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Cirocco, RM, Facelli, JM and Watling, JR (2018) A native parasitic plant affects the performance of an introduced host regardless of environmental variation across field sites. Functional Plant Biology, 45 (11). pp. 1128-1137. ISSN 1445-4408

DOI: https://doi.org/10.1071/FP17358

Publisher: CSIRO Publishing

Version: Accepted Version

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1	Title page
2	Running title: Native hemiparasite impacts invasive host in field
3	Title: A native parasitic plant affects the performance of an introduced host regardless
4	of environmental variation across field sites.
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12 13	All data presented is original and intellectual property of the authors with no part being published elsewhere.
14 15	The authors declare no conflicts of interest
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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 31 impacts on performance and growth of introduced host plants. However, most of this 32 evidence has been obtained from glasshouse studies. We investigated the effect of the native 33 Australian hemiparasite, Cassytha pubescens R. Br., on the physiology of the introduced 34 shrub Ulex europaeus L., at three field sites in South Australia. Parasite infection 35 significantly decreased Φ_{PSII} and maximum electron transport rates of U. europaeus across 36 sites. The impact of C. pubescens on photosynthetic performance of U. europaeus may have 37 38 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 39 had a significant impact on host F_v/F_m indicating chronic photoinhibition in response to 40 infection. The impact of infection on F_v/F_m was greatest at the wettest site, in line with a 41 previous experiment where C. pubescens had a greater impact on this host under high water 42 availability. At this site infected plants also had the highest foliar Fe and Al. δ^{13} C of infected 43 plants was significantly lower than uninfected plants at only one of the three sites. Unusually, 44 δ^{13} C of the parasite was either the same as or significantly higher than hosts. There were no 45 site effects on parasite F_v/F_m or Φ_{PSII} , however ETR_{max} and $\delta^{13}C$ did vary across sites. The 46 results suggest that this native parasite has negative effects on U. europaeus in the field, as 47 has previously been found for glasshouse studies. Thus, the survival and abundance of this 48 major introduced weed in Australia could be negatively affected by infection with C. 49 50 pubescens.

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

53 Introduction

54 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
- so example, they may enhance the ecosystem process of nutrient cycling, via their high quantity
- 57 and quality litter fall or even through indirect means by influencing soil microbial activity
- 58 (Bardgett *et al.* 2006; Quested 2008; Watson 2009). At the community level, the presence of
- 59 parasitic plants can increase the abundance of a range of fauna: insects, arachnids,
- 60 hymenoptera, detritivores and birds (Watson 2009; Hartley *et al.* 2015). Parasites can have
- 61 differential effects on host species, and thus impact on community structure. For instance, in
- 62 the presence of *Rhinanthus minor* L., the abundance of forbs relative to grasses significantly
- 63 increases (Bardgett et al. 2006; Hartley et al. 2015). This differential impact may be
- 64 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).
- 66 Once connected, there are still a number of factors that may alter the degree of impact of
- 67 parasites on their hosts, such as changes in abiotic conditions experienced by the association.
- 68 For example, in China the strong impact of the stem holoparasite *Cuscuta campestris* Yunker
- 69 on total biomass of the introduced host *Mikania micrantha* H.B.K. was more severe under
- ⁷⁰ high compared with low N conditions (Shen *et al.* 2013). The same outcome was reported in
- 71 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
- r3 significant impact of *S. hermonthica* on growth of *Sorghum bicolor* (L.) Moench cv. CSH1 at
- Value 74 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.
- 77 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.)
- 79 Tiegh. had no effect on water potentials or δ^{13} C of the host *Eucalyptus largiflorens* F. Muell..
- 80 Borowicz and Armstrong (2012) found that the effect of the root hemiparasite *Pedicularis*
- 81 *canadensis* L. on growth of the grass *Andropogon gerardii* Vitman was unaffected by light.
- 82 Through glasshouse experiments, it was found that neither light nor nitrogen influenced the
- 83 differential impact of the Australian native stem hemiparasite Cassytha pubescens R. Br. on
- 84 performance of introduced (*Ulex europaeus* L.) compared with native hosts (Cirocco *et al.*
- 85 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, 88 Cytisus scoparius L. Link, but not native host, Leptospermum myrsinoides Schltdl. (Prider et 89 al. 2009). However, it is unknown whether C. pubescens also affects physiology of U. 90 europaeus in the field, as reported for glasshouse experiments, or whether those effects will 91 be consistent across several sites which differ in environmental conditions. Physiological 92 measurements such as chlorophyll flourescence (Maxwell and Johnson 2000; Gurney et al. 93 94 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. 95 pubescens on U. europaeus physiology could be confirmed in the field and consistent across 96 locations, then there would be further evidence for the potential-use of this parasite as a 97 98 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 99 100 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, 101 102 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. 103 pubescens on the physiology of the introduced host U. europaeus at three field sites in South 104 Australia. It was hypothesised that the parasite would negatively affect the performance of U. 105 europaeus in the field, as has been observed previously in glasshouse studies (Cirocco et al. 106 2016a; Cirocco et al. 2016b; Cirocco et al. 2017). Our secondary hypothesis was that where 107 we found differences in host performance across the three sites in response to infection, these 108 could be explained by the differences in water, light or nutrients across sites. We also 109 predicted that in the field, C. pubescens would be more conservative in its water-use than the 110 host as previously found in a glasshouse study (Cirocco et al. 2016b). 111

112 Materials and methods

113 *Study species*

114 *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

- 117 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
- 120 which it obtains water and nutrients from its host's xylem. C. pubescens is a generalist
- 121 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*;
- 123 Cirocco *et al.* 2017).

124 *Study sites*

The study was conducted at three field sites, Engelbrook, Bradbury and Crafers in the Mt. 125 Lofty Ranges of South Australia. The Ranges lie east of the Adelaide plains in a north-south 126 direction and cover 5000 km² of which now only 10–18% supports remnant native vegetation 127 (Westphal et al. 2003). The climate is Mediterranean with mean annual rainfall of 789 mm, 128 most of which falls in winter (Australian Bureau of Meteorology: BoM 2011/2012). Mean 129 maximum temperatures in winter and summer are 9.5 and 23.4°C, respectively (BoM 130 2011/2012). The native vegetation of the study area is eucalypt dominated woodland with an 131 understorey of sclerophyllous shrubs and a ground layer of low lying shrubs, sedges and 132 grasses (Armstrong et al. 2003). Soils are generally sandy loams to sandy clays, shallow and 133 nutrient poor with a pH of 4-6 (Fogarty and Facelli 1999; Armstrong et al. 2003). 134

135 The elevation, slope and aspect at each site were: Engelbrook (330 m, 3° and East-West);

- 136 Bradbury (440 m, 31° and South-facing) and Crafers (492 m, 21.8° and North-facing). The
- 137 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*)
- 139 growing with as little over storey cover as possible (Supplementary Material Fig. S1).
- 140 Measurements were made on both infected and uninfected plants, and the parasite when
- 141 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
- 147 (Supplementary Material Fig. S3) were determined by Cuming Smith British Petroleum soil

and plant laboratory (Western Australia) using the techniques described in Supplementary

149 Material Fig. S3.

150 *Photosynthesis and water potential*

Pre-dawn (F_v/F_m) and midday (Φ_{PSII}) PSII efficiency, and maximum electron transport rates 151 (ETR_{max}) of *U. europaeus* spines and *C. pubescens* stems were measured with a portable, 152 pulse-modulated chlorophyll fluorometer (MINI-PAM, Walz, Effeltrich, Germany) fitted 153 with a leaf-clip (2030–B, Walz, Effeltrich, Germany). Measurements were made on the 154 youngest fully expanded shoot of uninfected U. europaeus plants, and likewise for infected 155 shoots of infected plants. Measurements for the parasite were made 15 cm from the growing 156 tip of individual stems. Φ_{PSII} measurements were made between 12–1:30 pm. Mean PPFDs 157 (umol m⁻² s⁻¹) (for uninfected and infected plants combined) during measurements of Φ_{PSII} 158 on U. europaeus at Engelbrook, Bradbury and Crafers were: 1230 ± 31 (n = 20), 1224 ± 25 (n159 = 10) and 1342 ± 21 (*n* = 20), respectively. Mean PPFDs (µmol m⁻² s⁻¹) during measurements 160 of Φ_{PSII} for *C. pubescens* (when infecting *U. europaeus*) at Engelbrook, Bradbury and Crafers 161 were: 1459 ± 28 (*n* =10), 1181 ± 39 (*n* = 5) and 1336 ± 26 (*n* = 10), respectively. Light 162 response curves were measured between 9 am-12 pm and used to determine ETR_{max} of all 163

164 plants (see Cirocco *et al.* 2017 for details).

165 Pre-dawn and midday shoot water potentials (Ψ) were determined on youngest fully

166 expanded shoots (freshly cut 15 cm from growing tip) of uninfected and infected U.

167 *europaeus* (Engelbrook and Crafers: n = 10; Bradbury: n = 5) using a Scholander-type

168 pressure chamber with a digital gauge (PMS Instrument Company, Albany, OR). Midday

169 water potential measurements were made between 12–2:30 pm. All physiological

170 measurements were made on the same day at Engelbrook, Bradbury and Crafers in March,

171 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

173 (Supplementary Material Fig. S2).

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

175 Stable carbon isotope discrimination (δ^{13} C) and nitrogen (N) concentration of host spines and

176 parasite stems were quantified via mass spectrometry (The University of Adelaide).

177 Additional elemental analysis of host spines and parasite stems was made using Radial View

178 Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) at Waite Analytical

- 179 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 180
- were made. This material was comparable in position and age with host shoots and parasite 181
- stems used for physiological measurements. Replication (uninfected and infected U. 182
- europaeus and the parasite) for carbon isotope, nitrogen and additional elemental analyses 183
- was n = 7-10 at Engelbrook, n = 5 at Bradbury and n = 10 at Crafers. 184

Statistical analyses 185

- The variances of the host data were homogeneous. The host's parameters were analysed 186
- using a two-way fixed effects ANOVA (since sites were not chosen randomly). The two-way 187
- ANOVA was used to determine whether there was an interaction between the C. pubescens 188
- infection status of the host and site. If an interaction was not detected, independent effects of 189
- 190 either infection (sites pooled) or site (uninfected and infected plants pooled) were considered.
- Parasite parameters (and soil characteristics: Supplementary Material Fig. S3), also 191
- presenting homogeneous variances, were analysed across sites using one-way ANOVAs. 192
- Significant effects for host and parasite parameters were only considered where the Tukey 193
- HSD test for pairwise comparisons of means also found a difference. All data were analysed 194
- with the software JMP Ver. 4.0.3 (SAS Institute Inc. 2000) and $\alpha = 0.05$. 195

196 Results

Host and parasite F_v/F_m , Φ_{PSII} and ETR_{max} 197

There was a significant interaction effect for infection \times site on F_v/F_m (Table 1). Infection 198 had a significant negative impact on F_v/F_m of U. europaeus at Bradbury and Crafers but not 199

at Engelbrook (Fig. 1*a*). While there was no significant interaction or site effect for Φ_{PSII} , it 200

was independently affected by infection (Table 1; Fig. 1b). Φ_{PSII} of infected plants was 201 approximately 40% less than that of uninfected plants, regardless of site (Fig. 1c). Site had no

- 203 effect on F_v/F_m or Φ_{PSII} of C. pubescens (P = 0.065 and 0.886, respectively; Fig. 1d, e;
- Supplementary Material Table S2). 204
- There was no significant interaction or independent site effect detected for ETR_{max} of U. 205
- europaeus, but it was significantly affected by infection (Table 1; Fig. 2a). On average, 206
- ETR_{max} of infected plants was 36% lower compared with that of uninfected plants, 207
- irrespective of site (Fig. 2b). ETR_{max} of C. pubescens was significantly different among sites 208
- (P = 0.008; Supplementary Material Table S2). ETR_{max} of the parasite at Crafers was 209

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

212 *Host PD and MD* Ψ

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

and Crafers was lower than that of respective uninfected plants (Table 1; Fig. 3*a*). A

- 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. 3b). 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 δ^{13} C of *U. europaeus* (Table
- 1). Infected plants at Crafers had significantly lower $\delta^{13}C$ (-22.6 ± 0.273‰) than respective
- uninfected plants ($-20.3 \pm 0.180\%$), while there was no effect of infection at the other two
- sites (Fig. 4*a*). There was a significant site effect on δ^{13} C of the parasite (*P* = 0.023), with
- values for *C. pubescens* at Crafers being significantly higher $(-20.9 \pm 0.172\%)$ than at
- $\frac{1}{2} = \frac{1}{2} = \frac{1}$
- Engelbrook (-22.1 \pm 0.279‰), while values at Bradbury were intermediate (-21.2 \pm 0.570‰)
- 227 (Fig. 4*b*; Supplementary Material Table S2). When infected plants were compared with
- parasites, there was a significant species × site interaction for δ^{13} C (*F*₂, 41 = 5.8, *P* = 0.006).
- Infected plants had significantly lower δ^{13} C than parasites at Bradbury and Crafers, while
- there was no difference between host and parasite at Engelbrook (Fig. 4c).

231 *Host and parasite nutrient concentrations*

- 232 There was no infection \times 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
- concentration of *U. europaeus* (Table 2). On average, infection with *C. pubescens* decreased
- nitrogen concentration of *U. europaeus* by 16%, across sites (Table 2). Infection had a large
- effect on aluminium and iron concentration of infected plants, with concentrations being
- approximately 60% and 30% higher, respectively, relative to uninfected plants (Table 2).
- Infection decreased potassium concentration of *U. europaeus* by 22%, across sites (Table 2).

239 There was also an independent effect of site on N, Al, K and Na concentration of U.

- 240 *europaeus* spines (Table 1). Nitrogen and potassium concentrations of plants at Engelbrook
- 241 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.
- 243 *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
- 245 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 (Table247 2).
- Nitrogen concentration of parasite stems was similar among sites (P = 0.121; Fig. 5*a*;
- 249 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. 5b; 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.
- 254 5*c*; Supplementary Material Table S2).

255 Discussion

256 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 257 258 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 259 three sites. Previously we have shown that C. pubescens significantly affected photosynthesis 260 of U. europaeus when grown under different nitrogen regimes (Cirocco et al. 2017) and 261 under either high or low light (Cirocco et al. 2016a). Photosynthesis of another introduced 262 host, Cytisus scoparius was significantly reduced by C. pubescens under ambient light 263 conditions in the glasshouse and also in the field (Prider et al. 2009; Shen et al. 2010). These 264 studies provide strong evidence that infection with C. pubescens has a negative impact on 265 photosynthesis in these introduced hosts. By contrast, C. pubescens had no effect on 266 photosynthesis of the native host Acacia paradoxa DC., irrespective of nitrogen addition to 267 the soil (Cirocco et al. 2017). The parasite did decrease photosynthesis of the native host 268 269 *Leptospermum myrsinoides* in the field and under high but not low light in the glasshouse.

270 Nevertheless, this effect did not translate into reductions in overall growth of this native host
271 (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 272 does not seem to be related to decreases in host stomatal conductance as $\delta^{13}C$ was actually 273 274 lower for infected plants (Fig. 4a). 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 275 (Tables 1, 2). This was also found for the C. pubescens-U. europaeus association in the 276 glasshouse (Cirocco et al. 2016b). Significant impacts of parasitic plants on host nitrogen 277 278 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 279 concentration of U. europaeus is a likely consequence of N removal by C. pubescens from 280

the host's xylem.

282 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 283 284 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 285 286 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 287 in foliar potassium are known to lead to increased potassium uptake by roots, which then 288 results in increased extrusion of protons to maintain charge balance in root cells. The 289 increased release of protons causes acidification of the rhizosphere (Houmani et al. 2015). 290 This response is thought to be the first line of defence against K deficiency (Houmani et al. 291 2015), however it is unknown whether foliar K levels in the infected plants in our study were 292 low enough to trigger this response. The decrease in potassium of infected plants is likely to 293 be a consequence of its removal by the parasite. Indeed, parasitic plants are well known to 294 accumulate potassium (Pate 1995), and in our study C. pubescens had around double the K 295 296 concentration of its hosts (Table 2; Fig. 5b). If parasitic plants can indirectly increase 297 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 298 299 concentrations of Al (for less than 60 min) can be toxic and impair root growth (Delhaize and Ryan 1995). 300

301 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 302 conditions across sites might influence the magnitude of the infection effect. For example, 303 previously we have found that the effect of C. pubescens is more pronounced when water 304 availability is high (Cirocco et al. 2016b), but was not influenced by soil N content or light 305 (Cirocco *et al.* 2016*a*; Cirocco *et al.* 2017). Using pre-dawn Ψ of uninfected plants as a proxy 306 for soil water availability (Fig. 3a), Bradbury was the wettest of the three sites, and the 307 308 infection effect on both ETR_{max} and F_v/F_m was greater here than at either of the other two sites (Figs 1a and 2a). These findings are consistent with the overall finding that C. 309 pubescens had a greater impact on growth of well watered plants in the glasshouse (Cirocco 310 et al. 2016b). Although Cirocco et al. (2016b) found that the strong decrease in F_v/F_m of U. 311 europaeus in response to the parasite was not influenced by water, there are also undoubtedly 312 multiple interacting factors in the field, so the combination of, for example, high water and 313 lower N at Bradbury might have influenced the impact of infection on U. europaeus. This 314 would run contrary to Tesitel *et al.* (2015) who found that *Rhinanthus alectorolophus* (Scop.) 315 Pollich (native to Europe) had a strong effect on F_v/F_m of the introduced crop maize relative 316 to wheat when water availability was low and nitrogen supply was high. Along with the 317 parasite strongly decreasing light-use efficiency of U. europaeus in this study (both at 318 Bradbury and Crafers), C. pubescens has also been found to significantly lower F_v/F_m of the 319 introduced host Cytisus scoparius under glassouse but not field conditions (Prider et al. 2009; 320 Shen *et al.* 2010). On the other hand, *C. pubescens* was found to have no impact on F_v/F_m of 321 the native host L. myrisnoides in both glasshouse and field settings (Prider et al. 2009; 322 323 Cirocco *et al.* 2015). In other cases, parasitic plants have been found to negatively affect $F_{\rm v}/F_{\rm m}$ of introduced (Mauromicale *et al.* 2008) but not native hosts (Hibberd *et al.* 1996). 324 Here, the greater decline in photosynthesis suggested by the ETR_{max} results, would result in 325 the plant being more likely to be exposed to excess light and ultimately chronic 326 photoinhibiton, as reflected by the decline in F_v/F_m (Demmig-Adams and Adams 2006). Both 327 lower rates of photosynthesis and chronic photoinhibtion in response to infection could 328 contribute to poor host growth (Gurney et al. 2002; Cirocco et al. 2016b). 329 Interestingly, there was no effect of infection on host $\delta^{13}C$ except at Crafers (Fig. 4*a*), where 330

infected plants had significantly lower δ^{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 δ^{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 δ^{13} C than infected plants at Bradbury 340 341 and Crafers but not those at Engelbrook (Fig. 4c). Similarly, Cirocco et al. (2016b) found that the parasite had significantly higher δ^{13} C than U. europaeus, regardless of water availability. 342 By contrast, Scalon and Wright (2015) using C isotope data for 93 mistletoe-host pairs from 343 around the world found that the parasites typically maintained lower $\delta^{13}C$ relative to their 344 hosts. When taking into account temperature, this difference held at warm sites while at cold 345 sites δ^{13} C did not vary between parasite and host (Scalon and Wright 2015). Bannister and 346 Strong (2001) investigating 158 mistletoe-host pairs in New Zealand found that 63% of 347 mistletoes had more negative δ^{13} C than their host, the other 37% had higher δ^{13} C than hosts. 348 They argued that the small difference in $\delta^{13}C$ between many mistletoe-host pairs may be 349 attributed to New Zealand's moist, temperate climate (Bannister and Strong 2001). Notably, 350 higher δ^{13} C in parasitic plants relative to their hosts might be due to heterotrophy (Cernusak 351 et al. 2004) and heterotrophic C gain in C. pubescens requires quantification. Our δ^{13} C 352 findings suggest that, as we hypothesised, C. pubescens is more conservative in its water-use 353 than its host, U. europaeus. Moreover, at least at Bradbury and Crafers, the parasite's main 354 355 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 356 357 conditions become drier as presumed at Crafers.

358 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)

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

380 Acknowledgements

Special thanks to Maria Johns (Bradbury), Professor Milton Hearn (Crafers) and Dr Russell
Sinclair and the National Trust of Australia (Engelbrook Reserve) for allowing me to work on
their sites in the field. Many thanks to Matthew Pearson and Dr Sonia Croft for assisting me
in the field and Dr Jane Prider, Professor Robert Reid for their expert advice and guidance.
Also, thank you to Nenah MacKenzie and Waite IRMS Facility (The University of Adelaide)
for expert analysis of our samples. This study was part funded by Nature Foundation SA Inc.
(61111804).

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

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

Table S1

	Factor	$F_{\rm v}/F_{\rm m}$	Φ_{PSII}	ETR _{max}	PD Ψ	MD Ψ	$\delta^{13}C$	Ν	Al	Fe	K	Na
	Ι	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
	$\mathbf{I}\times\mathbf{S}$	0.001	0.937	0.328	0.040	0.004	0.0001	0.860	0.336	0.368	0.327	0.103
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528 potassium (K, mg/kg) and sodium (Na, mg/kg) in *Ulex europaeus* spines when either 529 uninfected (–) or infected (+) with *Cassytha pubescens* at three field sites (Engelbrook:

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E; Bradbury: B; Crafers: C) in the Mt. Lofty Ranges of South Australia

Table 2. Concentrations of nitrogen (N, %), aluminium (Al, mg/kg), iron (Fe, mg/kg),

Data are means (\pm 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)

	Ν	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 ± 0.060 a	$30.9 \pm 2.42a$	$121 \pm 4a$	$9512\pm513a$	1900 ± 140
+	$1.4 \pm 0.076b$	$74.3\pm6.75b$	$170 \pm 9b$	$7386 \pm 567b$	2089 ± 142
Site					
Е	$1.9 \pm 0.062a$	$35.2 \pm 6.50a$	132 ± 9	$10588 \pm 626a$	$2335 \pm 147a$
В	$1.5 \pm 0.093b$	$70.4 \pm 10.9b$	155 ± 14	$8180\pm873b$	1917 ± 680ab
С	$1.4\pm0.048b$	$55.3 \pm 6.48ab$	148 ± 8	$6925\pm394b$	$1730 \pm 146b$

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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 (\pm 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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Fig. 2. (*a*) Maximum electron transport rates (ETR_{max}) 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 ETR_{max}. (*c*) ETR_{max} of *C. pubescens* infecting *U. europaeus* at the three sites. Data are means (\pm s.e.), different letters indicate significant differences and *n* = 10 (*a*, *c*) (except at Bradbury, *n* = 5); *n* = 25 (*b*).

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Fig. 3. (*a*) Pre-dawn and (*b*) midday 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 (\pm 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 δ^{13} C (‰) of *Ulex europaeus* when either uninfected (open bars) or infected

577 (light grey bars) with *Cassytha pubescens* at three field sites in the Mt. Lofty Ranges of South

578 Australia. (b) δ^{13} C of C. pubescens stems at the three sites. (c) δ^{13} C of both infected U.

579 *europaeus* (light grey bars) and parasite (black bars) at the three sites. Data are means $(\pm s.e.)$,

580 different letters indicate significant differences and n = 10 (*a*) (except at Bradbury, n = 5 and 581 n = 7 for infected plants at Engelbrook), n = 10 (*b*) (except at Bradbury, n = 5), n = as above 582 for (*c*).

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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
- 592 Australia. Data are means (\pm s.e.), different letters indicate significant differences and n = 10

593 (except at Bradbury, n = 5).

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