and sum of square values are presented in Supporting Information Table S2.

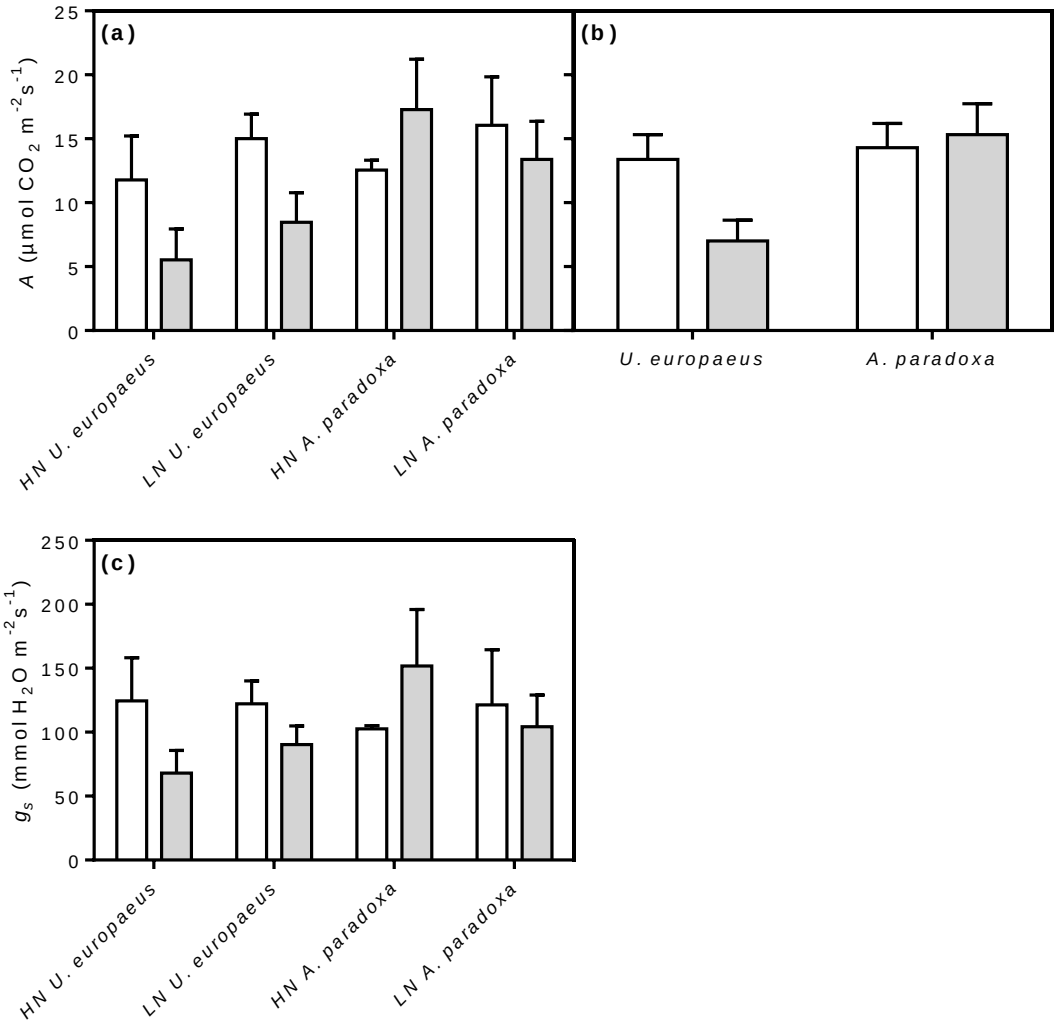

Fig. 2 (a) Photosynthetic rates (*A*) and (b) Species x infection interaction approaching significance (*P*=0.085) for *A*, and (c) stomatal conductance (g_s) of *Ulex europaeus* or *Acacia paradoxa* either uninfected (open bars) or infected (grey bars) with *Cassytha pubescens* and supplied (HN) or not supplied with nitrogen (LN). Data are means ± 1SE, *n*=4 (a, c) and *n*=8 (b).

Table 3 *P*-values from three-way ANOVA for the effects of host species (Sp), infection with *Cassytha pubescens* (I) and nitrogen supply (N) on total, shoot and root biomass, foliar area (FA), shoot/root ratio (S/R), nodule biomass (Nod), nodule biomass g^{-1} root biomass (Nod g^{-1} root biomass) and foliar nitrogen concentration [N] of *Ulex europaeus* and *Acacia paradoxa*

	Total	**Shoot**	**Root**	**FA**	**S/R**	**Nod**	**Nod g^{-1} root biomass**	**[N]**
Sp	**0.016**	**0.0008**	**0.0005**	**<0.0001**	**<0.0001**	**0.0005**	**<0.0001**	**<0.0001**
I	**<0.0001**	**<0.0001**	**<0.0001**	**0.003**	0.111	**0.001**	0.439	0.636
Sp x I	**0.016**	**0.033**	**0.004**	0.176	0.230	0.590	0.769	0.227
N	0.420	0.340	0.863	0.528	0.668	0.175	**0.040**	0.890
Sp x N	0.310	0.408	0.125	0.522	0.770	0.236	0.409	0.382
I x N	0.693	0.660	0.959	0.895	0.245	0.773	0.691	**0.017**
Sp x I x N	0.226	0.356	**0.035**	0.508	0.261	0.291	0.084	0.540
Block	**0.034**	**0.032**	0.275	0.156	0.207	0.612	0.986	0.281

Significant effects are in bold; *F* and sum of square values are presented in Supporting Information Table S3.

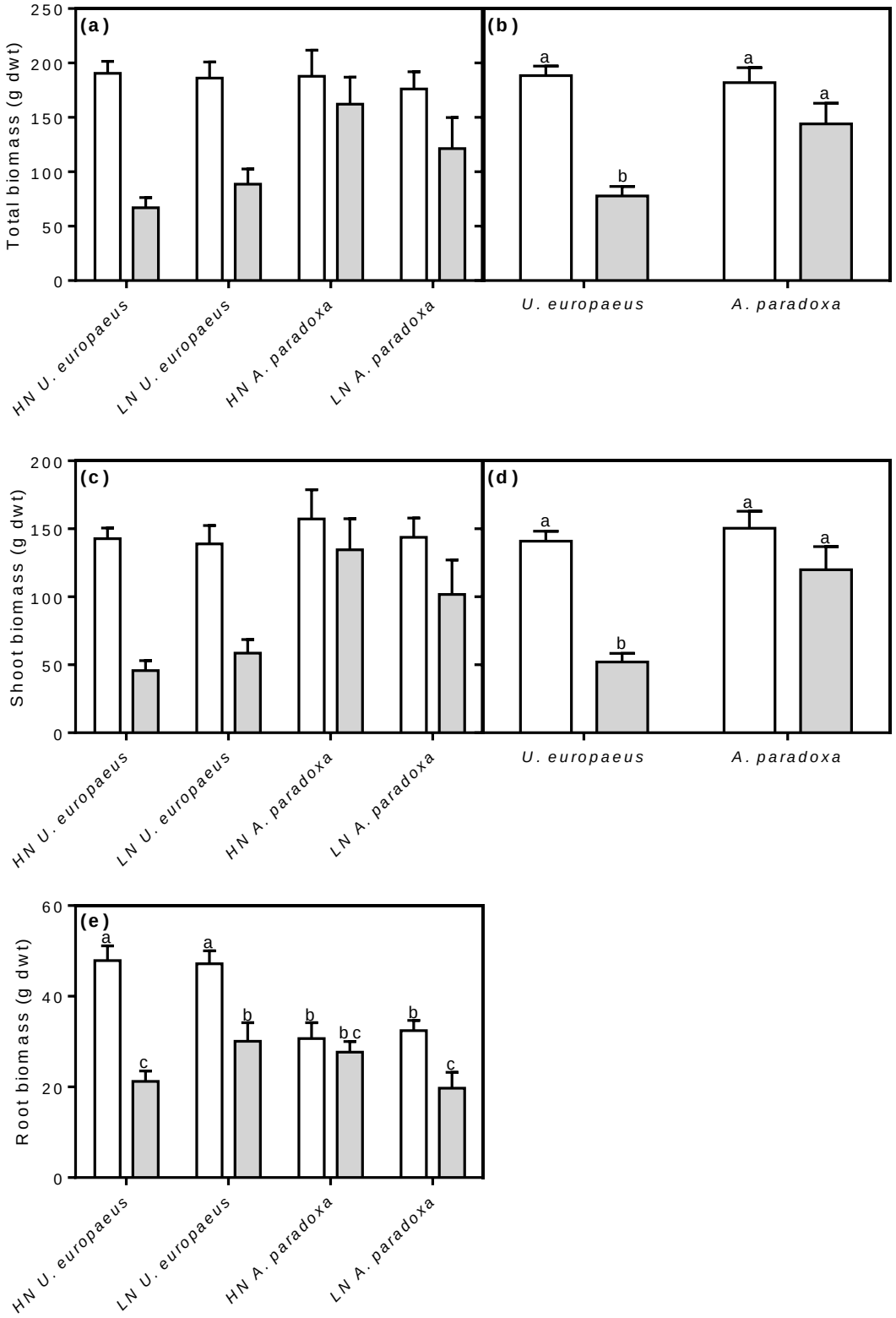
(a)
(b)
(c)
(d)
(e)
Total biomass (g dwt)
Shoot biomass (g dwt)
Root biomass (g dwt)
HN U. europaeus
LN U. europaeus
HN A. paradoxa
LN A. paradoxa
U. europaeus
A. paradoxa

Fig. 3 (a) Total, (c) shoot and (e) root biomass of *Ulex europaeus* or *Acacia paradoxa* either uninfected (open bars) or infected (grey bars) with *Cassytha pubescens* and supplied (HN) or not supplied (LN) with nitrogen. Species x infection effect on (b) total and (d) shoot biomass. Different letters denote significant differences, data are means ± 1SE, *n*=4–5 (a, c, e); *n*=19–20 (b, d).

Table 4 Foliar area (FA: cm^2), shoot/root ratio (S/R), nodule biomass (Nod: g dwt) and nodule biomass g^{-1} root biomass (Nod g^{-1} root biomass) of *Ulex europaeus* and *Acacia paradoxa* either uninfected (minus) or infected (plus) with *Cassytha pubescens* and supplied (HN) or not supplied (LN) with nitrogen

Treatment	FA	S/R	Nod	Nod g^{-1} root biomass
– HN *U. europaeus*	1175 ± 66	3.00 ± 0.07	2.68 ± 0.78	0.054 ± 0.013
– LN *U. europaeus*	1196 ± 90	2.96 ± 0.25	4.43 ± 0.40	0.094 ± 0.007
+ HN *U. europaeus*	462 ± 91	2.13 ± 0.16	1.08 ± 0.20	0.054 ± 0.011
+ LN *U. europaeus*	618 ± 96	1.92 ± 0.11	1.94 ± 0.38	0.069 ± 0.016
– HN *A. paradoxa*	3529 ± 639	5.19 ± 0.72	5.39 ± 0.93	0.177 ± 0.025
– LN *A. paradoxa*	3391 ± 739	4.45 ± 0.32	4.83 ± 0.49	0.150 ± 0.014
+ HN *A. paradoxa*	2521 ± 425	4.77 ± 0.56	3.51 ± 0.62	0.123 ± 0.014
+ LN *A. paradoxa*	1892 ± 513	5.02 ± 0.77	4.01 ± 0.96	0.211 ± 0.054
Infection effect				
uninfected	2323 ± 345a	3.90 ± 0.29	4.33 ± 0.39a	0.119 ± 0.013
infected	1346 ± 252b	3.38 ± 0.39	2.56 ± 0.38b	0.109 ± 0.018
Species effect				
U. europaeus	863 ± 85a	2.50 ± 0.13a	2.53 ± 0.36a	0.068 ± 0.007a
A. paradoxa	2883 ± 314b	4.85 ± 0.28b	4.46 ± 0.39b	0.163 ± 0.015b

No species x infection x nitrogen interaction for all parameters n=4–5; significant independent infection effect for FA and Nod; significant independent species effect for all parameters n=19–20. Different letters denote significant differences (vertically) and data are means ± 1SE.

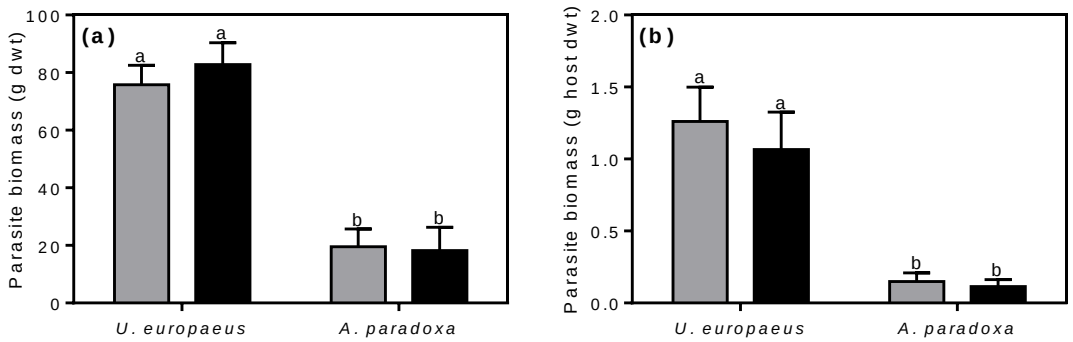

Fig. 4 (a) Parasite biomass and (b) parasite biomass per g host biomass of *Cassytha pubescens* when infecting *Ulex europaeus* or *Acacia paradoxa* supplied (dark grey bars) or not supplied (black bars) with nitrogen. Different letters denote significant differences between species, data are means ± 1SE, *n*=5 (a, b) (except *A. paradoxa* in no additional treatment, *n*=3).

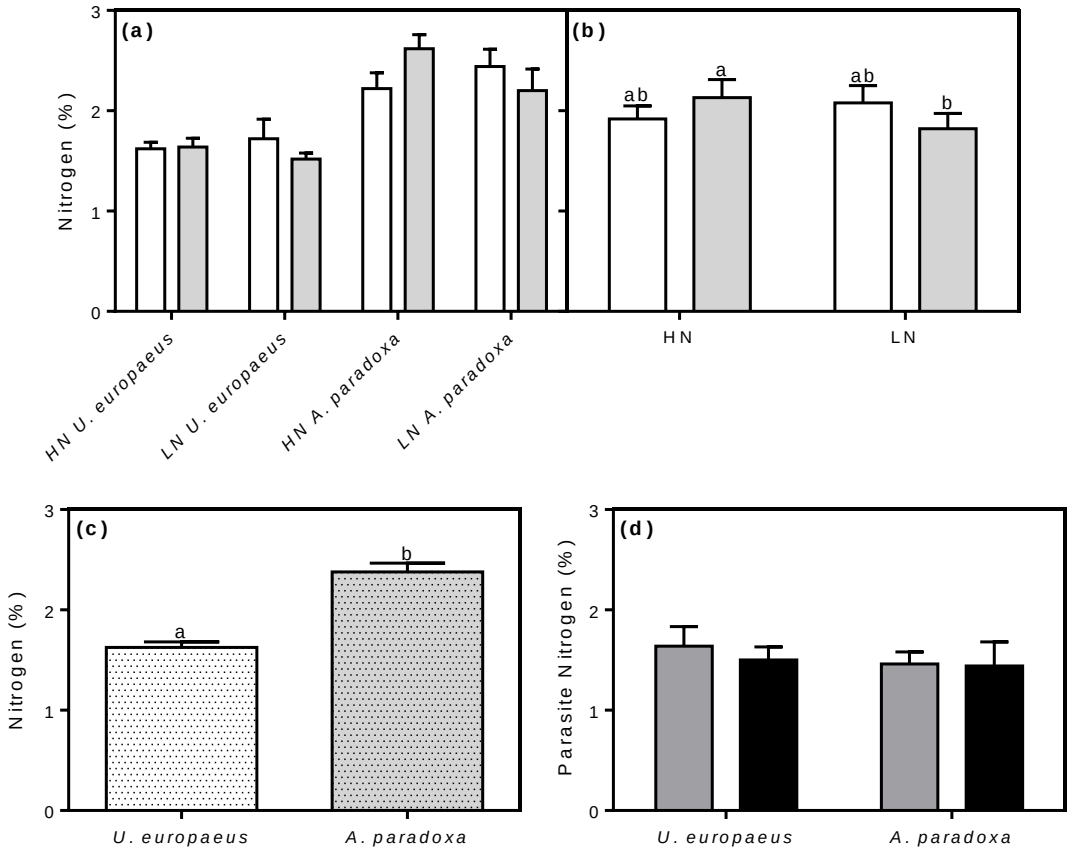

Fig. 5 (a) Foliar nitrogen concentration of *Ulex europaeus* or *Acacia paradoxa* either uninfected (open bars) or infected (grey bars) with *Cassytha pubescens* and supplied (HN) or not supplied (LN) with nitrogen. (b) Nitrogen x infection effect for host foliar nitrogen concentration. (c) Species effect for foliar nitrogen concentration of *U. europaeus* (dotted open bar) and *A. paradoxa* (dotted grey bar). (d) Stem nitrogen concentration of *C. pubescens* when infecting either host species supplied (dark grey bars) or not supplied (black bars) with nitrogen. Different letters denote significant differences, data are means ± 1SE, *n*=4–5 (a, d), *n*=9–10 (b) and *n*=19–20 (c).

***New Phytologist* Supporting Information**

Article title: **Does nitrogen affect the interaction between a native hemiparasite and its native or introduced leguminous hosts?**

Authors: **Robert M. Cirocco, José M. Facelli and Jennifer R. Watling**

Article acceptance date:

The following Supporting Information is available for this article:

Fig. S1 Photos of uninfected and infected hosts from the experiment.

Fig. S2 Rapid light response curves for host species and parasite supplied or not with extra nitrogen.

Table S1 Three-way ANOVA results for host photosynthesis and stomatal conductance.

Table S2 Two-way ANOVA results for parasite photosynthesis, biomass and nitrogen.

Table S3 Three-way ANOVA results for host growth measures, nodulation and nitrogen.

(a)

(b)

(c)

Fig. S1 Respectively, a) *Ulex europaeus* uninfected (left) or infected (right) with *Cassytha pubescens*. b) *Acacia paradoxa* uninfected (left) or uninfected (right) with *C. pubescens*. c) Vigorous growth of *C. pubescens* on *U. europaeus*. White scale bars on all photos represent 15-16 cm.

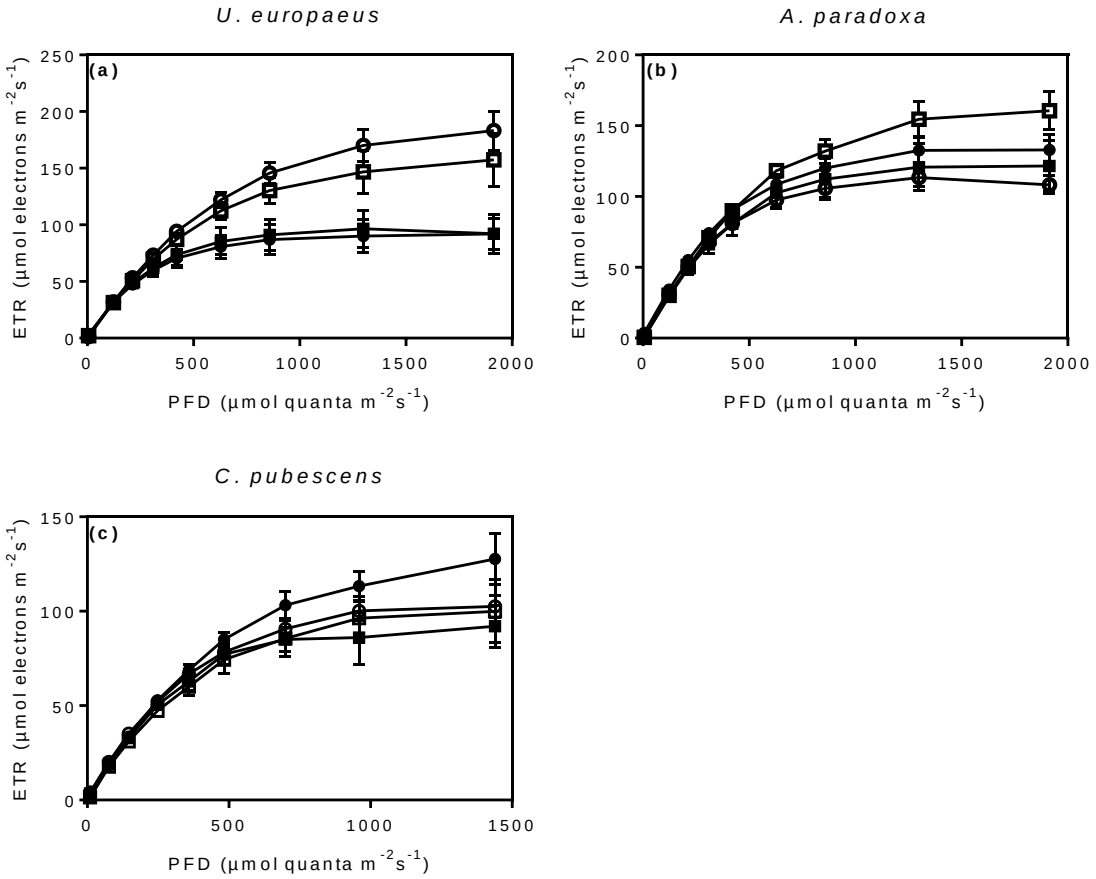

Fig. S2 Rapid light response curves for a) *Ulex europaeus* or b) *Acacia paradoxa* either uninfected (open symbols) or infected with *Cassytha pubescens* (closed symbols) and supplied (circles) or not supplied (squares) with extra nitrogen. c) Former of *Cassytha pubescens* when infecting either *U. europaeus* (circles) or *A. paradoxa* (squares) supplied (open symbol) or not supplied (closed symbol) with extra nitrogen. Data points are means ± 1SE and *n*=5–6 (a, b); *n*=4–6 (c).

Table S1 Results of three-way ANOVA for the effects of host species (Sp), infection with *Cassytha pubescens* (I) and nitrogen supply (N) on maximum electron transport rates (ETR_{max}), photosynthetic rates (*A*) and stomatal conductance (g_s) of *Ulex europaeus* and *Acacia paradoxa*.

	ETR_{max}	***A***	***g_s***
Sp	*0.005*	*5.09*	*0.845*
	7.03	171	2794
I	*15.2*	*1.72*	*0.479*
	20936	57.5	1582
Sp x I	*10.1*	*3.28*	*2.21*
	13913	110	7290
N	*0.003*	*0.497*	*0.012*
	4.63	16.7	38.3
Sp x N	*1.57*	*0.644*	*0.359*
	2168	21.6	1188
I x N	*0.373*	*0.878*	*0.264*
	514	29.5	872
Sp x I x N	*3.27*	*0.757*	*1.25*
	4504	25.4	4118
Block	*0.540*	*0.891*	*0.779*
	3723	89.7	7724
Error	45490	704	69426
df	1, 33	1, 21	1, 21

F and sum of square values are in italic and regular type, respectively.

Table S2 Results of two-way ANOVA for effects of host species (Sp) and nitrogen treatments (N) on maximum electron transport rates (ETR_{max}), parasite biomass, parasite biomass g^{-1} host biomass, and stem nitrogen concentration [N] of *Cassytha pubescens* infecting either *Ulex europaeus* or *Acacia paradoxa*.

	ETR_{max}	**Parasite biomass**	**Parasite biomass g^{-1} host**	**[N]**
Sp	*4.07*	*50.2*	*24.2*	*0.789*
	2294	13330	4.74	0.109
N	*0.041*	*0.252*	*0.297*	*0.353*
	23.0	66.9	0.058	0.049
Sp x N	*3.77*	*0.124*	*0.112*	*0.033*
	2126	32.9	0.022	0.005
Block	*2.21*	*0.686*	*0.842*	*1.02*
	7470	911	0.822	0.700
Error	6203	2391	1.76	1.38
df	1, 11	1, 9	1, 9	1, 10

F and sum of square values are in italic and regular type, respectively.

Table S3 Results of three-way ANOVA for the effects of host species (Sp), infection with *Cassytha pubescens* (I) and nitrogen supply (N) on total, shoot and root biomass, foliar area (FA), shoot/root ratio (S/R), nodule biomass (Nod), nodule biomass g^{-1} root biomass (Nod g^{-1} root biomass) and foliar nitrogen concentration [N] of *Ulex europaeus* and *Acacia paradoxa*.

	Total	**Shoot**	**Root**	**FA**	**S/R**	**Nod**	**Nod g^{-1} root biomass**	**[N]**
Sp	*6.67*	*14.4*	*16.0*	*46.8*	*59.9*	*15.9*	*33.6*	*46.9*
	8186	13623	689	35025265	48.4	32.1	7.94	4.47
I	*40.9*	*34.3*	*45.2*	*11.2*	*2.73*	*13.4*	*0.619*	*0.229*
	50191	32395	1940	8369537	2.20	26.98	0.146	0.022
Sp x I	*6.64*	*5.08*	*10.3*	*1.94*	*1.51*	*0.297*	*0.088*	*1.53*
	8156	4799	442	1450567	1.22	0.601	0.021	0.146
N	*0.673*	*0.945*	*0.030*	*0.410*	*0.189*	*1.95*	*4.70*	*0.019*
	826	893	1.30	306894	0.152	3.95	1.11	0.002
Sp x N	*1.07*	*0.709*	*2.53*	*0.421*	*0.088*	*1.47*	*0.705*	*0.791*
	1318	670	109	315192	0.071	2.98	0.167	0.075
I x N	*0.160*	*0.198*	*0.003*	*0.018*	*1.42*	*0.085*	*0.162*	*6.51*
	196	187	0.114	13417	1.15	0.172	0.038	0.620
Sp x I x N	*1.54*	*0.883*	*4.97*	*0.451*	*1.32*	*1.17*	*3.23*	*0.387*
	1891	834	213	337326	1.07	2.36	0.765	0.037
Block	*2.75*	*2.79*	*1.34*	*1.73*	*1.54*	*0.755*	*0.157*	*1.33*
	20284	15832	346	7745487	7.45	9.15	0.223	0.761
Error	30696	23620	1074	18701385	20.2	50.5	5.91	2.38

F and sum of square values are in italic and regular type, respectively, and *df*=1, 25 and block *df*=6, 25.

Chapter 5: Water

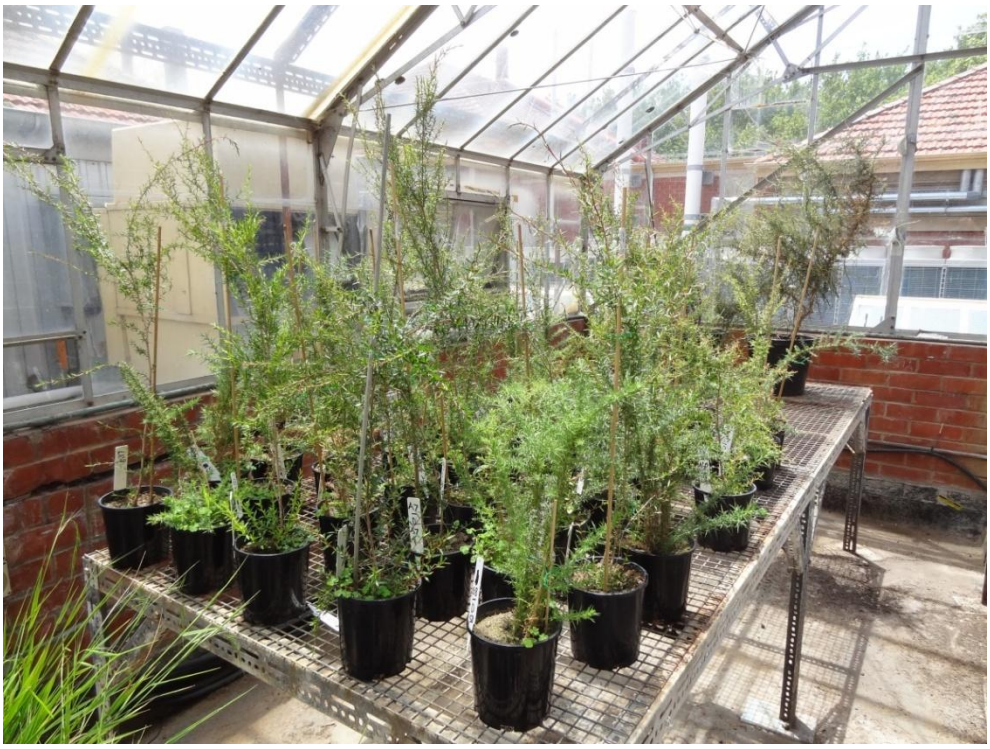

Fig. 1a. Photos for the water experiment that include the native host *Leptospermum continentale* which unfortunately did not become successfully infected with *Cassytha pubescens* in the time allocated for this process.

Statement of Authorship

Title of Paper	High water availability increases the negative impact of a native hemiparasite on its non-native host.
Publication Status	☑ Published ☐ Accepted for Publication ☐ Submitted for Publication ☐ Unpublished and Unsubmitted w ork w ritten in manuscript style
Publication Details	Cirocco, R. M., Facelli, J. M., & Watling, J. R. (2015). High water availability increases the negative impact of a native hemiparasite on its non-native host. Journal of Experimental Botany, erv548

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Contribution to the Paper	Co-conceived and designed the experiment, performed the experiment, analysed and interpreted the data, wrote manuscript and acted as corresponding author.
Overall percentage (%)	80
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.
Signature	Date 26/2/2016

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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High water availability increases the negative impact of a native hemiparasite on its non-native host

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Abstract

Environmental factors alter the impacts of parasitic plants on their hosts. However, there have been no controlled studies on how water availability modulates stem hemiparasites' effects on hosts. A glasshouse experiment was conducted to investigate the association between the Australian native stem hemiparasite *Cassytha pubescens* and the introduced host *Ulex europaeus* under high (HW) and low (LW) water supply. *Cassytha pubescens* had a significant, negative effect on the total biomass of *U. europaeus*, which was more severe in HW than LW. Regardless of watering treatment, infection significantly decreased shoot and root biomass, nodule biomass, nodule biomass per unit root biomass, F_v/F_m, and nitrogen concentration of *U. europaeus*. Host spine sodium concentration significantly increased in response to infection in LW but not HW conditions. Host water potential was significantly higher in HW than in LW, which may have allowed the parasite to maintain higher stomatal conductances in HW. In support of this, the $\delta^{13}C$ of the parasite was significantly lower in HW than in LW (and significantly higher than the host). *C. pubescens* also had significantly higher F_v/F_m and 66% higher biomass per unit host in the HW compared with the LW treatment. The data suggest that the enhanced performance of *C. pubescens* in HW resulted in higher parasite growth rates and thus a larger demand for resources from the host, leading to poorer host performance in HW compared with LW. *C. pubescens* should more negatively affect *U. europaeus* growth under wet conditions rather than under dry conditions in the field.

Key words: Biomass, carbon isotope, nitrogen, parasitic plant–host interactions, photoinhibition, sodium, water availability.

Introduction

Parasitic plants are an important and diverse functional group that can have significant impacts on all ecosystems inhabited by higher plants. For example, mistletoes have been identified as keystone species in a number of habitats where they contribute to biodiversity by providing habitat and food sources for a range of organisms including birds, which, in turn, pollinate flowers and aid seed dispersal of both hosts and mistletoes (Watson, 2001; van Ommeren and Whitham, 2002; Mathiasen *et al.*, 2008). Parasitic plants can also influence nutrient cycling in the ecosystems where they occur (March and Watson, 2007; Mathiasen *et al.*, 2008). For instance, in the nutrient-poor soils of the sub-arctic, litter of the root hemiparasite *Bartsia alpina*, can create fertile patches that enhance the growth of surrounding vegetation (Quested *et al.*, 2003; Press and Phoenix, 2005). Parasitic plants may also function as viable bio-controls as native hemi- and holoparasitic vines in Australia and China, respectively, have been found to have a much greater negative impact on growth of introduced (non-native) plants, compared with native host species (Prider *et al.*, 2009; Li *et al.*, 2012).

Differential impacts of parasites on native and introduced hosts may be driven by how effectively parasites connect to and remove resources from their host's vasculature via haustoria. The removal of host resources and subsequent effects on host performance are also influenced by a number of other factors including abiotic conditions. For instance, a high nitrogen supply has been found to dampen the effect of the stem holoparasite *Cuscuta reflexa* and the root hemiparasite *Striga hermonthica* on some hosts (Cechin and Press, 1993, 1994; Jeschke and Hilpert, 1997). While there are numerous studies on how nutrient supply affects the host–parasite relationship, there are surprisingly few studies investigating how water availability modulates the effects of the parasites on their hosts (Evans and Borowicz, 2013; Le *et al.*, 2015).

Using climate as a proxy for water availability, some studies have addressed water effects on associations involving mistletoes. In wetter environments, mistletoes tend not to maintain significantly higher transpiration rates or stomatal conductances than their hosts, which can affect their ability to withdraw resources from the host (Strong and Bannister, 2002). By contrast, in arid zones, mistletoes tend to have higher transpiration rates and stomatal conductances than their hosts, but they also track host transpiration (Ullmann *et al.*, 1985; Ehleringer *et al.*, 1986). Such co-ordination with the host may be necessary to prevent over-exploitation of water which would decrease the chances of survival for the

host, and thus the parasite, in more arid conditions (Ullmann *et al.*, 1985; Miller *et al.*, 2003). However, despite this co-ordination, there may be some conditions that are just too harsh for parasites successfully to establish on hosts. In a study of mistletoes infecting *Eucalyptus largiflorens* in semi-arid southern Australia, Miller *et al.* (2003) found that rates of mistletoe infection were higher in less stressed hosts growing in more hydrated conditions. They suggested that increasing water stress made *E. largiflorens* a less suitable host for mistletoes. This also raises the question of whether parasite performance is improved when growing on more hydrated hosts and whether, as a result, the parasite has a greater effect on host performance in these conditions.

To our knowledge, there have been no experimental studies of how water influences the effects of stem hemiparasites on hosts, mainly because mistletoes typically infect trees which would be difficult to use in controlled experiments. This study used a stem hemiparasite that infects shrubs and thus is suitable for such experimental manipulations. The results of a glasshouse experiment are reported here for the effects of the Australian native stem hemiparasite *Cassytha pubescens* on the physiology and growth of the introduced host *Ulex europaeus* in high water (HW) and low water (LW) conditions (see Supplementary Figs S1 and S2 at JXB online). Parasite performance in both treatments was also measured. It was predicted that *C. pubescens* would have a negative effect on this host and that it would be more pronounced in HW compared with LW treatment due to a better parasite performance when water availability was high.

Materials and methods

Study species

Ulex europaeus L. (Fabaceae) is a perennial, evergreen, leguminous shrub that reaches 1–4 m in height (Clements *et al.*, 2001; Tarayre *et al.*, 2007). Its stems and spines are both photosynthetic and it has few leaves (Clements *et al.*, 2001). It is native to Western Europe and North Africa but during the 20th century its range has expanded and it is now a highly noxious weed in Australia, New Zealand, Chile, Canada, Hawaii, and North America (Clements *et al.*, 2001). *Cassytha pubescens* R. Br. (Lauraceae) is a perennial, coiling hemiparasitic vine 0.5–1.5 mm thick that attaches to host stems and leaves via multiple haustoria (McLuckie, 1924; Weber, 1981). It has highly reduced leaves and its stems are photosynthetic (Prider *et al.*, 2009). It is widespread in south-eastern Australia and New

Zealand (Weber, 1981) and is frequently found infecting both native and introduced hosts (including *U. europaeus*) in South Australia (Prider *et al.*, 2009; Shen *et al.*, 2010).

Plant material and growth conditions

Ulex europaeus plants, all of around the same size (approximately 30 cm tall) and stage of development, were obtained from the field in early July 2013 (Mt. Lofty Ranges, South Australia: S 35º 00.456; E 138º 41.212). Each plant was transplanted into a 1.65 l pot filled with sandy loam. Randomly selected plants were infected with *C. pubescens* using the technique of Shen *et al.* (2010). Briefly, they were placed adjacent to large *U. europaeus* plants already infected with *C. pubescens*, allowing single stems of the parasite to attach to each new host. The connection with the donor host was severed in late November 2013, three months after infection was initiated. Newly attached *C. pubescens* were monitored for a further week to ensure that infection was successful. During the establishment of infection, all *U. europaeus* plants were provided with Nitrosol at rates recommended by the manufacturer (Rural Research Ltd, Auckland, New Zealand; NPK 8:3:6 wt. %). Individual plants, both infected and uninfected, were transplanted into 5.0 l pots in mid-December 2013 with the same sandy loam soil and provided with a single, recommended dose of Osmocote (Scotts-Sierra Horticultural Products, Marysville, OH, USA).

The experiment was carried out in an evaporatively cooled glasshouse at the University of Adelaide. Two watering regimes were established based on the field capacity of the soil which was determined using the filter-paper technique (Bouyoucos, 1929), but slightly modified as a vacuum was not required in this case. Briefly, 20 g of dry soil was made into a slurry using water and then poured into a filter paper and allowed to drain for 1 hr. The soil was then re-weighed and the field capacity (FC) calculated using the following formula:

$$FC = (S_W - S_D)/S_D$$

where S_W is the mass of the drained soil and S_D is the mass of the dry soil. In this case, the FC of the soil was 0.32. Thus, the mass of a 5.0 l pot of soil at 100% FC=1.32 × dry mass of soil in the pot (HW treatment=5.0 kg). Field capacity at 55% was 0.55 × 0.32=0.176. Thus, the mass of the 5.0 l pot at 55% FC was 1.176 × dry mass of soil in the pot (LW treatment=4.5 kg). Field capacity of 55% for the LW treatment was chosen because previous experiments in our laboratory (data not shown) had demonstrated that the parasite wilted below 55% while, by comparison, *U. europaeus* wilted at 40% FC. Uninfected and

infected plants were randomly allocated into the HW or LW treatments and there were four blocks containing all combinations of treatments. Pots in each treatment were weighed and watered accordingly, daily or every second day on cloudy days and re-randomized within each block fortnightly to negate small light differences in the glasshouse. Watering treatments ran from mid-February to mid-April 2014 when the plants were harvested.

Host and parasite chlorophyll a fluorescence

Photosynthetic light-use efficiency of *U. europaeus* and *C. pubescens* was measured using a portable, pulse-modulated chlorophyll fluorometer (Mini-PAM, Walz, Effeltrich, Germany) equipped with a leaf-clip (2030–B, Walz, Effeltrich, Germany). Pre-dawn (F_v/F_m) and midday (Φ_{PSII}) quantum yields (Genty *et al.*, 1989) were measured on *U. europaeus* spines, and also 15 cm from the growing tip of parasite stems 46 days after treatments had been imposed (DAT). Midday measurements were made on a sunny day between 12–1 pm at a photosynthetic photon flux density (PPFD) of approximately 1200 μmol m^{-2} s^{-1}.

Host water potentials

Midday shoot water potentials (Ψ) of *U. europaeus* were measured on freshly cut shoots using a Scholander-type pressure chamber with a digital gauge (PMS Instrument Company, Albany, OR). The balancing pressure was recorded once xylem sap had first appeared. Measurements were made between 1–2 pm (daylight saving time) on a sunny day 52 DAT. Water potential measurements on the parasite were not possible due to insufficient quantities of parasite tissue and also because the morphology of the parasite makes it very difficult to obtain Ψ measurements using a pressure chamber.

Host and parasite biomass, $\delta^{13}C$, nitrogen, and sodium concentration

The shedding of plant tissue in response to infection did not take place during the experiment (personal observations). Unfortunately, an initial harvest to enable quantification of host/parasite growth increments over the experimental period was not possible because of pre-experimental plant mortality leaving n=4. A final harvest was conducted 60 DAT with plants divided into spines (no leaves present), stems, roots, and nodules, and separated from parasite stems in the case of infected hosts. Both host and parasite material was oven-dried at 60 ºC for 6 d. The spine area was calculated using

previously determined positive linear relationships between spine weight and area for each treatment combination (all R >0.99) (Rolston and Robertson, 1976).

Stable carbon isotope composition and nitrogen concentration of host spines and parasite stems were determined using a Horizon isotope ratio mass spectrometer (Nu Instruments Ltd., Wrexham, UK) and a Euro elemental analyser (EuroVector, Tortona, Mil.) at the University of Adelaide. Sodium content of host spines and parasite stems was quantified with the Spectro CIROS CCD Radial Inductively Coupled Plasma Optical Emission Spectrometer (SPECTRO Analytical Instruments GmbH, Kleve, Germany) at Waite Analytical Services (University of Adelaide). All analyses were conducted on final harvest oven-dried material.

Statistical analysis

The variances of the data were homogenous and a two-way ANOVA was used to test for infection and water effects on *U. europaeus*. The additive effects of infection; comparisons between uninfected (uninfected HW and LW plants pooled) and infected (infected HW and LW plants pooled) plants, or the additive effects of water; comparisons between HW (uninfected and infected HW plants pooled) and LW (uninfected and infected LW plants pooled) plants were only considered if the interaction between infection × water was not significant. One-way ANOVA was conducted on *C. pubescens* data to test for any effects of water. Interactions and additive significant effects of infection or water generated by a Standard least squares model were only considered when pairwise comparisons of means were significant using a Tukey–Kramer HSD test. All data were analysed with the software JMP Ver. 4.0.3 (SAS Institute Inc., 2000) and α=0.05.

Results

Quantum yields of host and parasite

There was no interaction between infection × water for F_v/F_m or Φ_{PSII} of *U. europaeus* (Table 1; Fig. 1a, b). There was, however, an independent effect of infection on F_v/F_m but not on Φ_{PSII} (Table 1; Fig. 1a). On average, F_v/F_m of infected plants (0.775 ± 0.014) was 6% lower than that of uninfected plants (0.823 ± 0.006), regardless of watering treatment. There were no significant independent effects of watering on host F_v/F_m or Φ_{PSII} (Table 1).

F_v/F_m of *C. pubescens* was significantly affected by water (Table 2). F_v/F_m of the parasite in LW was 13% lower relative to that in HW conditions (Fig. 1c). There was no effect of

water on parasite Φ_{PSII} when measured under prevailing light conditions at midday (Table 2; Fig. 1d).

Host and parasite biomass

Infection had a differential impact on total biomass of *U. europaeus* in HW and LW (significant interaction, Table 3; Fig. 2a). Infection decreased total biomass of *U. europaeus* by 69% and 43% in the HW and LW treatments, respectively (Fig. 2a). Although there was a significant interaction for shoot biomass which followed a similar pattern, no significant difference was detected by the pairwise comparison (Table 3; Fig. 2b). Root biomass also followed a similar trend but no interaction was detected (Table 3; Fig. 2c). However, there were significant infection effects on both shoot and root biomass (g dwt) (Table 3; Fig. 2b, c). On average, shoot biomass of infected plants (18.3 ± 1.8) was approximately 60% lower compared with that of uninfected plants (47.3 ± 2.6), irrespective of watering treatment. In addition, root biomass of infected *U. europaeus* (9.6 ± 1.4) was 43% lower than that of uninfected plants (16.9 ± 0.8). There was a trend for the biomass of *C. pubescens* to be higher on HW than LW hosts and this difference was marginally significant on a per unit host biomass basis (P=0.069) (Table 2; Fig. 3a, b).

The spine area (SA) of *U. europaeus* was affected in a non-independent way by infection and water (significant interaction; Table 3). Infection decreased spine area by 83% and 51% in the HW and LW treatments, respectively (Table 4). There was no interaction detected for shoot/root ratio, nodule biomass or nodule biomass g^{-1} root biomass, and these parameters were affected only by infection (Table 3). The shoot/root ratio of infected plants was 28% lower compared with that of uninfected plants (Table 4). Nodule biomass of infected plants was an order of magnitude lower relative to that of uninfected plants, and infection decreased nodule biomass g^{-1} root biomass by 82% (Table 4).

Ψ, $\delta^{13}C$, and tissue N and Na concentrations

There was no interaction between infection × water or independent infection effect for Ψ of *U. europaeus*, but this parameter was affected by water treatment (Table 5). Water potentials of *U. europaeus* under LW were 28% lower than those of HW plants (Table 4). There was no significant interactive effect on $\delta^{13}C$ values of *U. europaeus* and, although the model detected a significant additive infection effect, the Tukey test did not find a difference (Tables 4, 5). There was a significant effect of water on $\delta^{13}C$ of *C. pubescens* (Table 2). Parasite $\delta^{13}C$ in LW (–26.7 ± 0.149‰) was 5% higher compared with that in

HW conditions (–28.2 ± 0.135‰) (significant water effect; Table 2). Also, the carbon isotope composition of *C. pubescens* was significantly higher (species effect, P <0.0001) than that of the uninfected and infected hosts in both water treatments (Table 4) (no species × water interaction).

There was no interactive effect of infection × water for spine nitrogen concentration of *U. europaeus*, but it was affected by infection (Table 5; Fig. 4a). On average, nitrogen concentration (%) of infected plants (1.92 ± 0.09) was 12% lower than that of uninfected plants (2.19 ± 0.06). By contrast, there was a significant interaction between infection × water on the sodium concentration of *U. europaeus* spines (Table 5). There was no effect of the parasite in HW conditions, whereas in LW, the sodium concentration increased by 65% in response to infection (Fig. 4b).

Water had no effect on the stem nitrogen concentration of *C. pubescens* (Table 2; Fig. 4c). By contrast, there was an effect of water on the sodium concentration of *C. pubescens* (Table 2). The sodium concentration of the parasite in LW was 2-fold higher relative to that in HW conditions (Fig. 4d).

Discussion

The hypothesis that *C. pubescens* would have a negative effect on *U. europaeus*, and that it would be more severe in the HW treatment was supported by the results presented here. Indeed, infection decreased total biomass of *U. europaeus* by nearly 30% more when plants were in HW compared with LW conditions. Similarly, Evans and Borowicz (2013) found that shoot and root biomass of *Verbesina alternifolia* were affected by the stem holoparasitic vine *Cuscuta gronovii*, and these effects were stronger in well-watered relative to dry conditions. Our finding may be due to hosts with a much higher water status (additive water effect; Table 2) possibly permitting higher transpiration rates in the parasite and thus greater resource uptake. This would lead to greater parasite growth and, in turn, further removal of resources from the host that could otherwise be used for photosynthesis and growth.

Following on, *C. pubescens* had higher biomass per unit of host biomass in HW compared with LW conditions, although this was only significant at α <0.07. Similarly, *Cuscuta gronovii* grew significantly larger in absolute and per unit host biomass terms in wet than in droughted treatments (Evans and Borowicz, 2015). As mentioned above, parasite growth in HW may have been greater because of increased resource removal from the host, but

also because of increased photosynthesis in the parasite. The decrease in parasite biomass per unit host under LW may be directly due to the relatively high Na concentration in *C. pubescens* in these conditions (Table 2; Figs 3b, 4d) (Taiz and Zeiger, 2002). It may also be due to the much lower F_v/F_m of the parasite in LW which is evidence of chronic photoinhibition in *C. pubescens*, compared with HW conditions (Demmig-Adams and Adams, 2006). Inoue *et al.* (2013) on the other hand, found no effect of water on F_v/F_m of *S. hermonthica* infecting sorghum, however, it should be kept in mind that drought treatments in this study only lasted 1–2 d. Here, the relatively high Na concentration in the parasite in LW may also directly explain the decrease in parasite F_v/F_m and or indirectly given that it may affect gas exchange, e.g. stomatal conductance (James *et al.*, 2002; Taiz and Zeiger, 2002; Parida and Das, 2005; Ranjbarfordoei *et al.*, 2006). The fact that $\delta^{13}C$ of *C. pubescens* was significantly higher in LW than in HW conditions does infer that the parasite maintained lower stomatal conductances in LW (Scalon and Wright, 2015). This may also have occurred if the parasite found it increasingly difficult to extract water from the hosts under the LW treatment, which could be likely given that host Ψ was significantly lower in these conditions (Table 4). Declines in parasite F_v/F_m in the LW treatment could also have occurred if stem N concentration was lower, however, this parameter was unaffected by watering treatment (Fig. 4c).

Infection had a negative effect on F_v/F_m of *U. europaeus*, regardless of water treatment. On the other hand, Le *et al.* (2015) found that a fluorescence parameter used as a proxy for F_v/F_m of *Mikania micrantha* was negatively affected by *Cuscuta australis* in droughted but not in well-watered treatments. Here, infection effects may, in part, be due to the negative effect of *C. pubescens* on the N concentration of *U. europaeus* (additive infection effect; Table 5; Fig. 4a). A similar explanation was provided for the strong decline in apparent quantum yield of *M. micrantha* in response to infection with *Cuscuta campestris* (Shen *et al.*, 2013). Moreover, depressions in F_v/F_m of some plant species have resulted from N deficiency (Verhoeven *et al.*, 1997; Huang *et al.*, 2004; Zhou *et al.*, 2006). Ultimately, our finding may be explained by the removal of N by the parasite. Infection negatively affecting host nitrogen would probably affect photosynthetic performance and should result in less carbohydrate which would explain significant infection effects on nodulation and nodulation per unit root biomass which might further limit the acquisition of N by infected plants.

Interestingly, infection had no effect on the Ψ of *U. europaeus*, in either HW or LW conditions. Similarly, Inoue *et al.* (2013) also found no effect of the root hemiparasite *S. hermonthica* on the relative water content of sorghum in either wet or dry treatments. The lack of an infection effect of host Ψ may be due to infected plants having lower stomatal conductances which would ameliorate their water status; but their more negative $\delta^{13}C$ does not support this notion. A more likely explanation may be related to significant reductions in host growth. All things being equal, a smaller infected plant requires less water than a larger uninfected plant to maintain similar water potentials. Further, although, infected hosts in LW received less water than smaller HW infected hosts, it is likely that the parasite also removed less water in these conditions due to stomatal limitations as inferred from the carbon isotope composition of the parasite mentioned earlier. In addition, infected LW hosts were significantly enriched in sodium (with respect to all other plants) which would make their osmotic potential and thus, water potential more negative. This would have the dual benefit of facilitating water uptake from the soil and impeding water removal by *C. pubescens* in this treatment. Infected LW plants did have the lowest water potentials, which is consistent with this argument.

This experiment clearly demonstrated that the impact of *C. pubescens* on total biomass of *U. europaeus* was more severe under conditions of high water availability. This may be due to a well-hydrated host resulting in a well-hydrated, healthy parasite that is capable of maintaining higher stomatal conductance ($\delta^{13}C$) and, hence, removing more resources from the host. Importantly, $\delta^{13}C$ of the parasite was significantly higher than that of both uninfected and infected *U. europaeus*, suggesting that the parasite was more conservative in its water use than the host. To our knowledge, this finding has not previously been reported for stem hemiparasitic plant–host associations. By contrast, Scalon and Wright (2015), looking at the $\delta^{13}C$ of 168 mistletoe–host pairs from 39 sites across the globe, in general, found the opposite to be true. This discrepancy between findings may be due to mistletoes mainly infecting trees that would have a much larger root system and hence have access to more water than plants in pots. Nevertheless, Scalon and Wright (2015) showed that mistletoes and their hosts save more water as moisture decreases. Here, the carbon isotope composition of the plants is in line with this, inferring that *C. pubescens* maintained lower stomatal conductances in LW (Scalon and Wright, 2015) and, in this case, even more so than the host. From the above, it was speculated that water supply, in conjunction with size of host roots and surface area of the parasite, may dictate the performance of *C. pubescens*. This was corroborated by the fact that *C. pubescens* was

observed to wilt (below 55% FC) well before *U. europaeus* (40% FC) (personal observations).

From the evidence, it is concluded that, when infected with *C. pubescens*, the growth of *U. europaeus* would decrease in mesic conditions more than in drier conditions. Nonetheless, even in times of prolonged drought, which are predicted as a consequence of climate change for many of the regions where *U. europaeus* occurs, the data clearly indicate that *C. pubescens* will still have a strong impact on the biomass of *U. europaeus*.

Supplementary data

Supplementary data can be found at JXB online.

Supplementary Fig. S1. Photos of the stem hemiparasite *Cassytha pubescens* growing on the introduced host *Ulex europaeus* in high (HW) and low (LW) water treatments.

Supplementary Fig. S2. Close-up photos of *C. pubescens* growing tips when infecting *U. europaeus* in HW and LW treatments.

Acknowledgements

Special thanks to Dr Jane Prider whose ‘mother plant’ I used for the infection process, Associate Professor Robert J Reid for help with determining the soil field capacity technique and with regard to my spine samples, Mark Rollog for his expert analysis with the IRMS, and Waite Analytical for their expert ICP-OES analysis. This work was supported by the Field Naturalists Society of South Australia Lirabenda Endowment Fund [61113283].

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Table 1. *Results of two-way ANOVA on the additive effects of infection with C. pubescens (I), watering treatment (W), and their interaction I×W on pre-dawn and midday quantum yields (F_v/F_m, Φ_{PSII}) of U. europaeus*

P, *F*, and sum of square values are in bold, italic, and regular type, respectively, and *df*=1, 9 for all parameters.

	$\boldsymbol{F_v/F_m}$	$\boldsymbol{\Phi_{PSII}}$
I	**0.019**	**0.121**
	8.14	*2.94*
	0.009	0.013
W	**0.743**	**0.299**
	0.114	*1.21*
	0.0001	0.005
I x W	**0.525**	**0.893**
	0.438	*0.019*
	0.0005	0.00009
Block	**0.663**	**0.896**
	0.546	*0.196*
	0.002	0.003
Error	0.010	0.040

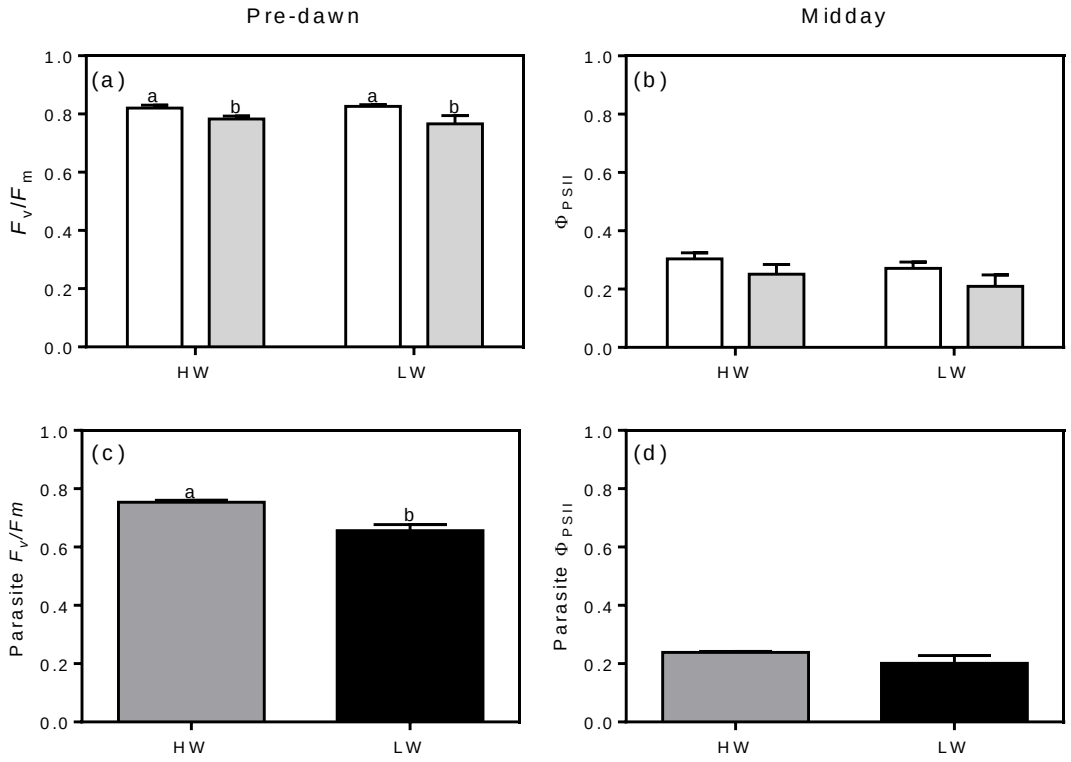

Fig. 1. (a) Pre-dawn (F_v/F_m) and (b) midday (Φ_{PSII}) quantum yields of *U. europaeus* uninfected (open bars) or infected (grey bars) with *C. pubescens* in high (HW) or low (LW) water conditions. (c) F_v/F_m and (d) Φ_{PSII} of *C. pubescens* infecting *U. europaeus* in HW (dark grey bars) or LW (black bars) conditions. Different letters denote significant differences, data are means (±1 SE) and n=4.

Table 2. *Results of one-way ANOVA on effects of watering treatment (W) on pre-dawn and midday quantum yields (F_v/F_m, Φ_{PSII}), carbon isotope composition ($\delta^{13}C$), stem nitrogen (N) and sodium (Na) concentration, parasite biomass, and parasite biomass g^{-1} host biomass of C. pubescens when infecting U. europaeus*

P, *F*, and sum of square values are in bold, italic, and regular type, respectively, and *df*=1, 3 for all parameters.

	F_v/F_m	Φ_{PSII}	$\delta^{13}C$	N	Na	Biomass	Biomass g^{-1} host biomass
W	**0.011**	**0.265**	**0.001**	**0.426**	**0.011**	**0.118**	**0.069**
	33.0	*1.87*	*135*	*0.843*	*32.7*	*4.71*	*7.78*
	0.019	0.003	4.62	0.061	94531250	59.8	0.382
Block	**0.264**	**0.550**	**0.155**	**0.337**	**0.465**	**0.333**	**0.297**
	2.23	*0.853*	*3.72*	*1.70*	*1.12*	*1.73*	*1.96*
	0.004	0.004	0.381	0.370	9693750	65.7	0.289
Error	0.002	0.005	0.103	0.218	8673750	38.1	0.147

Table 3. *Results of two-way ANOVA on the additive effects of infection with C. pubescens (I), watering treatment (W), and their interaction I×W on total, shoot, and root biomass, spine area (SA), shoot/root ratio (S/R), nodule biomass (Nod), and Nod g^{-1} root biomass of U. europaeus*

P, *F*, and sum of square values are in bold, italic, and regular type, respectively, and *df*=1, 9 for all parameters. Although the interaction for shoot biomass was significant, because the pairwise comparison did not detect these differences this effect was not considered.

	Total	**Shoot**	**Root**	**SA**	**S/R**	**Nod**	**Nod g^{-1} root**
I	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**0.005**	**0.0008**	**0.0006**
	186	*178*	*45.8*	*226*	*13.5*	*24.5*	*26.4*
	5263	3355	214	765822	2.46	0.295	0.0008
W	**0.132**	**0.733**	**0.008**	**0.049**	**0.051**	**0.035**	**0.032**
	2.74	*0.124*	*11.4*	*5.18*	*5.08*	*6.16*	*6.38*
	77.7	2.34	53.1	17508	0.922	0.074	0.0002
I x W	**0.006**	**0.007**	**0.092**	**0.003**	**0.429**	**0.081**	**0.075**
	12.9	*12.0*	*3.56*	*16.8*	*0.686*	*3.87*	*4.07*
	365	226	16.6	56658	0.125	0.047	0.0001
Block	**0.048**	**0.078**	**0.114**	**0.051**	**0.313**	**0.747**	**0.423**
	3.95	*3.17*	*2.63*	*3.82*	*1.37*	*0.415*	*1.03*
	336	179	36.8	38780	0.746	0.015	0.00009
Error	255	170	42.0	30448	1.63	0.109	0.0003

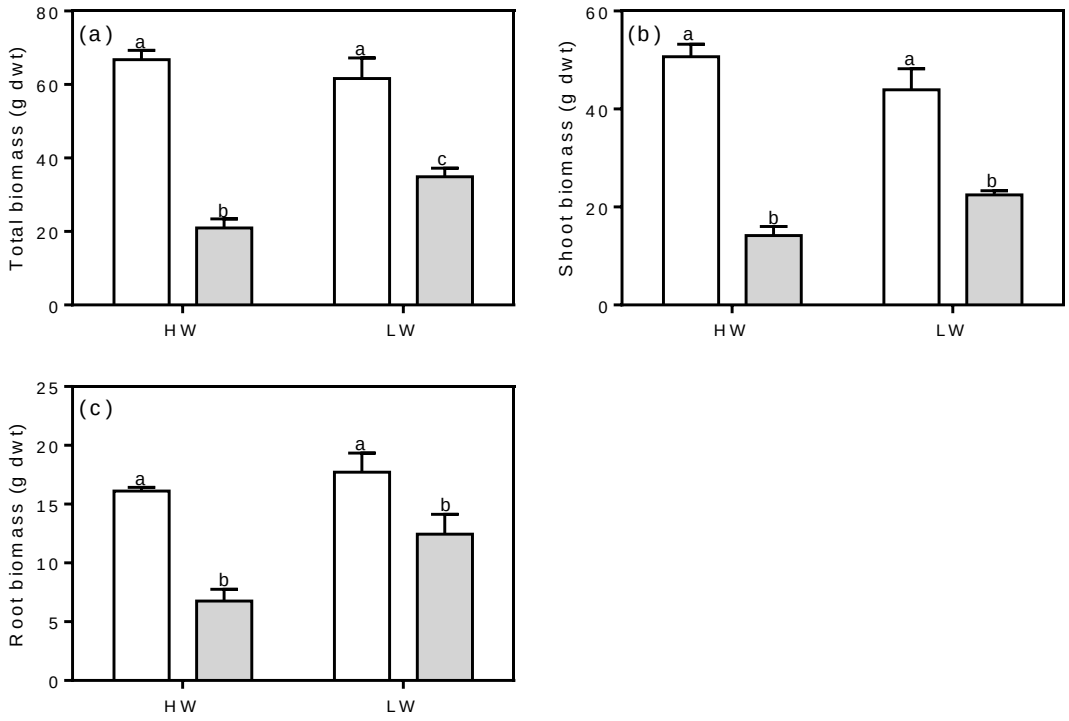

Fig. 2. (a) Total, (b) shoot, and (c) root biomass (g dwt) of *U. europaeus* either uninfected (open bars) or infected (grey bars) with *C. pubescens* in high (HW) or low (LW) water conditions. Different letters denote significant differences, data are means (±1 SE) and *n*=4.

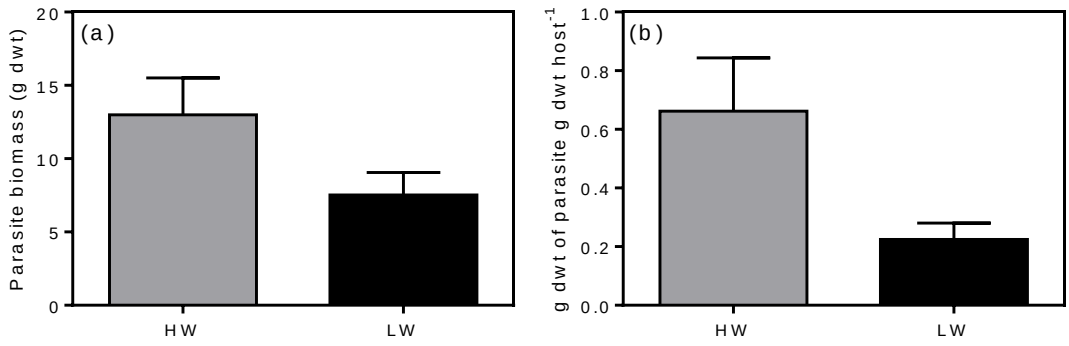

Fig. 3. (a) Parasite biomass (g dwt) and (b) parasite biomass supported per unit host biomass (g dwt g^{-1} dwt host biomass) of *C. pubescens* infecting *U. europaeus* in high (HW, dark grey bars) or low (LW, black bars) water conditions. No significant differences were detected, data are means (±1 SE) and *n*=4.

Table 4. *Spine area (SA, cm^2), shoot/root ratio (S/R), nodule biomass (Nod, g dwt), Nod g^{-1} root biomass, water potential (Ψ, MPa), and carbon isotope values ($\delta^{13}C$, ‰) of U. europaeus, either uninfected (–) or infected (+) with C. pubescens under high (HW) or low (LW) water supply*

Data are means (±1 SE) and letters denote significant differences for interaction between infection (I) × water (W) for SA (*n*=4), additive (I) effect for S/R, Nod, and Nod g^{-1} root, and additive (W) effect for Ψ (*n*=8). Additively, although the effect of (I) on $\delta^{13}C$ and (W) on S/R, Nod, Nod g^{-1} root, and $\delta^{13}C$ was significant, it was not considered because the pairwise comparison did not detect any difference.

	SA	**S/R**	**Nod**	**Nod g^{-1} root**	**Ψ**	**$\delta^{13}C$**
HW–	672.0 ± 31.7a	3.15 ± 0.170	0.180 ± 0.073	0.011 ± 0.004	−1.91 ± 0.075	−29.2 ± 0.372
LW–	619.1 ± 63.2a	2.49 ± 0.184	0.424 ± 0.069	0.024 ± 0.003	−2.67 ± 0.006	−28.2 ± 0.280
HW+	115.4 ± 17.8b	2.19 ± 0.310	0.016 ± 0.009	0.003 ± 0.002	−1.98 ± 0.043	−29.7 ± 0.627
LW+	300.6 ± 21.3c	1.89 ± 0.199	0.045 ± 0.012	0.004 ± 0.002	−2.76 ± 0.221	−29.5 ± 0.304
Infection						
–	–	2.82 ± 0.170a	0.302 ± 0.066a	0.017 ± 0.003a	−2.29 ± 0.148	−28.7 ± 0.290
+	–	2.04 ± 0.180b	0.030 ± 0.009b	0.003 ± 0.001b	−2.44 ± 0.199	−29.6 ± 0.326
Water						
HW	–	2.67 ± 0.244	0.098 ± 0.046	0.007 ± 0.003	−1.95 ± 0.042a	−29.5 ± 0.350
LW	–	2.19 ± 0.170	0.234 ± 0.079	0.014 ± 0.004	−2.71 ± 0.086b	−28.9 ± 0.309

Table 5. *Results of two-way ANOVA on the additive effects of infection with C. pubescens (I), watering treatment (W), and their interaction I×W on water potential (Ψ), carbon isotope values ($\delta^{13}C$), spine nitrogen and sodium concentrations of U. europaeus*

P, *F*, and sum of square values are in bold, italic, and regular type, respectively, and *df*=1, 9 for all parameters.

	Ψ	**$\delta^{13}C$**	**N**	**Na**
I	**0.245**	**0.044**	**0.044**	**0.116**
	1.55	*5.51*	*5.51*	*3.02*
	0.092	3.13	0.286	40322500
W	**<0.0001**	**0.129**	**0.221**	**0.058**
	47.4	*2.79*	*1.73*	*4.73*
	2.80	1.59	0.090	63202500
I x W	**0.546**	**0.322**	**0.865**	**0.032**
	0.394	*1.10*	*0.031*	*6.47*
	0.023	0.624	0.002	86490000
Block	**0.722**	**0.193**	**0.639**	**0.900**
	0.453	*1.94*	*0.586*	*0.191*
	0.080	3.31	0.091	7660000
Error	0.532	5.12	0.467	120245000

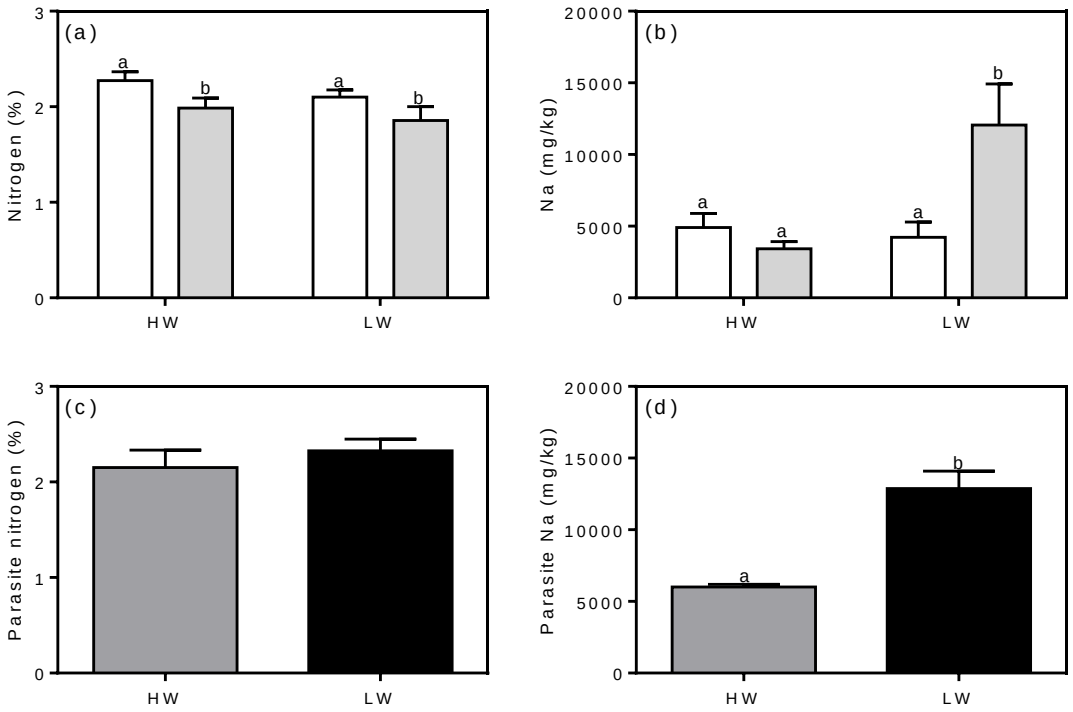

Fig. 4. (a) Spine nitrogen (% dwt) and (b) sodium (mg kg^{-1}) concentration of *U. europaeus* either uninfected (open bars) or infected (grey bars) with *C. pubescens* in high (HW) or low (LW) water conditions. (c) Stem nitrogen and (d) sodium concentration of *C. pubescens* infecting *U. europaeus* in HW (dark grey bars) or LW (black bars) conditions. Different letters denote significant differences, data are means (±1 SE) and *n*=4.

Water and native hemiparasite effects on an introduced host

Supplementary data, Journal of Experimental Botany

High water availability increases the negative impact of a native hemiparasite on its non-native host.

Robert M. Cirocco, José M. Facelli, Jennifer R. Watling

Figure S1. *Ulex europaeus* plants infected with the stem hemiparasite *Cassytha pubescens* (arrow) from the LW (left) or HW (right) treatments.

Figure S2. Close up of tips of the stem hemiparasite *C. pubescens* when infecting *U. europaeus* in the LW (top) or HW (bottom) treatments.

Chapter 6

Conclusion

In the past, much of the research on the impact of parasitic plants on their hosts has focused on very few species that are mainly of concern to agriculture; however, there are a few exceptional cases. For example, there have been evaluations on various aspects of native host:parasite associations in south/western Australia involving the mistletoe *Amyema preissii* (Miq.) Tiegh. and the root hemiparasites *Olax phyllanthi* (Labill.) R. Br. (Olacaceae) and *Santalum acuminatum* R. Br. A. DC. (Santalaceae) (Tennakoon and Pate, 1996; Tennakoon *et al.*, 1997a; Tennakoon *et al.*, 1997b). The lack of investigations in natural systems has limited our understanding of parasite effects on ecosystem function, and differential impacts of parasitic plants on various hosts which may control community structure (Press and Phoenix, 2005). One possible driver of these differential impacts is co-evolution between host and parasite. Over the past 10 or so years evidence has accumulated that indicates that native parasitic plants, both holo and hemiparasites, can have a much greater effect on growth and performance of introduced than native hosts. However, little is known about the mechanisms and processes behind these differential effects. Further, there have been surprisingly few studies that have investigated the influence of abiotic factors on parasite effects on their hosts. My PhD project which used the native Australian stem hemiparasite, *Cassytha pubescens* and a range of native and introduced hosts, addresses some of these gaps.

Summary of Main Findings

Light experiment (Ch. 2): It was predicted that as a result of parasite photosynthesis declining in low (LL) relative to high light (HL), *C. pubescens* would become more dependent on host carbon and have a greater effect on host growth in these conditions. However, light did not influence the effect of the perennial stem hemiparasite *C. pubescens* on total biomass of the introduced (*Ulex europaeus*) or native host (*Leptospermum myrsinoides*). Similarly, Borowicz and Armstrong (2012) found that shade did not influence the effect of the perennial root hemiparasite *Pedicularis canadensis* on biomass of the C_4 grass *Andropogon gerardii*. In addition, the biomass of *C. pubescens* per unit host biomass was also not influenced by light. My findings suggest that stem hemiparasites do

not increase their dependency on host carbon in low light to the point where host growth is more affected in these conditions.

Pigments (Ch. 3): In the light experiment it was found that infection with *C. pubescens* negatively affected midday electron transport rates of the native host *L. myrsinoides* in HL but not LL. Consequently, it was hypothesised that infected plants in HL would be exposed to excess light and would increase their xanthophyll capacity (VAZ/Chl) and engagement (de-epoxidation state) to prevent photodamage. Yet, it was found that VAZ/Chl and de-epoxidation state of the native *L. myrsinoides* were unaffected by infection with *C. pubescens*, irrespective of light conditions. To my knowledge, these are the first reports of their kind in the field of parasitic plants and may explain why I also found no signs of photodamage in *L. myrsinoides*. Ultimately, the lack of an infection effect on photoprotective capacity/engagement and PSII integrity of *L. myrsinoides* may also explain why overall growth of this native host is unaffected by this native parasite. By contrast, Shen *et al.* (2010) found signs of photodamage in the introduced host *Cytisus scoparius* in response to infection with *C. pubescens*. Investigations into the influence of abiotic factors on the effects of infection on pigment dynamics of less tolerant hosts (including *U. europaeus*) may be powerful tools in explaining some of the contributing factors responsible for their ultimate demise.

Nitrogen experiment (Ch. 4): Legumes increase their engagement with rhizobia at low versus high nitrogen supply and this comes at an additional carbon cost to the host. However, host carbon may already be in short supply due to infection effects on host photosynthesis. Thus, it was presumed that performance of leguminous hosts (particularly the introduced host) would be more negatively affected by *C. pubescens* when not supplied with nitrogen (LN). At LN, although root biomass of *Acacia paradoxa* was affected by infection, this native host responded by maintaining nodule biomass similar to that of uninfected plants. On the other hand, at LN, although nodule biomass of *U. europaeus* was affected by infection, root growth of this introduced host was less severely affected by *C. pubescens* in these conditions. Thus, both infected *A. paradoxa* and *U. europaeus* overcame nitrogen limitations at LN by different means, increasing nodulation and root growth, respectively. Consequently, nitrogen availability was not found to influence the effect of *C. pubescens* on total biomass of its leguminous hosts.

Future research would include investigating the influence of nitrogen on the effect of *C. pubescens* on non-leguminous hosts. The outcomes of such a study may be very different

to those reported here with nitrogen possibly influencing the effect of the parasite on host total biomass. While these non-leguminous hosts would not have the additional carbon burden of rhizobia at low N supply, they would also not have access to nitrogen from this external source and may be unable to cope with nitrogen removal by the parasite under low N supplements.

Water experiment (Ch. 5): It was predicted that *C. pubescens* would have a greater negative effect on *U. europaeus* in high (HW) compared with low water (LW) treatments due to improved parasite performance in these conditions. Confirming this hypothesis, total biomass of *U. europaeus* was significantly affected by *C. pubescens* in both treatments, but more severely in HW. As expected, this differential impact may be explained by increased parasite performance under HW likely resulting in more effective removal of host resources. In support, biomass of *C. pubescens* per unit host was significantly ($\alpha<0.07$) higher in HW relative to LW (I also observed browning of some *C. pubescens* tips when in LW, Ch. 5: supplementary data, Figure S2). F_v/F_m of the parasite was also significantly ($\alpha<0.05$) higher under HW. It is unlikely that the decrease in photosynthetic performance of *C. pubescens* in LW resulted from nitrogen limitations as parasite stem nitrogen concentration was similar between treatments. Rather, these decreases in photosynthetic performance and growth may be due decreases in stomatal conductance of the parasite in LW. This may be the case as the significantly higher carbon isotope composition ($\delta^{13}C$) of *C. pubescens* in LW relative to HW, suggests that the parasite maintained lower stomatal conductance in LW. All of the effects observed on *C. pubescens* may also be due to the realtively high sodium (Na) concentration found in the parasite under LW. One explanation for the high Na in *C. pubescens* is passive uptake as it reflects the significantly higher Na concentrations that were only detected in infected *U. europaeus* under LW. If the movement of Na was passive it might have been driven by osmotic accumulation (e.g. Na and K) at the haustorial interface rather than high rates of transpiration considering the inference that the parasite had lower stomatal conductance than the host.

Given my findings with this introduced host, it would be interesting to investigate the impact of water availability on the association between *C. pubescens* and native hosts. Notably, I noticed browning of parasite stem tips when infecting *L. myrsinoides* in the light experiment, analogous to that consistently observed for the parasite on *U. europaeus* in LW. As *L. myrsinoides* in the light experiment was well-watered, this browning of parasite

tips may have been due to an ineffective parasite haustorial connection and removal of resources from this native host. In low water if the native host responds similarly to infected *U. europaeus* in LW and lowers its water potential, it is possible that parasite may find it even more difficult to remove water from the native host and perform poorly relative to well-watered conditions. However, it is also possible that native hosts such as *L. myrsinoides* may decrease their stomatal conductance if droughted, which could improve the water potential of the host and consequently the parasite's ability to remove water by maintaining relatively higher transpiration rates and or osmotic loading, but this remains to be tested.

Broader Significance of My Findings

Impact of abiotic factors on the association

My studies have revealed that light and nitrogen supply (at least when hosts are legumes), within the ranges studied, are not important in modulating the effects of the stem hemiparasite *C. pubescens* on total biomass of the hosts investigated. By contrast, water was an important factor, with the parasite having a more severe effect on *U. europaeus* under well-watered conditions. Thus, by manipulating abiotic factors I demonstrated that in the case of water, performance and growth of *C. pubescens* was limited by resource supply to the host, such that the impact of infection on the host was different between treatments.

By contrast, in the light experiment, limiting light to both host and parasite did result in a similar impact of infection on the host between experimental conditions. Low light significantly limited both photosynthesis and growth of *U. europaeus* and thus, presumably supply of resources including carbon to the parasite. At the same time, photosynthesis and growth of *C. pubescens* on *U. europaeus* was also significantly lower in LL. The end result was that while both host and parasite were smaller in LL than HL, the relative effect of *C. pubescens* on *U. europaeus* growth in LL was the same as in HL. I hypothesised that in LL *C. pubescens* may have increased its dependence on the host for carbon, and that this would result in a greater relative impact on host growth in LL than in HL, but this was not the case. This finding suggests that the parasite is not controlling the allocation of resources from the host but rather the parasite's performance and impact is dictated by the host's ability to provide resources.

In the nitrogen experiment, not supplying additional nitrogen (LN) to the hosts also led to a similar impact of infection on host total biomass between nitrogen treatments. One potential way to overcome nitrogen limitations would be to increase nodulation, but this should come at a higher carbon cost which may impact on host growth, especially if the host was also infected with *C. pubescens*. However, infected *U. europaeus* at LN did not maintain nodule per root biomass (and likely did not incur this added carbon expense) relative to that of respective uninfected plants but rather increased root growth as a way of acquiring sufficient nitrogen. On the other hand, infected *A. paradoxa* at LN obtained adequate amounts of nitrogen by maintaining nodule per root biomass while probably offsetting this carbon cost with significant decreases in root growth compared with that of respective uninfected plants. The end result was that infected plants of both species presumably reconciled potentially higher carbon costs associated with rhizobia at LN, albeit by different means, while maintaining similar nitrogen concentrations relative to that of respective uninfected plants. Thus, it makes sense that I found no evidence of an increased impact of infection on overall host growth under LN. This was despite the fact that infected plants at LN (both species pooled) had lower foliar nitrogen concentration than infected plants supplied with nitrogen (HN). Evidently, this difference was too small to affect the nitrogen concentration, biomass and biomass per unit host of *C. pubescens* (i.e. parasite performance) and impact of the parasite on overall host growth between nitrogen treatments.

In contrast to nitrogen or light under my experimental conditions, limiting water supply did result in the impact of infection on the introduced host being different between treatments. Firstly, the parasite seems more sensitive to water availability than the host and thus, if water supply to the host is below a certain threshold the parasite will likely not survive. This was deduced from my observation that *C. pubescens* wilted below 55% field capacity while *U. europaeus* only began to wilt at 40% field capacity during the experimental set-up period of this experiment. This was supported by the finding that $\delta^{13}C$ of the parasite was significantly less negative than the host (also found in the field study at two of the three sites, Appendix 2: Fig. 4c), suggesting that *C. pubescens* was more conservative in its intrinsic water use efficiency than *U. europaeus*. This is a novel finding for stem hemiparasites as mistletoes typically have more negative $\delta^{13}C$ and thus, are generally less conservative in their water-use than their hosts (Scalon and Wright, 2015). Secondly, when water supply to the host was decreased, *C. pubescens* became even more conservative in its

water-use as indicated by its significantly higher $\delta^{13}C$ in LW versus HW. Higher $\delta^{13}C$ is generally linked to water stress, which can be a consequence of either an arid environment or high salinity (see Farquhar *et al.*, 1989; Lambers *et al.*, 2008). Thus, it is plausible to infer from the $\delta^{13}C$ that the parasite maintained lower stomatal conductance in LW realtive to HW. This along with my finding of significantly higher Na concentrations in *C. pubescens* under LW may explain poor parasite performance including decreases in F_v/F_m and growth. Consequently, parasite infection had a less severe impact on total biomass of the host, *U. europaeus*, in LW versus HW. These findings suggest that limiting water supply to the soil and thus, host controls parasite performance as *C. pubescens* is seemingly not able to effectively increase its demand of water from the host in LW conditions.

There is a poor understanding of resource extraction mechanisms used by some stem hemiparasites such as *Cassytha* (see Těšitel *et al.*, 2010). Typically, parasitic plants maintain a lower water potential than their hosts, to enable extraction of water and other nutrients. This can be achieved by high transpiration rates (often higher than their hosts), and in a field study, Prider *et al.* (2009) reported that *C. pubescens* had higher transpiration rates than its hosts (*L. myrsinoides* and *C. scoparius*). However, data for this field study was collected on a single occasion and thus, comparisons of plant responses to differing water availabilities over time could not be made. In my study I found that $\delta^{13}C$ of *C. pubescens* was significantly higher than its host, especially under LW suggesting lower stomatal conductance relative to the host, and also that *Cassytha* responded to low water availability by possibly reducing stomatal conductance. This together with the relatively high Na of the parasite under LW suggests that under less favourable water conditions, *C. pubescens* was possibly relying on osmotic accumulation to maintain a water potential gradient with its host. Osmotic loading in the form of proline accumulation in the haustorial tissue of *S. acuminatum* has also been suggested as an important means by which this root hemiparasite acquires resources from its hosts (Tennakoon *et al.*, 1997c), especially considering the parasite was found to consistently transpire much less than the native host *Acacia rostellifera* Benth. (Tennakoon *et al.*, 1997b). Interestingly, osmotic accumulation is also reported for mistletoes in temperate zones where lower leaf to air VPDs can make it more difficult for the parasite to maintain a favourable water potential gradient by maintaining high transpiration rates (Bannister and Strong, 2001; Strong and Bannister, 2002). However, in my study lower transpiration rates in the parasite were likely

to be a response to water stress, rather than a consequence of lower leaf to air VPDs. My result, that $\delta^{13}C$ of *C. pubescens* was much higher than the host especially under LW, is opposite to what has been found for mistletoes growing in similar conditions (i.e. arid or semi-arid environments). That is, mistletoes in more arid environments tend to have much lower $\delta^{13}C$ than their hosts, presumably maintaining higher stomatal conductance and transpiration rates than hosts as main means of extracting resources (Ullmann *et al.*, 1985; Ehleringer *et al.*, 1986; see Bannister and Strong, 2001).

In summary with regard to water, when light and nutrients are not limiting, but soil water is restricted, the photosynthetic performance and growth of *C. pubescens* suffered. My finding of a significant decrease in F_v/F_m of *C. pubescens* in LW may be the first report of its kind with regard to how a parasitic plant responds to changes in water availability. Inoue *et al.* (2013) found no decline in F_v/F_m of the root hemiparasite *Striga hermonthica* infecting *Sorghum bicolor* when water availability was low. However, it is very difficult to compare this finding with my own as water treatments in their study only lasted 1-2 days. Nevertheless, in this short time they did find that stomatal conductance of *S. hermonthica* significantly declined in response low water conditions. Similarly, using $\delta^{13}C$ as a proxy for stomatal aperture, I found that *C. pubescens* likely had lower stomatal conductance in response to low soil water availability. It is interesting that both a perennial stem hemiparasite and an annual root hemiparasite responded to low water availability with lower stomatal conductance. As far as I am aware there are no controlled studies on the influence of water on other stem hemiparasites (e.g. mistletoes), or on how water availability influences the effect of root hemi or holoparasites on host growth, thus no generalisations can be made. Comparisons, however, can be made with the stem holoparasite *Cuscuta gronovii*, which has also been reported to grow more vigorously and have a greater effect on host growth in well-watered conditions (Evans and Borowicz, 2013, 2015). Increased growth of these hemi and holoparasites could be the cause of greater impact on the host in well-watered conditions, especially for parasites with indeterminate growth like *Cassytha* and *Cuscuta*.

Experiments looking at the effects of parasites under different combinations of abiotic factors will reveal more about the impact of infection on hosts under varying abiotic conditions. However, it should be kept in mind that water and nutrients are inextricably linked, with water affecting movement and supply of nutrients to the host, as well as between the host and the parasite (e.g. Těšitel *et al.*, 2015). Information on physiology

(including nutrient composition) and growth of both host and parasite across these treatment combinations would provide deep insights into the association, including whether the parasite can preferentially increase its demand for a particular resource, when the presence of another abiotic factor is altered.

Impact of *C. pubescens* on host performance

Biomass

Previous studies on *Cassytha* in Australia (e.g. Prider *et al*., 2009; Shen *et al*., 2010) and *Cuscuta* in China (e.g. Yu *et al.*, 2009; Yu *et al.*, 2011; Li *et al.*, 2012) have reported that native parasites grow better on, and affect introduced hosts much more than, native hosts. In my studies, I also found that *C. pubescens* consistently grew more vigorously and had a greater effect on total biomass of introduced versus native hosts. My findings also showed that that this differential impact on total biomass of introduced relative to native hosts is unaffected by varying abiotic conditions. Thus, *C. pubescens* may be an effective management tool in helping eradicate major invasive weeds in areas of ranging light, nitrogen (at least for legumes) and water availability.

As both *Cassytha* and *Cuscuta* are vines with indeterminate growth, increases in their biomass (and number of haustorial attachments) should also translate into a greater ability to remove resources from the host. Thus, increases in growth of *C. pubescens* may be a useful predictor of how strongly the parasite impacts on host growth. Indeed, increasing *C. pubescens* biomass per unit host biomass predicts 60% of the negative effect of infection on host growth (Fig. 1). Similarly, under high versus low water supply, *Cuscuta gronovii* achieved significantly greater biomass per unit *Verbesina alternifolia* and impact on growth of this host in these conditions (Evans and Borowicz, 2015).

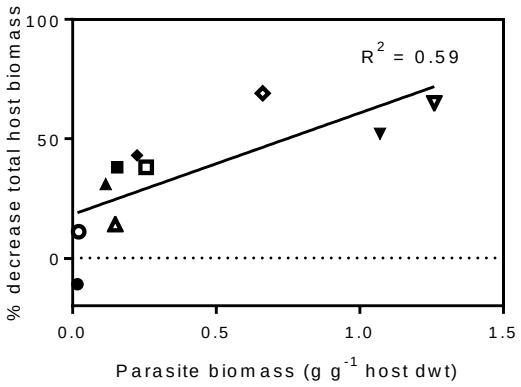

Fig. 1. The relationship between percentage decrease in host biomass and the biomass of

C. pubescens per unit host biomass across all experiments in my study. Data are means; high and low supply are open and closed symbols, respectively. Circles and squares represent *L. myrsinoides* and *U. europaeus* in the light experiment, respectively; upward and downward triangles represent *A. paradoxa* and *U. europaeus* in the nitrogen experiment, respectively, and diamonds represent *U. europaeus* in the water experiment. The line does not deviate significantly from linearity and the slope is significantly different from zero ($F_{1, 8} = 11.3$, $P = 0.010$ and Y = 42.53*X + 18.29).

Photosynthetic performance

In most of my studies, infection with *C. pubescens* had little or no effect on photosynthetic performance of native hosts, but did have a clear and consistent negative effect on that of *U. europaeus*. In the light experiment (Ch. 2), however, photosynthesis of the native *L. myrsinoides* was 43% lower in infected plants, which is similar to the 37% decrease found for the same host infected with *C. pubescens* in the field (Prider *et al.*, 2009). Prider *et al.* (2009) found that the impact on photosynthetic performance did not translate into effects on leaf biomass of *L. myrsinoides*. Similarly, I found no effect on total biomass of this host in my study, despite the impact on photosynthesis. It is possible that these effects on host photosynthesis only occurred later on in the association, and thus had limited impact on growth. This has been suggested for the *Striga asiatica-Sorghum arundinaceum* association, in which host photosynthesis was affected but not biomass accumulation (Gurney *et al.*, 2002). Following on, midday electron transport rates of *L. myrsinoides* in both HL and LL were unaffected by infection when measured three weeks before the end of an approximately 15 week experiment (Appendix 3).

In contrast to infection effects on photosynthesis of *L. myrsinoides*, I observed no impact of infection on photosynthesis of *A. paradoxa,* whereas I found that *C. pubescens* negatively affected photosynthetic performance of *U. europaeus* across all experiments reported in my thesis and also a subsequent field study (Table 1, Appendix 2: Table 2). Similarly, *C. pubescens* has been found to negatively affect photosynthesis of the introduced host *Cytisus scoparius* by approximately 30% and 50% in field and glasshouse studies, respectively (Prider *et al.*, 2009; Shen *et al.*, 2010). In these studies the lower rates of photosynthesis were most likely caused by infection effects on host stomatal conductance (Shen *et al.*, 2010). Although not significant, I also observed decreases in stomatal conductance resulting from infection of *U. europaeus* with *C. pubescens*. In addition, infection was found to have a significant negative effect on F_v/F_m of *U.*

europaeus in both the water experiment (Ch. 5) and field study (Appendix 2). These findings indicate that *U. europaeus* was showing signs of chronic photoinhibition as a consequence of infection. A similar result was observed for *C. scoparius* infected with *C. pubescens* (Shen *et al.*, 2010) and is consistent with the suggestion that introduced hosts are more impacted by infection with *C. pubescens* than native hosts. The decreases in F_v/F_m of *U. europaeus* may be due to the negative effect of *C. pubescens* on spine nitrogen concentration of this host in the water experiment (Ch. 5: Table 5) and field study (Appendix 2: Tables 3, 4; and or parasite-induced decreases in pre-dawn water potential as found at two of the three field sites, Appendix 2: Fig. 3a). These infection effects on host nitrogen and water-status are the first reports of their kind for associations involving *C. pubescens* and provide insights on why introduced hosts show low tolerance to this native parasite. Further, if lower nitrogen is a consistent response for introduced hosts due to *C. pubescens* infection, this might explain why flowering and seed set of *C. scoparius* were suppressed by *C. pubescens* in the field (Prider *et al.*, 2011).

Moreover, the negative effects of *C. pubescens* on photosynthetic performance of *U. europaeus* may be due to more effective removal of resources such as nitrogen and water from introduced compared with native hosts. This notion is supported by earlier work by Tsang (2010) using radioactive phosphate (^{32}P) that found *C. pubescens* effectively removed ^{32}P from *C. scoparius* but not from the native host *Acacia myrtifolia* (Sm.) Willd.. This was attributed to a more effective haustorial connection to the introduced host (Tsang, 2010). Histological investigations of haustorial connections on a range of introduced and native hosts are needed to confirm this more broadly. In tandem with quantifications of resource flux across the haustorial interface, this will further clarify the mechanisms behind these differential impacts on host physiology and growth.

Table 1. Effect of infection with *C. pubescens* on electron transport rates of *U. europaeus*.

	ETR
U. europaeus	% decrease
Light Exp. (Ch. 2)	24
Nitrogen Exp. (Ch. 4)	46
Water Exp. (data not shown)	32
Field Study (Appendix 2)	42

Potential applications of *C. pubescens* for weed control

Previously, *C. pubescens* has been shown to negatively affect the introduced *C. scoparius* but not the native host *L. myrsinoides* and hence, implicated as having practical applications for controlling weeds (Prider *et al.*, 2009). I also found that *C. pubescens* negatively affected the major invasive weed *U. europaeus* but not the native host *A. paradoxa*, species not yet studied. Thus, in addition to my finding that the native host *L. myrsinoides* also shows tolerance to the parasite, which is consistent with previous reports, my project provides strong novel evidence that supports the potential use of *C. pubescens* as a native bio-control against major invasive weeds in Australia. Indeed, as found for *Cytisus scoparius* my results consistently show that *C. pubescens* has a strong negative effect on growth and physiology of *U. europaeus*. This is of significance for control of *U. europaeus* which is classified as one of the top 20 worst weeds in Australia (Thorp and Lynch, 2000). Costs to agriculture and forestry for controlling *U. europaeus* have been estimated at >AUS$5 million annually (Thorp and Lynch, 2000), and there are additional impacts on native biodiversity such as displacement of native flora and fauna and natural ecosystem dysfunction. My research has also contributed novel information on how the effect of the parasite on its hosts may vary with abiotic conditions. The differential impact of *C. pubescens* on introduced hosts such as *U. europaeus*, could contribute to their control and thus reduce the financial burden and facilitate long-term recovery of native biodiversity including threatened species. Further, it would be a particularly useful management tool in difficult terrain which may be impossible to access with heavy machinery. *Cassytha pubescens* is also potentially less environmentally damaging than using herbicides, particularly in areas feeding into aquatic systems. Nevertheless, more research is needed on the impacts of *C. pubescens* on other introduced and native hosts in the field and glasshouse, over what distance vectors disperse the parasite's fruit and what triggers parasite seed germination. This information is vital in gauging whether *C. pubescens* will be effective in limiting the abundance and spread of these major weeds, without negatively impacting native species across a range of abiotic and biotic conditions, before it could be applied in this way.

Wider ecological significance

My results provide a tool to predict and explain the potential effect of *C. pubescens* on survival of introduced hosts and thus, their abundance and distribution in the field under different environmental conditions. For example, based on the results of the light

experiment (Ch. 2), I would expect the impact of *C. pubescens* on survival of *U. europaeus* in the field to be similar in areas of both high and low light availability. Along with other introduced hosts such as *C. scoparius* (Prider *et al.*, 2009), *U. europaeus* is found in both open and more shaded areas of eucalypt dominated woodland in many parts of southern Australia. Similarly, the results of the nitrogen experiment (Ch. 4) suggest that negative impacts of infection on introduced leguminous hosts will not be affected by variation in soil nitrogen concentrations. In contrast, the results of the water availability experiment (Ch. 5) suggest that soil water will influence the effect of infection on introduced hosts. In drier areas, *U. europaeus* would be less impacted by infection with *C. pubescens* than in wetter regions. This could have implications for the spread of *U. europaeus* if rainfall declines as a consequence of climate change. A further prediction is that *C. pubescens* may have a stronger effect on the distribution of *U. europaeus* and other introduced hosts in wetter areas, for example parts of south-eastern Australia, including Tasmania, and much of New Zealand, compared with drier parts of southern Australia. In addition to the abiotic factors investigated here, it should be noted that, in the field, the vigorous growth of *C. pubescens* on introduced hosts can result in dense matting covering the host (Fig. 2). In addition to the direct effect of shading rather than its interaction (or lack of) with infection, the weight of this dense matting may place a high mechanical pressure on the host. Thus, especially in wetter areas, where parasite growth is likely to be enhanced, these additional stressors may increase the deleterious effect of *C. pubescens* on introduced hosts.

Invasion theory

My work also has significance for ecological theory regarding invasion success, which has not previously considered associations between parasitic plants and their hosts. The enemy-release hypothesis states that introduced species will be successful colonisers due to leaving behind the bulk of their native enemies (Keane and Crawley, 2002; Morrison and Hay, 2011). On the other hand, the biotic resistance/naïve invader hypothesis suggests that successful colonisation by exotic species is restricted by the presence of novel, enemies that are native to the newly invaded habitat (Levine *et al.*, 2004; Verhoeven *et al.*, 2009). My results support the biotic resistance/naïve invader hypothesis, in that the native hemiparasite, *C. pubescens*, had a greater impact on an introduced host than native hosts. Further, by manipulating abiotic factors, I have also provided evidence on how the impact of the native enemy (*C. pubescens*) may change as a consequence of environmental variability, something poorly represented in the literature (Maron and Vilà, 2001). For

example, in the light experiment, I demonstrated that the relative impact of *C. pubescens* on *U. europaeus* was similar in sun and shade. In contrast, the beetle (*Chrysolina quadrigemina*) used as a bio-control for St. Johns wort (*Hypericum perforatum*) in the western United States was not as effective in the shade due to the fact of the beetle having low performance in these conditions (see Maron and Vilà, 2001).

My findings show that *C. pubescens* has low virulence with native hosts, and has likely co-evolved, while it has high virulence on introduced hosts. This phenomenon has also been reported for other parasite-host associations. The protozoa *Trypanosoma brucei* for example, has a greater effect on introduced versus native ruminants in East Africa (Allison, 1982). High virulence in parasites can be disadvantageous if it results in significant reductions in host populations, unless the parasite can find another host. This may not be an issue for *C. pubescens* as being a perennial vine with indeterminate growth it may have a better chance of transmission to a new host if it kills the former.

Fig. 2. *Cassytha pubescens* 'infection front' (arrow) moving over a thicket of *Ulex europaeus* at Crafers in the Mt. Lofty Ranges of South Australia (please refer to Table 1 in Appendix 2 for site details). Dead *U. europaeus* lie beneath the dense matting of dead *C. pubescens*.

Final conclusions

My project has made significant contributions to the field of parasitic plants. It has provided evidence of the mechanisms (e.g. lowered host nitrogen and water-status, electron transport and photosynthetic rates, stomatal conductance, PSII efficiency, F_v/F_m as signs of chronic photoinhibition and more vigorous parasite growth) underlying the differential effects of *C. pubescens* on introduced versus native hosts. In addition, the manipulations of abiotic factors have contributed to our ability to predict the impact of *C. pubescens* on its hosts in the field, as well as in response to impacts of climate change. My work has also provided knowledge on using a native stem hemiparasite as a biological agent to suppress exotic shrubs under various abiotic conditions which is rarely found in the literature. Indeed, the information generated here has made a significant contribution to the field of parasitic plants in general. For example, to the best of my knowledge the results of the nitrogen experiment (Ch. 4) are the first of their kind with regard to the influence of low versus high rhizobial nodulation on parasite effects on hosts' photosynthesis and growth. Another major finding was that *C. pubescens* did not perform as well and had less of an impact on growth *U. europaeus* under low water conditions, which strategically implies that the parasite should be a more effective bio-control agent in areas of high water availability. My understanding is that this is the only information currently recorded on how water influences growth of hemiparasites and their effects on host growth which is a significant contribution to the field considering that hemiparasites constitute around 90% of all parasitic plants. Furthermore, the poorer parasite performance under low water availability may be the reason why *C. pubescens* is not commonly found in semi-arid regions and does not occur at all in arid environments, despite the presence of suitable hosts.

My research found that the perennial stem hemiparasite, *C. pubescens*, differentially impacted introduced relative to native hosts, and similar findings have been reported for root hemiparasites of the genus *Striga* (Gurney *et al.*, 2002) and stem holoparasites of the genus *Cuscuta* (Li *et al.*, 2012). That is, the differential effect of native parasites on introduced versus native hosts appears independent of whether the parasite is stem/root, holo or hemiparasite having an annual or perennial life cycle. In addition, previous work on *C. pubescens* has only investigated one introduced (*C. scoparius*) and one native (*L. myrsinoides*) host (Prider *et al.*, 2009; Shen *et al.*, 2010). My research expanded the range of hosts to the introduced *U. europaeus* and the native *A. paradoxa*. These two hosts are

also from the same family (Fabaceae), and yet I still found a differential effect. This further confirms the possibility that native hosts have co-evolved tolerance or resistance to infection with *C. pubescens*.

In conclusion, my PhD studies have not only helped to explain the mechanisms underpinning the differential impact of *C. pubescens* on introduced compared with native hosts, but have shown the ability to utilize *C. pubescens* as a native bio-control agent against major introduced weeds in Australia and possibly other countries (including New Zealand which also has several introduced hosts of the parasite e.g. *U. europaeus*), under a range of light, nitrogen and water conditions. My project provided evidence on the influence of abiotic factors on stem hemiparasite effects on host physiology and growth (including root biomass) under controlled conditions, from my understanding information that has been completely missing from the literature. Finally, my research has also contributed to debates on invasion theory, by adding further evidence in support of the biotic resistance/naïve invader hypothesis.

Future directions

In addition to the knowledge gaps and avenues for future research already mentioned, it would also be important to conduct field trials to determine advisable methods of parasite deployment. This would involve exploring various ways of implementing *C. pubescens* on thickets of introduced shrubs (e.g. slashed versus non-slashed infestations), monitoring how long the parasite takes to progress and potentially kill major introduced weeds, and evaluate its success (i.e. effective control agent while posing no significant threat to non-target native species). Field trials could also be conducted at locations that vary in rainfall, assessing if the parasite's greater impact on the introduced host in high water conditions found in the glasshouse (Ch. 5) is externally validated in the field. Glasshouse studies would include determining if *C. pubescens* has a greater impact on introduced hosts when they are small compared with when they are large and measuring the parasite's ability to spread from introduced to native or from native to native host. Also, quantifying the parasite association with a range of hosts across seasons in various environmental settings would be ideal, and provide a great understanding of these relationships, including whether the phenomenon of $\delta^{13}C$ of *C. pubescens* being less negative than its host holds regardless of time, space and host species (Pate *et al.*, 1990; Tennakoon *et al.*, 1997b). To end, another key line of enquiry would be to elucidate whether chemical signalling between *C. pubescens* and its hosts occurs (Cameron pers. comm.).

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Appendix 1

Sum of square and *F* values for pigment experiment

Table A1. Two-way ANOVA results for the effect of infection with *Cassytha pubescens* (I), light (L) and their interaction (I x L) on xanthophyll cycle pool (VAZ), total chlorophyll (Chl), total carotenoids (Car), lutein, chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*) and chlorophyll *a*/*b* ratio (Chl *a*/*b*) of *Leptospermum myrsinoides*. *F* and sum of square values are in *italics* and regular type, respectively.

	VAZ	Chl	Car	Lutein	Chl *a*	Chl *b*	Chl *a*/*b*
I	*9.04*	*6.24*	*6.21*	*3.88*	*7.24*	*3.58*	*9.03*
	112	84767	1298	224	52662	3803	0.305
L	*0.401*	*1.39*	*0.444*	*1.27*	*0.102*	*11.4*	*52.1*
	4.98	18833	92.9	73.5	739	12110	1.76
I x L	*0.478*	*0.190*	*0.217*	*0.394*	*0.460*	*0.046*	*4.83*
	5.94	2582	45.4	22.7	3343	49.1	0.163
Block	*44.4*	*4.25*	*6.62*	*2.56*	*1.56*	*16.9*	*33.1*
	551	57773	1384	148	11329	17935	1.12
Error	720	788427	12130	3350	421706	61586	1.96

Table A2. Two-way ANOVA results for the effect of infection with *Cassytha pubescens* (I), light (L) and their interaction (I x L) on xanthophyll pool per unit chlorophyll (VAZ/Chl), total carotenoids per unit chlorophyll (Car/Chl), pre-dawn (PD) and midday (MD) de-epoxidation states (A+Z/VAZ), PD and MD quantum yields (F_v/F_m and Φ_{PSII}, respectively) of *Leptospermum myrsinoides*. *F* and sum of square values are in *italics* and regular type, respectively.

	VAZ/Chl	Car/Chl	PD A+Z/VAZ	MD A+Z/VAZ	F_v/F_m	Φ_{PSII}
I	*0.253*	*1.46*	*0.140*	*0.587*	*0.303*	*0.771*
	0.0006	0.023	1.61	38.6	0.0001	0.004
L	*4.29*	*1.86*	*129*	*162*	*14.0*	*102*
	0.011	0.029	1486	10600	0.007	0.582
I x L	*2.36*	*4.01*	*0.997*	*0.013*	*0.012*	*10.7*
	0.006	0.063	11.5	0.879	0.000006	0.061
Block	*41.7*	*0.257*	*8.68*	*24.4*	*0.013*	*0.045*
	0.106	0.004	100	1600	0.000006	0.0003
Error	0.147	0.912	312	1707	0.015	0.188

Pigments

Table A3. One-way ANOVA results for the effect of light (L) on xanthophyll pool (VAZ), total chlorophyll (Chl), total carotenoids (Car), lutein, lutein expoxide (Lx), chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), and chlorophyll *a*/*b* ratio (Chl *a*/*b*) of *Cassytha pubescens* when infecting *Leptospermum myrsinoides*. *F* and sum of square values are in *italics* and regular type, respectively.

	VAZ	Chl	Car	Lutein	Lx	Chl *a*	Chl *b*	Chl *a*/*b*
L	*4.48*	*0.463*	*0.086*	*0.186*	*0.077*	*0.364*	*0.751*	*2.35*
	53.8	4593	24.4	18.5	0.147	1852	612	0.090
Block	*1.97*	*1.40*	*1.44*	*1.05*	*5.82*	*1.41*	*1.36*	*0.004*
	23.6	13927	408	105	11.1	7187	1105	0.0001
Error	180	148841	4250	1497	28.7	76322	12217	0.571

Table A4. One-way ANOVA results for the effect of light (L) on xanthophyll pool per unit total chlorophyll (VAZ/Chl), total carotenoids per unit total chlorophyll (Car/Chl), lutein epoxide per unit total chlorophyll (Lx/Chl), pre-dawn (PD) and midday (MD) de-epoxidation state, (A+Z/VAZ), PD and MD Lx/Chl, PD and MD quantum yields (F_v/F_m and Φ_{PSII}, respectively), of *Cassytha pubescens* when infecting *Leptospermum myrsinoides*. *F* and sum of square values are in *italics* and regular type, respectively.

	VAZ/Chl	Car/Chl	Lx/Chl	PD A+Z/VAZ	MD A+Z/VAZ	PD Lx/Chl	MD Lx/Chl	F_v/F_m	Φ_{PSII}
L	*19.9*	*3.24*	*0.122*	*1.14*	*6.55*	*0.023*	*0.276*	*0.784*	*5.09*
	0.107	0.238	0.0004	265	1256	0.0001	0.001	0.0002	0.018
Block	*2.00*	*0.239*	*3.98*	*0.132*	*0.396*	*2.75*	*0.814*	*0.0001*	*1.85*
	0.011	0.018	0.012	30.7	75.9	0.008	0.003	0.00000002	0.007
Error	0.081	1.11	0.044	1395	1151	0.018	0.024	0.002	0.025

Appendix 2

The effect of *Cassytha pubescens* on *Ulex europaeus* in the field

Materials and methods

Study sites

The study was conducted in three field sites (Table 1) located in the Mt. Lofty Ranges of South Australia. The Ranges lie east of the Adelaide plains in a north-south direction and cover 5000 km^2 of which only 10-18 % supports remnant native vegetation (Westphal *et al.*, 2003). The climate is Mediterranean, with an annual rainfall of 700−1100 mm and mean winter (June-August) and summer (December-February) rainfall of approximately 400 and 53 mm, respectively (Fogarty and Facelli, 1999; Prider *et al.*, 2009). Mean winter and summer maximum temperatures, respectively, are 12.9 ºC and 26.8 ºC (Fogarty and Facelli, 1999). The vegetation of the area has an over storey dominated by eucalypts with an understorey of sclerophyllous shrubs and a ground layer of low lying shrubs, sedges and grasses (Prider *et al.*, 2009). Soils are generally sandy loams to sandy clays, shallow and nutrient poor with a pH of 5−6 or less herein (see Fogarty and Facelli, 1999; Prider *et al.*, 2009).

Study species

Ulex europaeus L. (Fabaceae) is a leguminous evergreen spiny shrub 0.6 to 2 m tall that is native to Western Europe and Northern Africa (Clements *et al.*, 2001). It quickly establishes in disturbed areas and has become a major introduced weed in many parts of the world including Australia (Clements *et al.*, 2001). *Cassytha pubescens* R. Br. (Lauraceae) is a stem hemiparasitic vine native to Australia (McLuckie, 1924). It has no true roots or leaves, and its stems (0.5−2 mm in diameter) coil around the host producing numerous haustoria through which it obtains water and nutrients from its host xylem. *C. pubescens* is a generalist parasite and in its native range, has often been observed infecting *U. europaeus* an association that has been extensively studied in the glasshouse (Britton, 2002; Cirocco *et al.*, 2015, 2016a, 2016b).

Field Study

Experimental design

The main differences among the three field sites were that they varied in relief, aspect and intensity of infection with *C. pubescens* (Table 1). The maximum amount of replicates possible was chosen at each site and selected according to two criteria: a) having similar size and similar levels of infection and b) growing with as little canopy cover as possible (Table 1). Measurements were made on infected and uninfected plants (including the parasite) interspaced and growing within 30 m of each other at all three sites, so any conclusions made about site effects are only restricted to this area within each study site. Maximum photosynthetic photon flux densities (PPFD) and temperature were recorded on days when physiological measurements were conducted at each site using LI-1400 data loggers fitted with a quantum (LI-190 SA) and relative humidity/air temperature sensor (1400-104) (LI-COR, Lincoln NEB., Table 1). All soil characteristics at each site were determined by CSBP soil and plant laboratory (Western Australia, Table 1).

Photosynthetic performance and shoot water potentials

Pre-dawn (F_v/F_m) and midday (Φ_{PSII}) quantum yields and midday electron transport rates (ETR) of *U. europaeus* spines and *C. pubescens* stems were measured with a portable, pulse-modulated chlorophyll fluorometer (MINI-PAM, Walz, Effeltrich, Germany) fitted with a leaf-clip (2030–B, Walz, Effeltrich, Germany). Measurements were taken on sunny days in late March-early May 2013 which is the end of the dry season. PPFD's (μmol m^{-2} s^{-1}) averaged across sites for both Φ_{PSII} and midday ETR of *U. europaeus* and *C. pubescens* were 1273 ± 17 (*n*=50) and 1354 ± 27 (*n*=25), respectively. Pre-dawn and midday shoot water potentials (Ψ) were determined on freshly cut shoots of *U. europaeus* using a Scholander-type pressure chamber with a digital gauge (PMS Instrument Company, Albany, OR). Measurements were taken on sunny days in late March-early May 2013.

Carbon isotope ($\delta^{13}C$) and elemental analyses

Carbon isotope composition ($\delta^{13}C$) and nitrogen (N) concentration of host spines and parasite stems were quantified via mass spectroscopy at Waite IRMS Facility (The University of Adelaide). Elemental analysis of host spines (Al, Fe, K, and Na) and parasite stems (K and Na) was obtained using Radial View Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) at Waite Analytical Services (The University of Adelaide). All analyses were conducted on harvested, oven-dried material (60 ºC for six days) collected in late March-early May 2013 on days when measurements were made.

Field Study

Statistical analyses

The variances of the host data were homogeneous. The host's parameters were analysed using a two-way fixed effects ANOVA (since sites were not chosen at random). The two-way ANOVA was used to determine whether there was an interaction between the *C. pubescens* infection status of the host and site. If an interaction was not detected, independent effects of either infection (sites pooled) or site (uninfected and infected plants pooled) were considered. Parasite parameters, also presenting homogeneous variances, were analysed across sites using one-way ANOVAs. Significant effects for host and parasite parameters were only considered where the Tukey HSD test for pairwise comparisons of means also found a difference. All data were analysed with the software JMP Ver. 4.0.3 (SAS Institute Inc. 2000) and α=0.05.

Results

Host and parasite F_v/F_m, Φ_{PSII} and midday ETR

There was a significant interaction for infection x site on F_v/F_m (Table 2). Infection had a significant negative impact on F_v/F_m of *U. europaeus* at Bradbury and Crafers but not at Engelbrook (Fig. 1a). While there was no significant interaction or site effect for Φ_{PSII}, it was independently affected by infection (Table 2, Fig. 1b). Φ_{PSII} of infected plants were approximately 40% less than those of uninfected plants, regardless of site (Fig. 1c). Site had no effect on F_v/F_m or Φ_{PSII} of *C. pubescens* (P=0.065 and 0.886, respectively; Fig. 1d, e).

There was no significant interaction or independent site effect detected for midday ETR of *U. europaeus*, but it was significantly affected by infection (Table 2, Fig. 2a). On average, midday ETR of infected plants were 42% lower compared with those of uninfected plants, irrespective of site (Fig. 2b). Midday ETR of *C. pubescens* did not differ significantly among sites (P=0.289, Fig. 2c).

Host PD and MD Ψ

An interaction was detected for shoot water potentials of *U. europaeus* at pre-dawn, however, the pairwise comparison found no differences; although not significant this parameter of infected plants at Bradbury and Crafers was lower than that of respective uninfected plants (Table 2, Fig. 3a). Midday shoot water potentials of *U. europaeus* were also affected in a non-independent way (significant interaction; Table 2). In terms of

infection having a negative effect within sites, although not significant, again this parameter of infected plants at both Bradbury and Crafers was lower than that of respective uninfected plants (Fig. 3b).

Host and parasite $\delta^{13}C$

There was a significant interaction for infection x site on carbon isotope composition of *U. europaeus* (Table 2). With respect to uninfected plants at Crafers, $\delta^{13}C$ (‰) was significantly higher than that of all other combinations including that of infected plants at this site (Fig. 4a). There was a significant effect of site on $\delta^{13}C$ of the parasite (P=0.023). Carbon isotope composition of *C. pubescens* at Crafers was significantly higher than that at Engelbrook with both sites sharing similar $\delta^{13}C$ with that at Bradbury (Fig. 4b). There was a significant interaction between infected plants/parasite x site for $\delta^{13}C$ ($F_{2,\ 41}$=5.8, P=0.006). The differences were between infected plants and parasites located at Bradbury and Crafers with $\delta^{13}C$ of *C. pubescens* being significantly higher relative to that of infected plants at both sites (Fig. 4c).

Host and parasite nutrient concentrations

There was no interaction for infection x site on nutrient concentrations of *U. europaeus* spines (Tables 3, 4). There was however, an independent effect of infection on N, Al, Fe and K concentration of *U. europaeus* (Table 3). On average, infection with *C. pubescens* decreased nitrogen concentration of *U. europaeus* by 16%, across sites (Table 4). Interestingly, aluminium and iron concentration of infected plants were approximately 60% and 30% higher relative to that of uninfected plants, respectively (Table 4). Infection decreased potassium concentration of *U. europaeus* by 22%, irrespective of site (Table 4).

There was also an independent effect of site on N, Al, K and Na concentration of *U. europaeus* spines (Table 3). Nitrogen and potassium concentration of plants at Engelbrook were significantly higher compared with those of plants at both Bradbury and Crafers which were not significantly different from each other (Table 4). Aluminium concentration of *U. europaeus* spines at Engelbrook was significantly lower than that of plants at Bradbury with values at both these sites not being significantly different from Al of plants at Crafers (Table 4). Sodium of *U. europaeus* at Engelbrook was 26% higher relative to that at Crafers with concentrations of plants at both these sites not differing from Na of plants at Bradbury (Table 4).

Field Study

Nitrogen concentration of parasite stems was similar among sites (P=0.121, Fig. 5a). Potassium of *C. pubescens* stems was significantly higher at Engelbrook compared with Crafers, with parasite values at these two sites being similar to those at Bradbury (site effect; P=0.042, Fig. 5b). Sodium concentration of *C. pubescens* stems at Crafers was significantly higher than those of the other two sites which did not differ significantly from each other (site effect; P=0.0002, Fig. 5c).

Table 1. Location, relief, aspect, climate, size of *U. europaeus*, level of *C. pubescens* infection and soil characteristics from three study sites located in the Mt. Lofty Ranges of South Australia in mid-autumn 2013.

	Engelbrook	Bradbury	Crafers
Latitude	S35º 01.278	S35º 3.130	S35º 00.456
Longitude	E138º 45.992	E138º 43.412	E138º 41.212
Elevation (m)	330	440	492
Relief	gully	31º	21.8º
Aspect	N/A	South	North
Max PPFD (μmol m^{-2} s^{-1}) on day of measurement	1708.7	505.6	1587.9
Max temperature (ºC) on day of measurement	30.00	30.18	26.22
Size of *U. europaeus*	m	s - m	m
Intensity of infection	m	h	h
Soil ammonium (mg/kg)	13.60 ± 2.34	6.80 ± 1.39	19.60 ± 11.19
Soil nitrate (mg/kg)	11.00 ± 5.45	2.00 ± 0.00	8.40 ± 1.60
Soil pH_{CaCl2}	4.40 ± 0.30	4.28 ± 0.04	4.42 ± 0.10
Soil conductivity (dS/m)	0.19 ± 0.04	0.04 ± 0.01	0.08 ± 0.01
Soil organic carbon (%)	4.90 ± 0.18	1.98 ± 0.23	2.81 ± 0.27

m = medium, s = small and h = heavy

Table 2. Two-way ANOVA results (*P*-values) for the effect of infection with *Cassytha pubescens* (I) and three field sites in the Mt. Lofty Ranges of South Australia (S) on pre-dawn and midday quantum yields (F_v/F_m, Φ_{PSII}), midday electron transport rates (ETR), pre-dawn (PD) and midday (MD) shoot water potentials (Ψ) and carbon isotope composition ($\delta^{13}C$) of *Ulex europaeus* spines. Significant effects are in bold.

Factor	F_v/F_m	Φ_{PSII}	ETR	PD Ψ	MD Ψ	$\delta^{13}C$
I	**0.0002**	**0.0003**	**0.0004**	0.376	0.731	**0.001**
S	**<0.0001**	0.107	0.193	0.169	**0.0006**	**0.0002**
I x S	**0.001**	0.937	0.971	**0.040**	**0.004**	**0.0001**

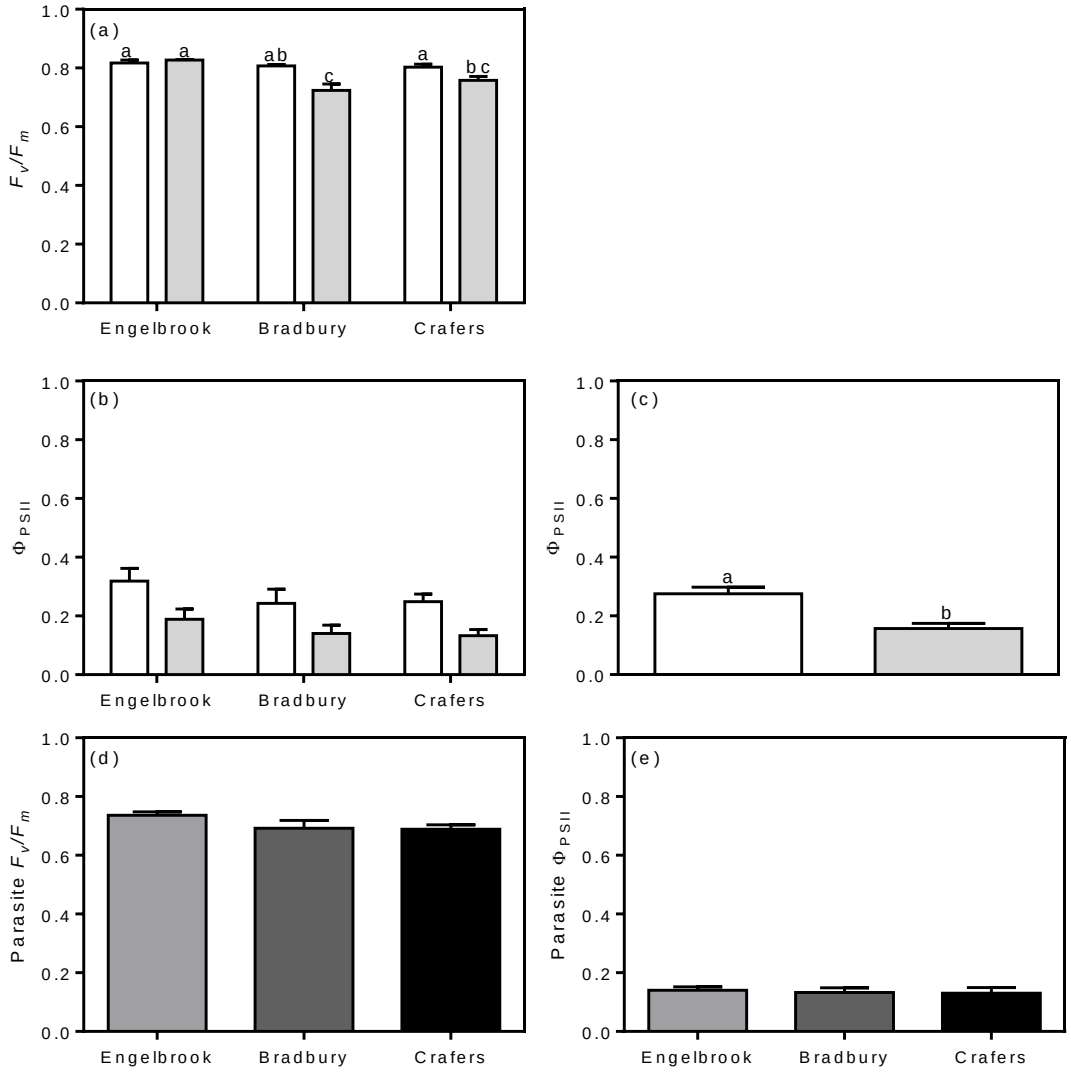

Fig. 1. (a) Pre-dawn (F_v/F_m) and (b) midday (Φ_{PSII}) quantum yields of *Ulex europaeus* either uninfected (open bars) or infected (light grey bars) with *Cassytha pubescens* at three field sites in the Mt. Lofty Ranges of South Australia. (c) Independent infection effect on host Φ_{PSII}. (d) F_v/F_m and (e) Φ_{PSII} of *C. pubescens* infecting *U. europaeus* at the three sites. Data are means (±1SE), different letters indicate significant differences and n=10 (a, b, d, e) (except at Bradbury, n=5); n=25 (c).

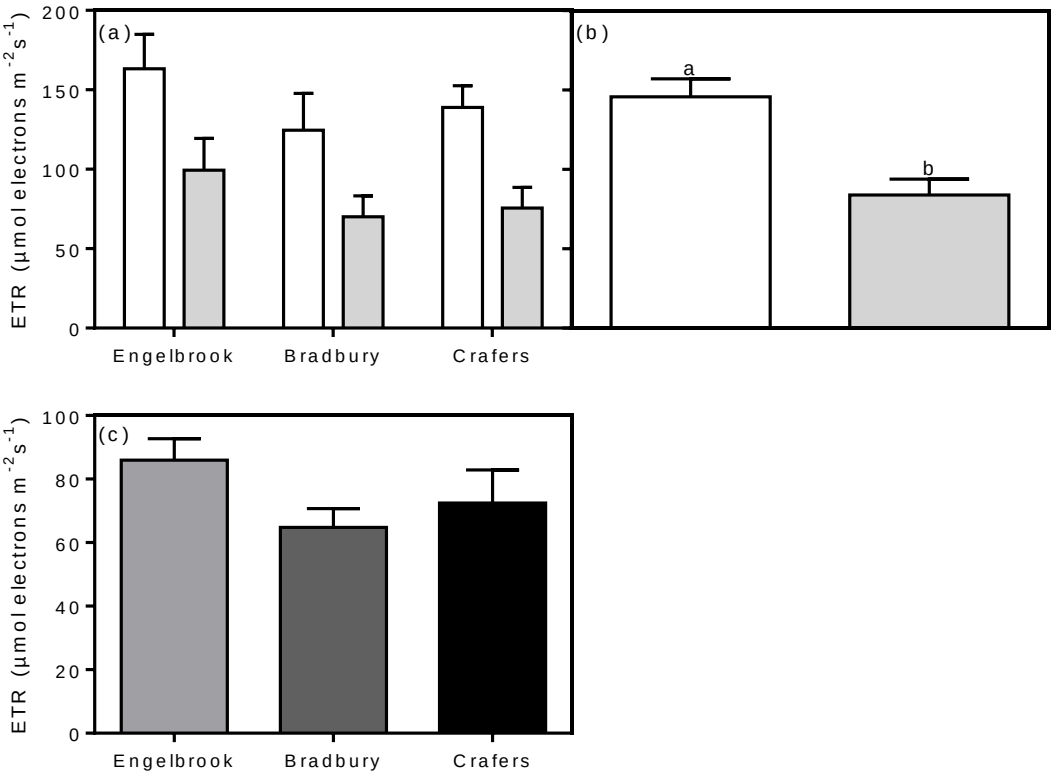

Fig. 2. (a) Midday electron transport rates (ETR) of *Ulex europaeus* either uninfected (open bars) or infected (light grey bars) with *Cassytha pubescens* at three field sites in the Mt. Lofty Ranges of South Australia. (b) Independent infection effect on host midday ETR. (c) Midday ETR of *C. pubescens* infecting *U. europaeus* at the three sites. Data are means (±1SE), different letters indicate significant differences and *n*=10 (a, c) (except at Bradbury, *n*=5); *n*=25 (b).

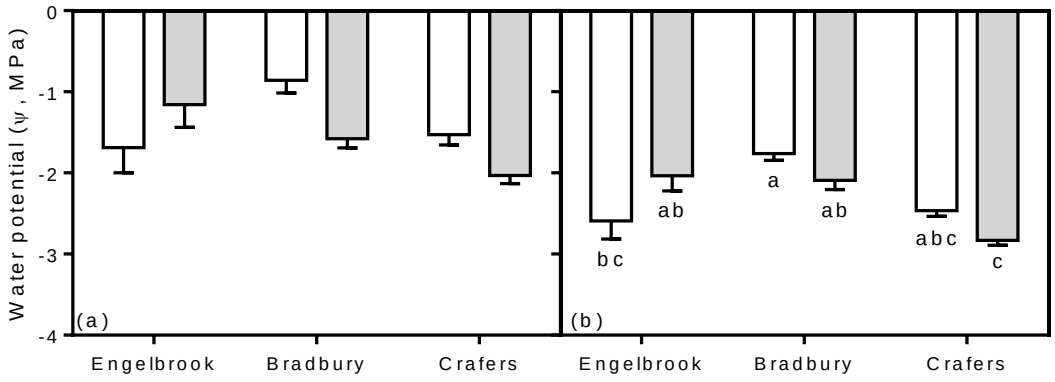

Fig. 3. Pre-dawn (a) and midday (b) shoot water potentials of *Ulex europaeus* either uninfected (open bars) or infected (light grey bars) with *Cassytha pubescens* at three field sites in the Mt. Lofty Ranges of South Australia. Data are means (±1SE), different letters indicate significant differences and *n*=10 (a, b) (except at Bradbury, *n*=5).

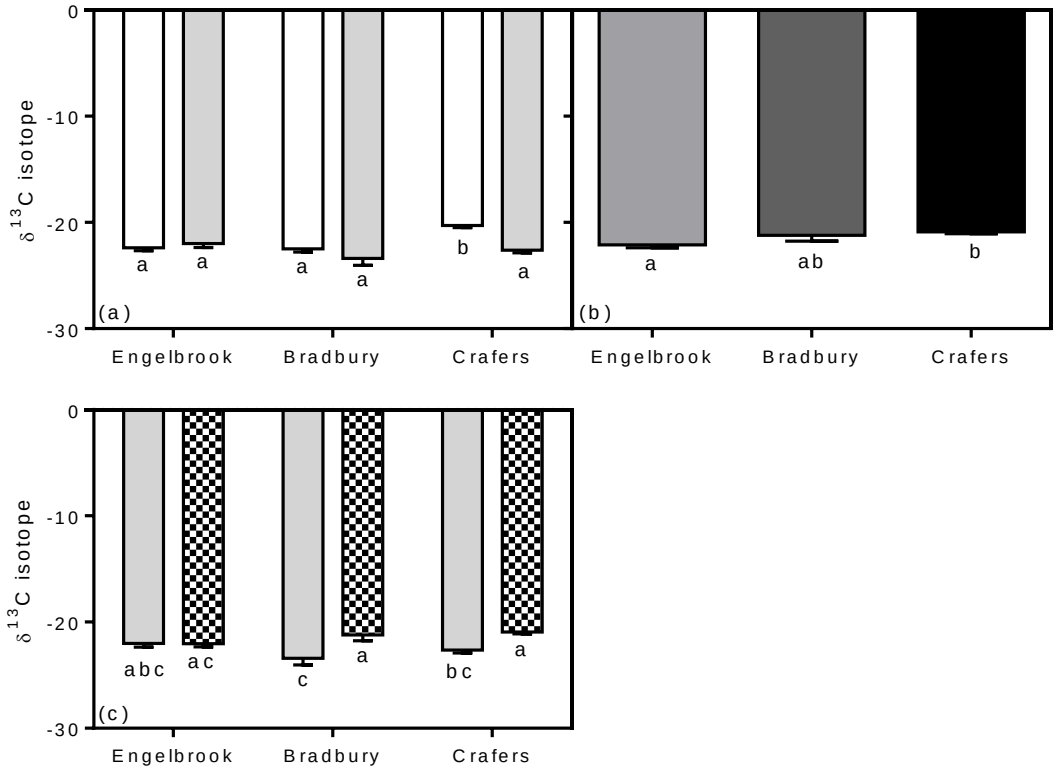

Fig. 4. (a) Spine carbon isotope composition (‰) of *Ulex europaeus* either uninfected (open bars) or infected (light grey bars) with *Cassytha pubescens* at three field sites in the Mt. Lofty Ranges of South Australia. (b) Carbon isotope composition of *C. pubescens* stems at the three sites. (c) Carbon isotope composition of both infected *U. europaeus* (light grey bars) and parasite (checker bars) at the three sites. Data are means (±1SE), different letters indicate significant differences and *n*=10 (a) (except at Bradbury, *n*=5 and *n*=7 for infected plants at Engelbrook), *n*=10 (b) (except at Bradbury, *n*=5), *n*=as above for (c).

Table 3. Two-way ANOVA results (*P*-values) for the effect of infection with *Cassytha pubescens* (I) and three field sites in the Mt. Lofty Ranges of South Australia (S) on nitrogen (N), aluminium (Al), iron (Fe), potassium (K) and sodium (Na) concentration of *Ulex europaeus* spines. Significant effects are in bold.

Factor	N	Al	Fe	K	Na
I	**0.001**	**<0.0001**	**<0.0001**	**0.008**	0.256
S	**<0.0001**	**0.001**	0.230	**<0.0001**	**0.025**
I x S	0.860	0.336	0.368	0.327	0.103

Table 4. Spine nitrogen (N, %), aluminium (Al, mg/kg), iron (Fe, mg/kg), potassium (K, mg/kg) and sodium (Na, mg/kg) concentration of *Ulex europaeus* either uninfected (–) or infected (+) with *Cassytha pubescens* at three field sites (Engelbrook: E; Bradbury: B; Crafers: C) in the Mt. Lofty Ranges of South Australia. Data are means (±1SE), different letters indicate significant differences for independent infection (I) effect on N, Al, Fe and K (uninfected *n*=25; infected *n*=22) and independent site (S) effect on N, Al, K and Na (E, *n*=17; B, *n*=10; C, *n*=20). There were no I x S interactions detected; *n*=10 (except at Bradbury, *n*=5 and *n*=7 for infected plants at Engelbrook).

	N	Al	Fe	K	Na
E–	2.0 ± 0.058	20.9 ± 0.94	117 ± 7	11880 ± 474	2449 ± 189
E+	1.8 ± 0.116	55.4 ± 12.4	153 ± 18	8743 ± 1045	2171 ± 235
B–	1.6 ± 0.086	41.3 ± 3.79	120 ± 3	8700 ± 1078	1762 ± 168
B+	1.3 ± 0.133	99.6 ± 9.93	191 ± 16	7660 ± 1461	2072 ± 410
C–	1.5 ± 0.044	35.8 ± 3.89	125 ± 7	7550 ± 428	1420 ± 171
C+	1.2 ± 0.073	74.7 ± 8.82	172 ± 11	6300 ± 621	2040 ± 199
Infection					
–	1.7 ± 0.060a	30.9 ± 2.42a	121 ± 4a	9512 ± 513a	1900 ± 140
+	1.4 ± 0.076b	74.3 ± 6.75b	170 ± 9b	7386 ± 567b	2089 ± 142
Site					
E	1.9 ± 0.062a	35.2 ± 6.50a	132 ± 9	10588 ± 626a	2335 ± 147a
B	1.5 ± 0.093b	70.4 ± 10.9b	155 ± 14	8180 ± 873b	1917 ± 680ab
C	1.4 ± 0.048b	55.3 ± 6.48ab	148 ± 8	6925 ± 394b	1730 ± 146b

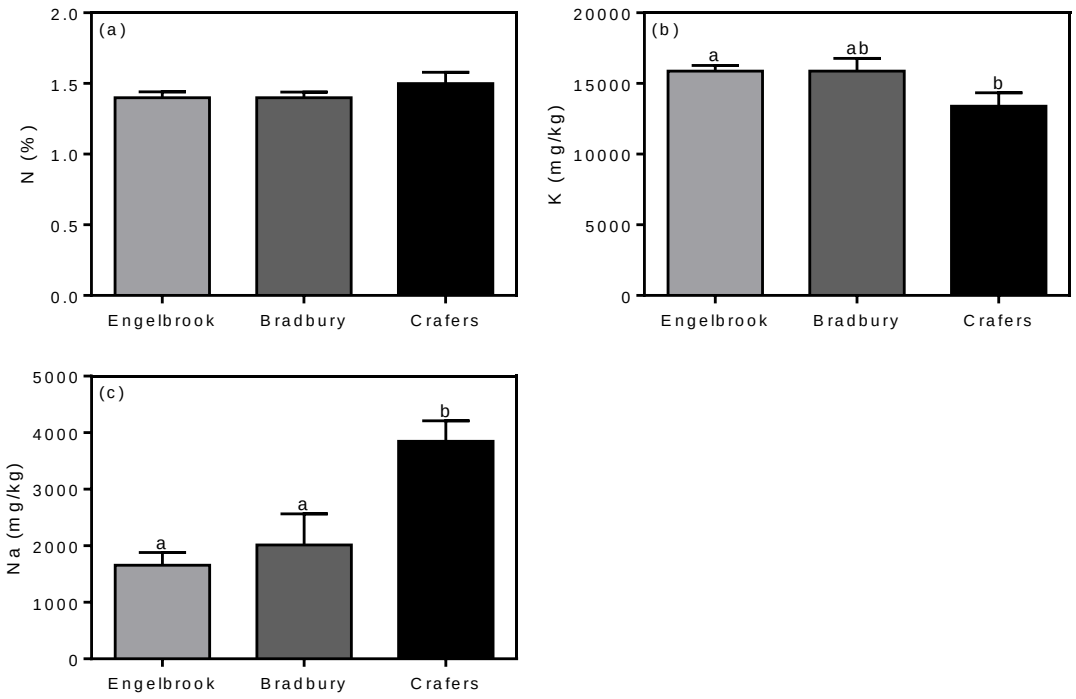

Fig. 5. (a) Nitrogen, (b) potassium and (c) sodium concentration of *Cassytha pubescens* stems infecting *Ulex europaeus* at three field sites in the Mt. Lofty Ranges of South Australia. Data are means (±1SE), different letters indicate significant differences and *n*=10 (except at Bradbury, *n*=5).

Appendix 3

Midday ETR of *L. myrsinoides* three weeks prior to final measurments

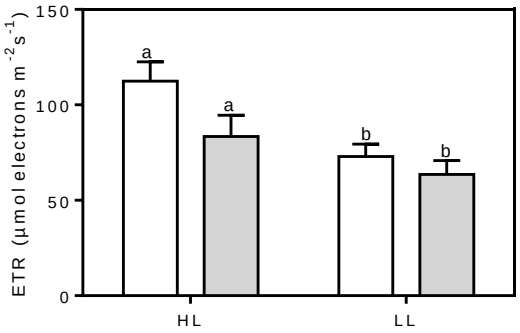

Fig. 1. *In situ* midday electron transport rates (ETRs) of *L. myrsinoides* grown in high (HL) or low light (LL), and uninfected (open bars) or infected (grey bars) with *C. pubescens*. Measurments were taken 3 weeks before the end of light experiment 1. No significant interaction between light x infection ($P = 0.465$) or independent infection effect ($P = 0.097$). There was a significant light effect ($P = 0.002$) as indicated by the different letters. Bars are means (±1 s.e.) and $n = 10$ (except infected LL plants, $n = 8$).