Title page

Running title: Native hemiparasite impacts invasive host in field

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A native parasitic plant affects the performance of an introduced host regardless of environmental variation across field sites.

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Abstract. There is increasing evidence that native hemiparasitic plants can have significant impacts on performance and growth of introduced host plants. However, most of this evidence has been obtained from glasshouse studies. We investigated the effect of the native Australian hemiparasite, *Cassytha pubescens* R. Br., on the physiology of the introduced shrub *Ulex europaeus* L., at three field sites in South Australia. Parasite infection significantly decreased Φ_{PSII} and maximum electron transport rates of *U. europaeus* across sites. The impact of *C. pubescens* on photosynthetic performance of *U. europaeus* may have been due to infected plants having significantly lower nitrogen and potassium, but higher iron and aluminium than uninfected plants at all three sites. At two of the three sites *C. pubescens* had a significant impact on host F_v/F_m indicating chronic photoinhibition in response to infection. The impact of infection on F_v/F_m was greatest at the wettest site, in line with a previous experiment where *C. pubescens* had a greater impact on this host under high water availability. At this site infected plants also had the highest foliar Fe and Al. $\delta^{13}C$ of infected plants was significantly lower than uninfected plants at only one of the three sites. Unusually, $\delta^{13}C$ of the parasite was either the same as or significantly higher than hosts. There were no site effects on parasite F_v/F_m or Φ_{PSII}, however ETR_{max} and $\delta^{13}C$ did vary across sites. The results suggest that this native parasite has negative effects on *U. europaeus* in the field, as has previously been found for glasshouse studies. Thus, the survival and abundance of this major introduced weed in Australia could be negatively affected by infection with *C. pubescens*.

Additional keywords: Carbon isotopes, nitrogen nutrition, photosynthesis, quantum yield, water potential.

Introduction

Parasitic plants are an important group globally, with both direct and indirect effects on their hosts and also on the ecological systems in which they occur (Press and Phoenix 2005). For example, they may enhance the ecosystem process of nutrient cycling, via their high quantity and quality litter fall or even through indirect means by influencing soil microbial activity (Bardgett *et al.* 2006; Quested 2008; Watson 2009). At the community level, the presence of parasitic plants can increase the abundance of a range of fauna: insects, arachnids, hymenoptera, detritivores and birds (Watson 2009; Hartley *et al.* 2015). Parasites can have differential effects on host species, and thus impact on community structure. For instance, in the presence of *Rhinanthus minor* L., the abundance of forbs relative to grasses significantly increases (Bardgett *et al.* 2006; Hartley *et al.* 2015). This differential impact may be explained by the parasite's haustoria connecting more effectively to the vasculature of grasses over forbs (Cameron *et al.* 2006; Cameron and Seel 2007; Rümer *et al.* 2007).

Once connected, there are still a number of factors that may alter the degree of impact of parasites on their hosts, such as changes in abiotic conditions experienced by the association. For example, in China the strong impact of the stem holoparasite *Cuscuta campestris* Yunker on total biomass of the introduced host *Mikania micrantha* H.B.K. was more severe under high compared with low N conditions (Shen *et al.* 2013). The same outcome was reported in Africa for the root hemiparasite *Striga hermonthica* (Del.) Benth. when infecting *Oryza sativa* L. (Cechin and Press 1994). By contrast, Cechin and Press (1993) found that the significant impact of *S. hermonthica* on growth of *Sorghum bicolor* (L.) Moench cv. CSH1 at low N was ameliorated by high N supply. On the other hand, at a single field location in Africa, Gurney *et al.* (1995) found that nitrogen had no influence on the effect of *S. hermonthica* on growth of maize and sorghum cultivars.

In southern Australia across six field sites that varied significantly in soil salinity, Miller *et al.* (2003) found that the stem hemiparasitic mistletoe *Amyema miquelii* (Lehm. ex Miq.) Tiegh. had no effect on water potentials or $\delta^{13}C$ of the host *Eucalyptus largiflorens* F. Muell.. Borowicz and Armstrong (2012) found that the effect of the root hemiparasite *Pedicularis canadensis* L. on growth of the grass *Andropogon gerardii* Vitman was unaffected by light. Through glasshouse experiments, it was found that neither light nor nitrogen influenced the differential impact of the Australian native stem hemiparasite *Cassytha pubescens* R. Br. on performance of introduced (*Ulex europaeus* L.) compared with native hosts (Cirocco *et al.* 2016*a*; Cirocco *et al.* 2017). By contrast, the significant effect of *C. pubescens* on growth of

U. europaeus was much stronger under high rather than low water supply (Cirocco *et al.* 2016*b*).

In the field, *C. pubescens* has been shown to negatively affect growth of the introduced host, *Cytisus scoparius* L. Link, but not native host, *Leptospermum myrsinoides* Schltdl. (Prider *et al.* 2009). However, it is unknown whether *C. pubescens* also affects physiology of *U. europaeus* in the field, as reported for glasshouse experiments, or whether those effects will be consistent across several sites which differ in environmental conditions. Physiological measurements such as chlorophyll flourescence (Maxwell and Johnson 2000; Gurney *et al.* 2002; Cirocco *et al.* 2015) can be used as strong indicators of early declines in host health where biomass comparisons in the field are otherwise not feasible. If the impact of *C. pubescens* on *U. europaeus* physiology could be confirmed in the field and consistent across locations, then there would be further evidence for the potential-use of this parasite as a native bio-control against major invasive shrubby weeds in Australia. This is of great importance as *U. europaeus* is considered one of the top 20 worst weeds in Australia because it has become so difficult to manage with conventional methods (Thorp and Lynch 2000). In addition, there is a need to understand the effects of *C. pubescens* on community structure, and thus more field studies on specific hosts are needed (Demey *et al.* 2015).

Here, we investigated the impact of the Australian native stem hemiparasitic vine *C. pubescens* on the physiology of the introduced host *U. europaeus* at three field sites in South Australia. It was hypothesised that the parasite would negatively affect the performance of *U. europaeus* in the field, as has been observed previously in glasshouse studies (Cirocco *et al.* 2016*a*; Cirocco *et al.* 2016*b*; Cirocco *et al.* 2017). Our secondary hypothesis was that where we found differences in host performance across the three sites in response to infection, these could be explained by the differences in water, light or nutrients across sites. We also predicted that in the field, *C. pubescens* would be more conservative in its water-use than the host as previously found in a glasshouse study (Cirocco *et al.* 2016*b*).

Materials and methods

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 establishes quickly 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's xylem. *C. pubescens* is a generalist parasite and in its native range, has been observed infecting *U. europaeus,* an association that has been extensively studied in the glasshouse (Cirocco *et al.* 2016*a*; Cirocco *et al.* 2016*b*; Cirocco *et al.* 2017).

Study sites

The study was conducted at three field sites, Engelbrook, Bradbury and Crafers 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 now only 10−18% supports remnant native vegetation (Westphal *et al.* 2003). The climate is Mediterranean with mean annual rainfall of 789 mm, most of which falls in winter (Australian Bureau of Meteorology: BoM 2011/2012). Mean maximum temperatures in winter and summer are 9.5 and 23.4°C, respectively (BoM 2011/2012). The native vegetation of the study area is eucalypt dominated woodland with an understorey of sclerophyllous shrubs and a ground layer of low lying shrubs, sedges and grasses (Armstrong *et al.* 2003). Soils are generally sandy loams to sandy clays, shallow and nutrient poor with a pH of 4−6 (Fogarty and Facelli 1999; Armstrong *et al.* 2003).

The elevation, slope and aspect at each site were: Engelbrook (330 m, 3° and East-West); Bradbury (440 m, 31º and South-facing) and Crafers (492 m, 21.8º and North-facing). The maximum number of replicate plants possible was chosen at each site and selected according to two criteria: *a*) having similar size and levels of infection (around 30−50% cover), and *b*) growing with as little over storey cover as possible (Supplementary Material Fig. S1). Measurements were made on both infected and uninfected plants, and the parasite when present. Photosynthetic photon flux densities (PPFD), temperature and relative humidity were recorded on days when physiological measurements were conducted using LI-1400 data loggers fitted with a quantum sensor (LI-190 SA) and relative humidity/air temperature sensor (1400-104) (LI-COR, Lincoln NEB., Supplementary Material Fig. S2). At each site, soil was sampled from the top 60 cm of the profile using an auger at five different locations spanning the area where plants were measured. All soil characteristics at each site (Supplementary Material Fig. S3) were determined by Cuming Smith British Petroleum soil

and plant laboratory (Western Australia) using the techniques described in Supplementary Material Fig. S3.

Photosynthesis and water potential

Pre-dawn (F_v/F_m) and midday (Φ_{PSII}) PSII efficiency, and maximum electron transport rates (ETR_{max}) 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 made on the youngest fully expanded shoot of uninfected *U. europaeus* plants, and likewise for infected shoots of infected plants. Measurements for the parasite were made 15 cm from the growing tip of individual stems. Φ_{PSII} measurements were made between 12–1:30 pm. Mean PPFDs (μmol m^{-2} s^{-1}) (for uninfected and infected plants combined) during measurements of Φ_{PSII} on *U. europaeus* at Engelbrook, Bradbury and Crafers were: 1230 ± 31 (n = 20), 1224 ± 25 (n = 10) and 1342 ± 21 (n = 20), respectively. Mean PPFDs (μmol m^{-2} s^{-1}) during measurements of Φ_{PSII} for *C. pubescens* (when infecting *U. europaeus*) at Engelbrook, Bradbury and Crafers were: 1459 ± 28 (n =10), 1181 ± 39 (n = 5) and 1336 ± 26 (n = 10), respectively. Light response curves were measured between 9 am–12 pm and used to determine ETR_{max} of all plants (see Cirocco *et al.* 2017 for details).

Pre-dawn and midday shoot water potentials (Ψ) were determined on youngest fully expanded shoots (freshly cut 15 cm from growing tip) of uninfected and infected *U. europaeus* (Engelbrook and Crafers: n = 10; Bradbury: n = 5) using a Scholander-type pressure chamber with a digital gauge (PMS Instrument Company, Albany, OR). Midday water potential measurements were made between 12–2:30 pm. All physiological measurements were made on the same day at Engelbrook, Bradbury and Crafers in March, April and May, respectively. Nonetheless, all measurements were made at the end of the dry season on sunny days where PPFD was both saturating, and also similar for all plants (Supplementary Material Fig. S2).

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

Stable carbon isotope discrimination ($\delta^{13}C$) and nitrogen (N) concentration of host spines and parasite stems were quantified via mass spectrometry (The University of Adelaide). Additional elemental analysis of host spines and parasite stems was made 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°C for six days) collected on the same days when physiological measurements were made. This material was comparable in position and age with host shoots and parasite stems used for physiological measurements. Replication (uninfected and infected *U. europaeus* and the parasite) for carbon isotope, nitrogen and additional elemental analyses was $n = 7–10$ at Engelbrook, $n = 5$ at Bradbury and $n = 10$ at Crafers.

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 randomly). 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 (and soil characteristics: Supplementary Material Fig. S3), 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 $\alpha = 0.05$.

Results

Host and parasite F_v/F_m, Φ_{PSII} and ETR_{max}

There was a significant interaction effect for infection × site on F_v/F_m (Table 1). Infection had a significant negative impact on F_v/F_m of *U. europaeus* at Bradbury and Crafers but not at Engelbrook (Fig. 1*a*). While there was no significant interaction or site effect for Φ_{PSII}, it was independently affected by infection (Table 1; Fig. 1*b*). Φ_{PSII} of infected plants was approximately 40% less than that of uninfected plants, regardless of site (Fig. 1*c*). Site had no effect on F_v/F_m or Φ_{PSII} of *C. pubescens* ($P = 0.065$ and 0.886, respectively; Fig. 1*d*, *e*; Supplementary Material Table S2).

There was no significant interaction or independent site effect detected for ETR_{max} of *U. europaeus*, but it was significantly affected by infection (Table 1; Fig. 2*a*). On average, ETR_{max} of infected plants was 36% lower compared with that of uninfected plants, irrespective of site (Fig. 2*b*). ETR_{max} of *C. pubescens* was significantly different among sites ($P = 0.008$; Supplementary Material Table S2). ETR_{max} of the parasite at Crafers was

210 significantly higher than those at the other two sites which did not significantly differ from
211 each other (Fig. 2*c*).

212 *Host PD and MD Ψ*

213 An interaction was detected for shoot Ψ of *U. europaeus* at pre-dawn, however, the pairwise
214 comparison found no differences. Although not significant, Ψ of infected plants at Bradbury
215 and Crafers was lower than that of respective uninfected plants (Table 1; Fig. 3*a*). A
216 significant interaction effect was also detected for midday shoot Ψ of *U. europaeus* (Table 1).
217 Infection had a negative effect on host Ψ, although not significant, at both Bradbury and
218 Crafers (Fig. 3*b*). The lowest Ψ at midday was recorded in infected plants at Crafers (–2.83 ±
219 0.062 MPa), and the highest was in uninfected plants at Bradbury (–1.76 ± 0.085 MPa).

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

221 There was a significant interaction effect for infection × site on $\delta^{13}C$ of *U. europaeus* (Table
222 1). Infected plants at Crafers had significantly lower $\delta^{13}C$ (–22.6 ± 0.273‰) than respective
223 uninfected plants (–20.3 ± 0.180‰), while there was no effect of infection at the other two
224 sites (Fig. 4*a*). There was a significant site effect on $\delta^{13}C$ of the parasite ($P = 0.023$), with
225 values for *C. pubescens* at Crafers being significantly higher (–20.9 ± 0.172‰) than at
226 Engelbrook (–22.1 ± 0.279‰), while values at Bradbury were intermediate (–21.2 ± 0.570‰)
227 (Fig. 4*b*; Supplementary Material Table S2). When infected plants were compared with
228 parasites, there was a significant species × site interaction for $\delta^{13}C$ ($F_{2, 41} = 5.8$, $P = 0.006$).
229 Infected plants had significantly lower $\delta^{13}C$ than parasites at Bradbury and Crafers, while
230 there was no difference between host and parasite at Engelbrook (Fig. 4*c*).

231 *Host and parasite nutrient concentrations*

232 There was no infection × site interaction on nutrient concentrations of *U. europaeus* spines
233 (Tables 1, 2). There was, however, an independent effect of infection on N, Al, Fe and K
234 concentration of *U. europaeus* (Table 2). On average, infection with *C. pubescens* decreased
235 nitrogen concentration of *U. europaeus* by 16%, across sites (Table 2). Infection had a large
236 effect on aluminium and iron concentration of infected plants, with concentrations being
237 approximately 60% and 30% higher, respectively, relative to uninfected plants (Table 2).
238 Infection decreased potassium concentration of *U. europaeus* by 22%, across sites (Table 2).

There was also an independent effect of site on N, Al, K and Na concentration of *U. europaeus* spines (Table 1). Nitrogen and potassium concentrations 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 2). 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 2). 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 2).

Nitrogen concentration of parasite stems was similar among sites ($P = 0.121$; Fig. 5*a*; Supplementary Material Table S2). 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. 5*b*; Supplementary Material Table S2). 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. 5*c*; Supplementary Material Table S2).

Discussion

Based on previous glasshouse studies, we hypothesised that *C. pubescens* would have a negative impact on performance of *U. europaeus* in the field, regardless of variation in environmental conditions across our study sites. This was supported by our results, which showed that ETR_{max} was significantly lower, by around a third, in infected plants across all three sites. Previously we have shown that *C. pubescens* significantly affected photosynthesis of *U. europaeus* when grown under different nitrogen regimes (Cirocco *et al.* 2017) and under either high or low light (Cirocco *et al.* 2016*a*). Photosynthesis of another introduced host, *Cytisus scoparius* was significantly reduced by *C. pubescens* under ambient light conditions in the glasshouse and also in the field (Prider *et al.* 2009; Shen *et al.* 2010). These studies provide strong evidence that infection with *C. pubescens* has a negative impact on photosynthesis in these introduced hosts. By contrast, *C. pubescens* had no effect on photosynthesis of the native host *Acacia paradoxa* DC., irrespective of nitrogen addition to the soil (Cirocco *et al.* 2017). The parasite did decrease photosynthesis of the native host *Leptospermum myrsinoides* in the field and under high but not low light in the glasshouse.

Nevertheless, this effect did not translate into reductions in overall growth of this native host (Prider *et al.* 2009; Cirocco *et al.* 2015; Cirocco *et al.* 2016*a*).

In the present study, the effect of infection on photosynthetic performance of *U. europaeus* does not seem to be related to decreases in host stomatal conductance as $\delta^{13}C$ was actually lower for infected plants (Fig. 4*a*). Rather, the parasite impact on host ETR_{max} may be due to the significant negative effect *C. pubescens* had on host nitrogen concentration at all sites (Tables 1, 2). This was also found for the *C. pubescens*-*U. europaeus* association in the glasshouse (Cirocco *et al.* 2016*b*). Significant impacts of parasitic plants on host nitrogen status have also been reported for a number of other host-parasite relationships (Watling and Press 2000; Meinzer *et al.* 2004; Shen *et al.* 2013). The effect of infection on nitrogen concentration of *U. europaeus* is a likely consequence of N removal by *C. pubescens* from the host's xylem.

In addition to having lower N concentrations, infected *U. europaeus* were significantly enriched in Al and Fe compared with uninfected plants across all sites (Tables 1, 2). One explanation for this may be that infection led to increased acidification of the rhizosphere which would increase the mobility of Al and Fe ions for uptake by roots (Haynes 1990). This increased acidification of the rhizosphere may have occurred in response to the negative effect of infection on plant potassium concentrations across all sites (Tables 1, 2). Decreases in foliar potassium are known to lead to increased potassium uptake by roots, which then results in increased extrusion of protons to maintain charge balance in root cells. The increased release of protons causes acidification of the rhizosphere (Houmani *et al.* 2015). This response is thought to be the first line of defence against K deficiency (Houmani *et al.* 2015), however it is unknown whether foliar K levels in the infected plants in our study were low enough to trigger this response. The decrease in potassium of infected plants is likely to be a consequence of its removal by the parasite. Indeed, parasitic plants are well known to accumulate potassium (Pate 1995), and in our study *C. pubescens* had around double the K concentration of its hosts (Table 2; Fig. 5*b*). If parasitic plants can indirectly increase rhizosphere acidification via lowering host K, then increased Fe and Al uptake could have consequences for host plant performance. For example, plant exposure to micromolar concentrations of Al (for less than 60 min) can be toxic and impair root growth (Delhaize and Ryan 1995).

While our results demonstrate that infection with *C. pubescens* has a negative impact on *U. europaeus* in the field regardless of site, we also expected that variations in environmental conditions across sites might influence the magnitude of the infection effect. For example, previously we have found that the effect of *C. pubescens* is more pronounced when water availability is high (Cirocco *et al.* 2016*b*), but was not influenced by soil N content or light (Cirocco *et al.* 2016*a*; Cirocco *et al.* 2017). Using pre-dawn Ψ of uninfected plants as a proxy for soil water availability (Fig. 3*a*), Bradbury was the wettest of the three sites, and the infection effect on both ETR_{max} and F_v/F_m was greater here than at either of the other two sites (Figs 1*a* and 2*a*). These findings are consistent with the overall finding that *C. pubescens* had a greater impact on growth of well watered plants in the glasshouse (Cirocco *et al.* 2016*b*). Although Cirocco *et al.* (2016*b*) found that the strong decrease in F_v/F_m of *U. europaeus* in response to the parasite was not influenced by water, there are also undoubtedly multiple interacting factors in the field, so the combination of, for example, high water and lower N at Bradbury might have influenced the impact of infection on *U. europaeus*. This would run contrary to Tesitel *et al.* (2015) who found that *Rhinanthus alectorolophus* (Scop.) Pollich (native to Europe) had a strong effect on F_v/F_m of the introduced crop maize relative to wheat when water availability was low and nitrogen supply was high. Along with the parasite strongly decreasing light-use efficiency of *U. europaeus* in this study (both at Bradbury and Crafers), *C. pubescens* has also been found to significantly lower F_v/F_m of the introduced host *Cytisus scoparius* under glassouse but not field conditions (Prider *et al.* 2009; Shen *et al.* 2010). On the other hand, *C. pubescens* was found to have no impact on F_v/F_m of the native host *L. myrisnoides* in both glasshouse and field settings (Prider *et al.* 2009; Cirocco *et al.* 2015). In other cases, parasitic plants have been found to negatively affect F_v/F_m of introduced (Mauromicale *et al.* 2008) but not native hosts (Hibberd *et al.* 1996). Here, the greater decline in photosynthesis suggested by the ETR_{max} results, would result in the plant being more likely to be exposed to excess light and ultimately chronic photoinhibiton, as reflected by the decline in F_v/F_m (Demmig-Adams and Adams 2006). Both lower rates of photosynthesis and chronic photoinhibtion in response to infection could contribute to poor host growth (Gurney *et al.* 2002; Cirocco *et al.* 2016*b*).

Interestingly, there was no effect of infection on host $\delta^{13}C$ except at Crafers (Fig. 4*a*), where infected plants had significantly lower $\delta^{13}C$ than uninfected plants. Midday Ψ of infected plants at Crafers was also lower than at the other two sites (Fig. 3*b*), which may have been a consequence of greater stomatal conductance as suggested by the $\delta^{13}C$ results. The Crafers

site has a north-facing aspect, which in the southern-hemisphere is more exposed to sun, and would be expected to have the greatest evaporation rates. These plants also accumulated more sodium, relative to uninfected plants, than at the other two sites, which may also have contributed to the lower midday Ψ (Table 2). Maintaining a low midday Ψ would make it more difficult for the parasite to remove water while also facilitating host water uptake from the soil at this site.

We found that *C. pubescens* had significantly higher $\delta^{13}C$ than infected plants at Bradbury and Crafers but not those at Engelbrook (Fig. 4*c*). Similarly, Cirocco *et al.* (2016*b*) found that the parasite had significantly higher $\delta^{13}C$ than *U. europaeus*, regardless of water availability. By contrast, Scalon and Wright (2015) using C isotope data for 93 mistletoe-host pairs from around the world found that the parasites typically maintained lower $\delta^{13}C$ relative to their hosts. When taking into account temperature, this difference held at warm sites while at cold sites $\delta^{13}C$ did not vary between parasite and host (Scalon and Wright 2015). Bannister and Strong (2001) investigating 158 mistletoe-host pairs in New Zealand found that 63% of mistletoes had more negative $\delta^{13}C$ than their host, the other 37% had higher $\delta^{13}C$ than hosts. They argued that the small difference in $\delta^{13}C$ between many mistletoe-host pairs may be attributed to New Zealand's moist, temperate climate (Bannister and Strong 2001). Notably, higher $\delta^{13}C$ in parasitic plants relative to their hosts might be due to heterotrophy (Cernusak *et al.* 2004) and heterotrophic C gain in *C. pubescens* requires quantification. Our $\delta^{13}C$ findings suggest that, as we hypothesised, *C. pubescens* is more conservative in its water-use than its host, *U. europaeus*. Moreover, at least at Bradbury and Crafers, the parasite's main mode of resource extraction might be osmotic accumulation (high parasite K relative to host as mentioned), rather than maintaining higher transpiration rates than the host, particularly as conditions become drier as presumed at Crafers.

Conclusion

We aknowledge that there is a possibility that some variables measured in this study might not be independent. However the low *P* values for all but one of the host parameters discussed minimise the possibility of basing our conclusion on spurious significant effects. Also, while non-manipulative field studies such as ours cannot conclusively demonstrate cause and effect, the combination of the field data reported here and the controlled experimental evidence (e.g. Cirocco *et al.* 2016*a*; Cirocco *et al.* 2016*b*; Cirocco *et al.* 2017) strongly suggest that the native hemiparasite *C. pubescens* has consistent negative impacts on

366 the performance of the introduced host *U. europaeus*. We also showed that the parasite is
367 likely to have a greater effect on the host at wetter sites, as was found in the glasshouse.
368 Although this study cannot confirm the chain of physiological effects triggered by infection,
369 the data suggest that *C. pubescens* negatively impacts *U. europaeus* by severely affecting
370 host nitrogen and perhaps K, Al and Fe-status, likely leading to suppressed photosynthesis
371 and ultimately chronic photoinhibition. As a result, thiswould negatively affect the C budget
372 and thus, growth and reproduction of this host. The question remains, why *C. pubescens* did
373 not have as great an effect on *U. europaeus* at Engelbrook as at the other two sites. This
374 discrepancy may be due to plants at Engelbrook being infected with *C. pubescens* for a
375 shorter time relative to the other two sites, as was reported by the landowners. Regardless, we
376 have provided additional evidence of the physiological mechanisms that underpin the effect
377 of *C. pubescens* on this introduced host in the field, thus, helping confirm the potential-use of
378 *C. pubescens* as a native bio-control against this major introduced weed, and possibly others,
379 in Australia.

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Table 1. Two-way ANOVA results (*P*-values) for the effect of infection with *Cassytha pubescens* (I) and site (S) on pre-dawn and midday PSII efficiency (F_v/F_m, Φ_{PSII}), maximum electron transport rates (ETR_{max}), pre-dawn (PD) and midday (MD) shoot water potentials (Ψ), carbon isotope composition ($\delta^{13}C$), nitrogen (N), aluminium (Al), iron (Fe), potassium (K) and sodium (Na) concentration of *Ulex europaeus* spines. Significant effects are in bold

Degrees of freedom, *F* and sum of square values are presented in Supplementary Material Table S1

Factor	F_v/F_m	Φ_{PSII}	ETR_{max}	PD Ψ	MD Ψ	$\delta^{13}C$	N	Al	Fe	K	Na
I	**0.0002**	**0.0003**	**0.0002**	0.376	0.731	**0.001**	**0.001**	**<0.0001**	**<0.0001**	**0.008**	0.256
S	**<0.0001**	0.107	0.664	0.169	**0.0006**	**0.0002**	**<0.0001**	**0.001**	0.230	**<0.0001**	**0.025**
I × S	**0.001**	0.937	0.328	**0.040**	**0.004**	**0.0001**	0.860	0.336	0.368	0.327	0.103

Table 2. Concentrations of nitrogen (N, %), aluminium (Al, mg/kg), iron (Fe, mg/kg), potassium (K, mg/kg) and sodium (Na, mg/kg) in *Ulex europaeus* spines when 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 (± s.e.), 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

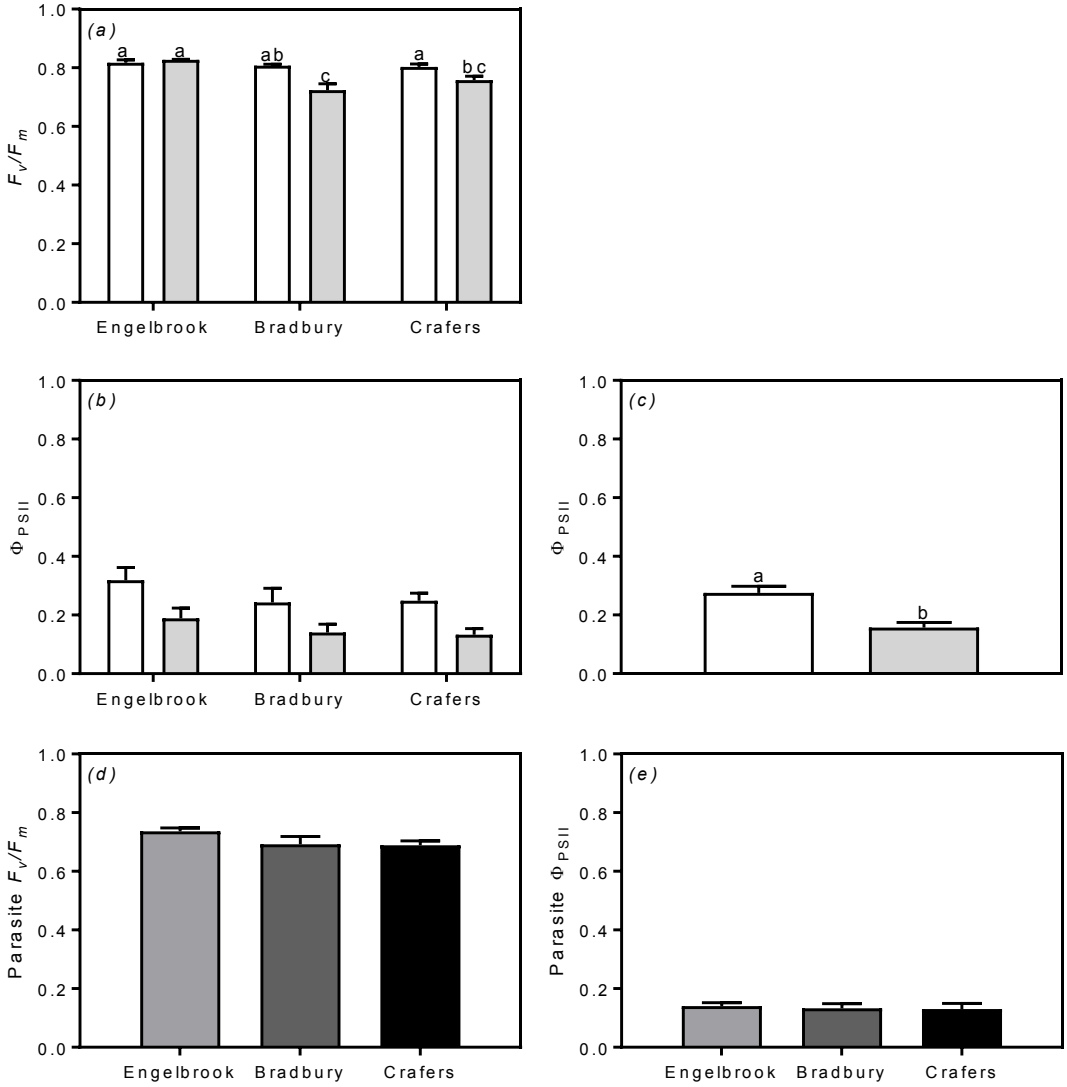
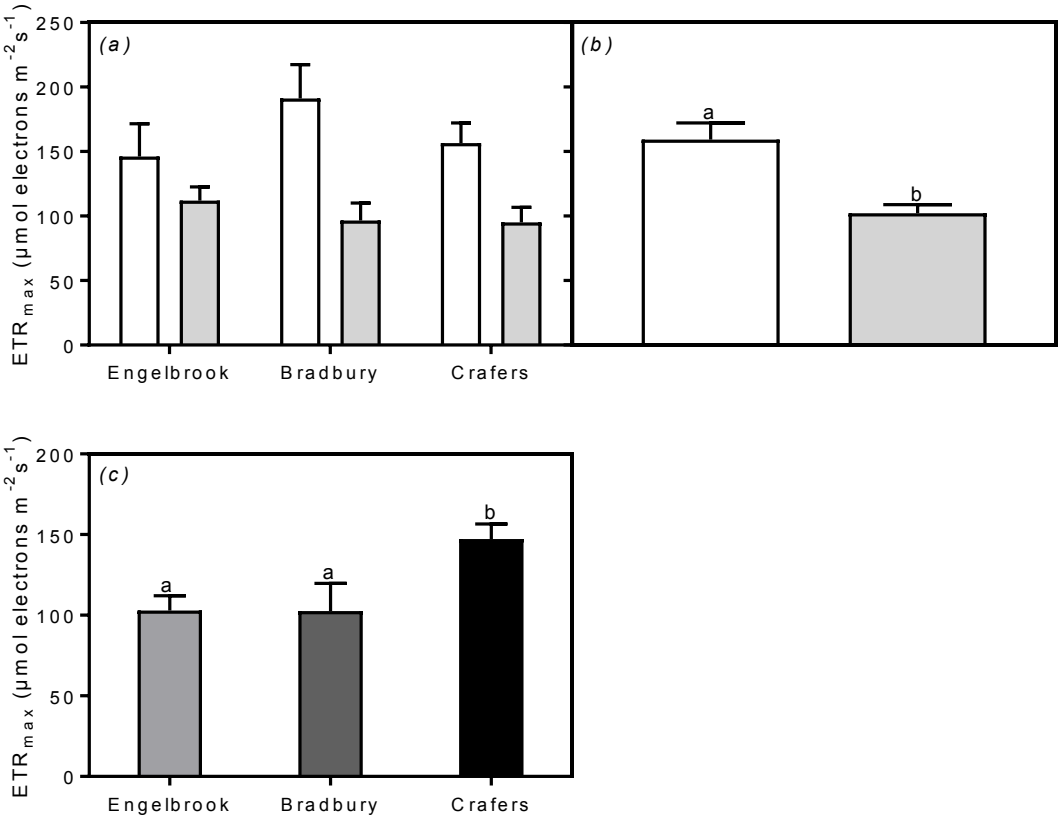


Fig. 1. (*a*) Pre-dawn (F_v/F_m) and (*b*) midday (Φ_{PSII}) PSII efficiency of *Ulex europaeus* either uninfected (open bars) or infected (light grey bars) with *Cassytha pubescens* at three field sites in the Mt. Lofty Ranges of South Australia. (*c*) Independent infection effect on host Φ_{PSII}.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



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553 **Fig. 2.** (*a*) Maximum electron transport rates (ETR_{max}) of *Ulex europaeus* either uninfected
554 (open bars) or infected (light grey bars) with *Cassytha pubescens* at three field sites in the Mt.
555 Lofty Ranges of South Australia. (*b*) Independent infection effect on host ETR_{max}. (*c*) ETR_{max}
556 of *C. pubescens* infecting *U. europaeus* at the three sites. Data are means (± s.e.), different
557 letters indicate significant differences and $n = 10$ (*a*, *c*) (except at Bradbury, $n = 5$); $n = 25$
558 (*b*).

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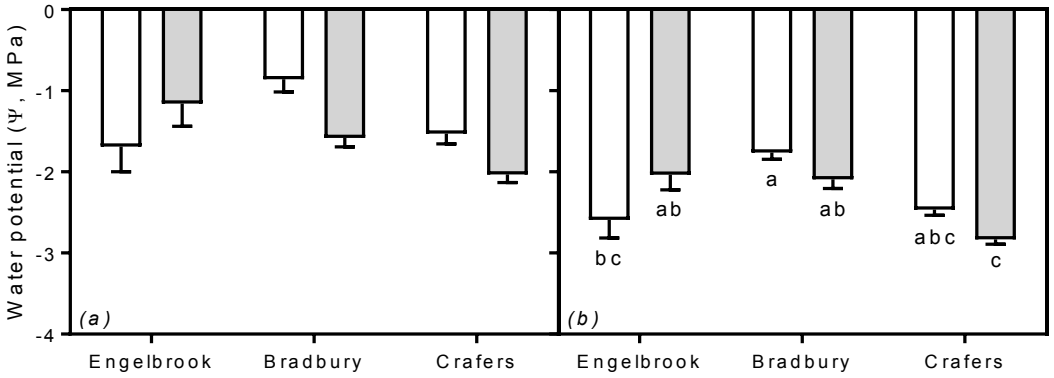

566

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

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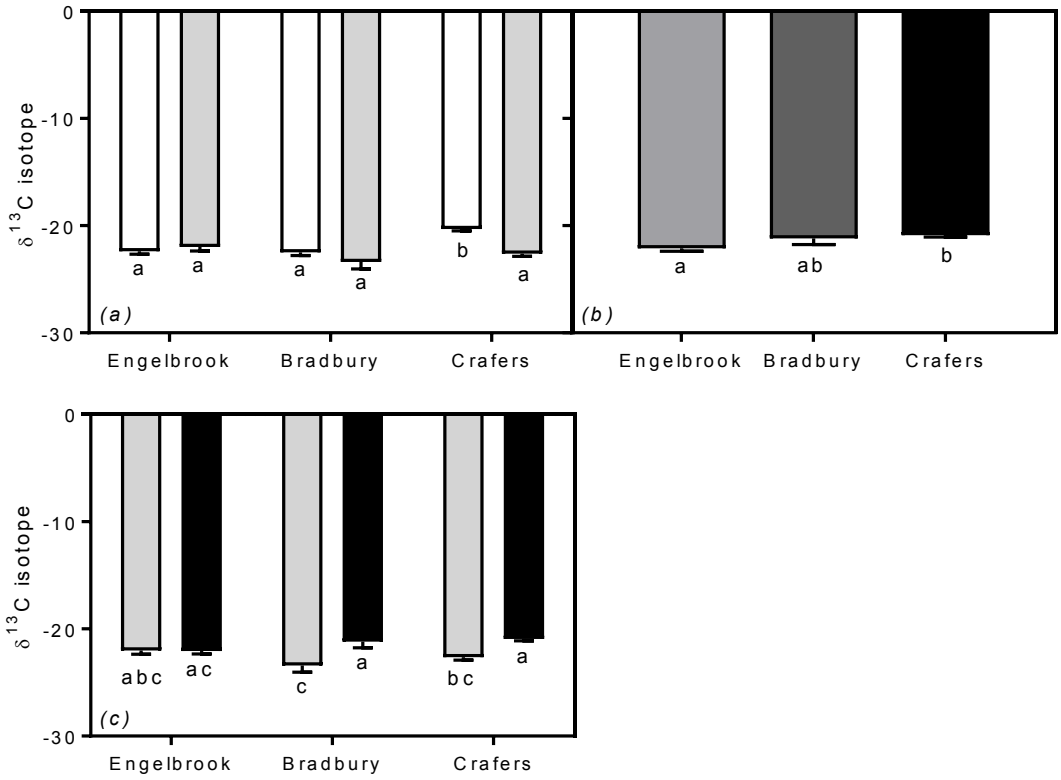

Fig. 4. (*a*) Spine $\delta^{13}C$ (‰) of *Ulex europaeus* when either uninfected (open bars) or infected (light grey bars) with *Cassytha pubescens* at three field sites in the Mt. Lofty Ranges of South Australia. (*b*) $\delta^{13}C$ of *C. pubescens* stems at the three sites. (*c*) $\delta^{13}C$ of both infected *U. europaeus* (light grey bars) and parasite (black bars) at the three sites. Data are means (± s.e.), 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*).

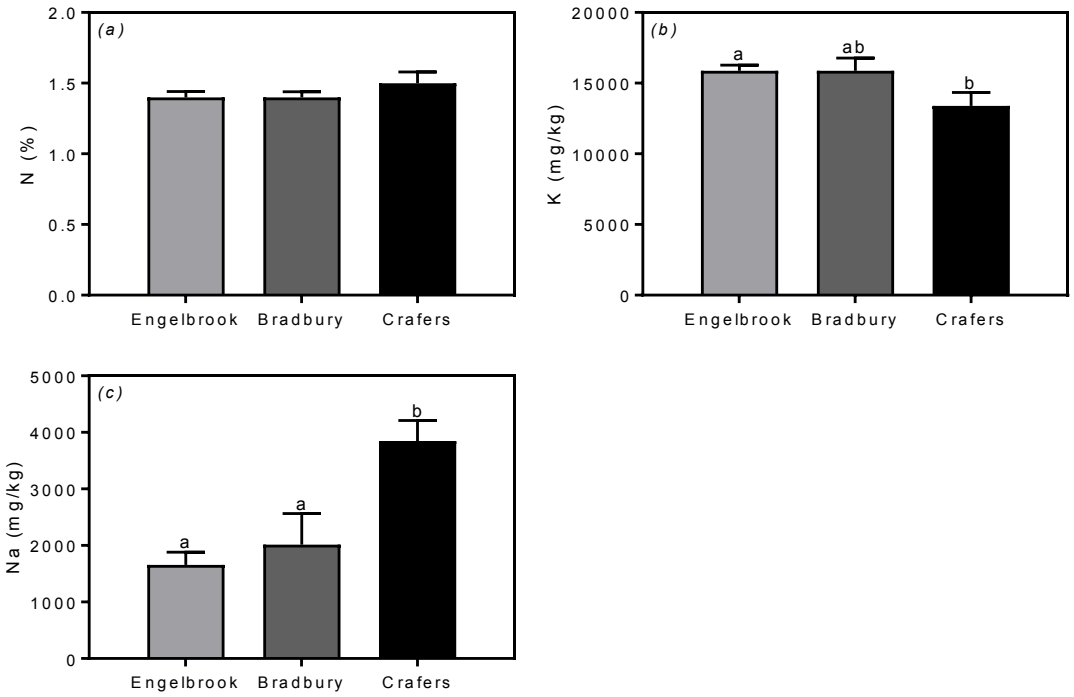

Fig. 5. (*a*) Nitrogen, (*b*) potassium and (*c*) sodium concentration of *Cassytha pubescens* stems infecting *Ulex europaeus* at three field sites in the Mt. Lofty Ranges of South Australia. Data are means (± s.e.), different letters indicate significant differences and $n = 10$ (except at Bradbury,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