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

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31 **Abstract.** There is increasing evidence that native hemiparasitic plants can have significant
32 impacts on performance and growth of introduced host plants. However, most of this
33 evidence has been obtained from glasshouse studies. We investigated the effect of the native
34 Australian hemiparasite, *Cassytha pubescens* R. Br., on the physiology of the introduced
35 shrub *Ulex europaeus* L., at three field sites in South Australia. Parasite infection
36 significantly decreased Φ_{PSII} and maximum electron transport rates of *U. europaeus* across
37 sites. The impact of *C. pubescens* on photosynthetic performance of *U. europaeus* may have
38 been due to infected plants having significantly lower nitrogen and potassium, but higher iron
39 and aluminium than uninfected plants at all three sites. At two of the three sites *C. pubescens*
40 had a significant impact on host F_v/F_m indicating chronic photoinhibition in response to
41 infection. The impact of infection on F_v/F_m was greatest at the wettest site, in line with a
42 previous experiment where *C. pubescens* had a greater impact on this host under high water
43 availability. At this site infected plants also had the highest foliar Fe and Al. $\delta^{13}\text{C}$ of infected
44 plants was significantly lower than uninfected plants at only one of the three sites. Unusually,
45 $\delta^{13}\text{C}$ of the parasite was either the same as or significantly higher than hosts. There were no
46 site effects on parasite F_v/F_m or Φ_{PSII} , however ETR_{max} and $\delta^{13}\text{C}$ did vary across sites. The
47 results suggest that this native parasite has negative effects on *U. europaeus* in the field, as
48 has previously been found for glasshouse studies. Thus, the survival and abundance of this
49 major introduced weed in Australia could be negatively affected by infection with *C.*
50 *pubescens*.

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

53 **Introduction**

54 Parasitic plants are an important group globally, with both direct and indirect effects on their
55 hosts and also on the ecological systems in which they occur (Press and Phoenix 2005). For
56 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
65 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*
72 *sativa* L. (Cechin and Press 1994). By contrast, Cechin and Press (1993) found that the
73 significant impact of *S. hermonthica* on growth of *Sorghum bicolor* (L.) Moench cv. CSH1 at
74 low N was ameliorated by high N supply. On the other hand, at a single field location in
75 Africa, Gurney *et al.* (1995) found that nitrogen had no influence on the effect of *S.*
76 *hermonthica* on growth of maize and sorghum cultivars.

77 In southern Australia across six field sites that varied significantly in soil salinity, Miller *et*
78 *al.* (2003) found that the stem hemiparasitic mistletoe *Amyema miquelii* (Lehm. ex Miq.)
79 Tiegh. had no effect on water potentials or $\delta^{13}\text{C}$ 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 2016a; Girocco *et al.* 2017). By contrast, the significant effect of *C. pubescens* on growth of

86 *U. europaeus* was much stronger under high rather than low water supply (Cirocco *et al.*
87 2016b).

88 In the field, *C. pubescens* has been shown to negatively affect growth of the introduced host,
89 *Cytisus scoparius* L. Link, but not native host, *Leptospermum myrsinoides* Schltdl. (Prider *et*
90 *al.* 2009). However, it is unknown whether *C. pubescens* also affects physiology of *U.*
91 *europaeus* in the field, as reported for glasshouse experiments, or whether those effects will
92 be consistent across several sites which differ in environmental conditions. Physiological
93 measurements such as chlorophyll fluorescence (Maxwell and Johnson 2000; Gurney *et al.*
94 2002; Girocco *et al.* 2015) can be used as strong indicators of early declines in host health
95 where biomass comparisons in the field are otherwise not feasible. If the impact of *C.*
96 *pubescens* on *U. europaeus* physiology could be confirmed in the field and consistent across
97 locations, then there would be further evidence for the potential-use of this parasite as a
98 native bio-control against major invasive shrubby weeds in Australia. This is of great
99 importance as *U. europaeus* is considered one of the top 20 worst weeds in Australia because
100 it has become so difficult to manage with conventional methods (Thorp and Lynch 2000). In
101 addition, there is a need to understand the effects of *C. pubescens* on community structure,
102 and thus more field studies on specific hosts are needed (Demey *et al.* 2015).

103 Here, we investigated the impact of the Australian native stem hemiparasitic vine *C.*
104 *pubescens* on the physiology of the introduced host *U. europaeus* at three field sites in South
105 Australia. It was hypothesised that the parasite would negatively affect the performance of *U.*
106 *europaeus* in the field, as has been observed previously in glasshouse studies (Cirocco *et al.*
107 2016a; Girocco *et al.* 2016b; Girocco *et al.* 2017). Our secondary hypothesis was that where
108 we found differences in host performance across the three sites in response to infection, these
109 could be explained by the differences in water, light or nutrients across sites. We also
110 predicted that in the field, *C. pubescens* would be more conservative in its water-use than the
111 host as previously found in a glasshouse study (Cirocco *et al.* 2016b).

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
115 native to Western Europe and Northern Africa (Clements *et al.* 2001). It establishes quickly
116 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
118 hemiparasitic vine native to Australia (McLuckie 1924). It has no true roots or leaves, and its
119 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
122 has been extensively studied in the glasshouse (Cirocco *et al.* 2016a; *Cirocco et al.* 2016b;
123 *Cirocco et al.* 2017).

124 *Study sites*

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

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
138 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
142 recorded on days when physiological measurements were conducted using LI-1400 data
143 loggers fitted with a quantum sensor (LI-190 SA) and relative humidity/air temperature
144 sensor (1400-104) (LI-COR, Lincoln NEB., Supplementary Material Fig. S2). At each site,
145 soil was sampled from the top 60 cm of the profile using an auger at five different locations
146 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

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

150 *Photosynthesis and water potential*

151 Pre-dawn (F_v/F_m) and midday (Φ_{PSII}) PSII efficiency, and maximum electron transport rates
152 (ETR_{max}) of *U. europaeus* spines and *C. pubescens* stems were measured with a portable,
153 pulse-modulated chlorophyll fluorometer (MINI-PAM, Walz, Effeltrich, Germany) fitted
154 with a leaf-clip (2030-B, Walz, Effeltrich, Germany). Measurements were made on the
155 youngest fully expanded shoot of uninfected *U. europaeus* plants, and likewise for infected
156 shoots of infected plants. Measurements for the parasite were made 15 cm from the growing
157 tip of individual stems. Φ_{PSII} measurements were made between 12–1:30 pm. Mean PPFs
158 ($\mu\text{mol m}^{-2} \text{s}^{-1}$) (for uninfected and infected plants combined) during measurements of Φ_{PSII}
159 on *U. europaeus* at Engelbrook, Bradbury and Crafers were: 1230 ± 31 ($n = 20$), 1224 ± 25 (n
160 $= 10$) and 1342 ± 21 ($n = 20$), respectively. Mean PPFs ($\mu\text{mol m}^{-2} \text{s}^{-1}$) during measurements
161 of Φ_{PSII} for *C. pubescens* (when infecting *U. europaeus*) at Engelbrook, Bradbury and Crafers
162 were: 1459 ± 28 ($n = 10$), 1181 ± 39 ($n = 5$) and 1336 ± 26 ($n = 10$), respectively. Light
163 response curves were measured between 9 am–12 pm and used to determine ETR_{max} of all
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
172 season on sunny days where PPF was both saturating, and also similar for all plants
173 (Supplementary Material Fig. S2).

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

175 Stable carbon isotope discrimination ($\delta^{13}\text{C}$) 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
180 material (60°C for six days) collected on the same days when physiological measurements
181 were made. This material was comparable in position and age with host shoots and parasite
182 stems used for physiological measurements. Replication (uninfected and infected *U.*
183 *europaeus* and the parasite) for carbon isotope, nitrogen and additional elemental analyses
184 was $n = 7-10$ at Engelbrook, $n = 5$ at Bradbury and $n = 10$ at Crafers.

185 *Statistical analyses*

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

196 **Results**

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

198 There was a significant interaction effect for infection \times site on F_v/F_m (Table 1). Infection
199 had a significant negative impact on F_v/F_m of *U. europaeus* at Bradbury and Crafers but not
200 at Engelbrook (Fig. 1a). While there was no significant interaction or site effect for Φ_{PSII} , it
201 was independently affected by infection (Table 1; Fig. 1b). Φ_{PSII} of infected plants was
202 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;
204 Supplementary Material Table S2).

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

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. 3a). 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. 3b). The lowest Ψ at midday was recorded in infected plants at Crafers ($-2.83 \pm$
219 0.062 MPa), and the highest was in uninfected plants at Bradbury (-1.76 ± 0.085 MPa).

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

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

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
242 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
244 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
246 concentrations of plants at both these sites not differing from Na of plants at Bradbury (Table
247 2).

248 Nitrogen concentration of parasite stems was similar among sites ($P = 0.121$; Fig. 5a;
249 Supplementary Material Table S2). Potassium of *C. pubescens* stems was significantly higher
250 at Engelbrook compared with Crafers, with parasite values at these two sites being similar to
251 those at Bradbury (site effect; $P = 0.042$; Fig. 5b; Supplementary Material Table S2). Sodium
252 concentration of *C. pubescens* stems at Crafers was significantly higher than those of the
253 other two sites which did not differ significantly from each other (site effect; $P = 0.0002$; Fig.
254 5c; Supplementary Material Table S2).

255 **Discussion**

256 Based on previous glasshouse studies, we hypothesised that *C. pubescens* would have a
257 negative impact on performance of *U. europaeus* in the field, regardless of variation in
258 environmental conditions across our study sites. This was supported by our results, which
259 showed that ETR_{max} was significantly lower, by around a third, in infected plants across all
260 three sites. Previously we have shown that *C. pubescens* significantly affected photosynthesis
261 of *U. europaeus* when grown under different nitrogen regimes (Cirocco *et al.* 2017) and
262 under either high or low light (Cirocco *et al.* 2016a). Photosynthesis of another introduced
263 host, *Cytisus scoparius* was significantly reduced by *C. pubescens* under ambient light
264 conditions in the glasshouse and also in the field (Prider *et al.* 2009; Shen *et al.* 2010). These
265 studies provide strong evidence that infection with *C. pubescens* has a negative impact on
266 photosynthesis in these introduced hosts. By contrast, *C. pubescens* had no effect on
267 photosynthesis of the native host *Acacia paradoxa* DC., irrespective of nitrogen addition to
268 the soil (Cirocco *et al.* 2017). The parasite did decrease photosynthesis of the native host
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.* 2016a).

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

282 In addition to having lower N concentrations, infected *U. europaeus* were significantly
283 enriched in Al and Fe compared with uninfected plants across all sites (Tables 1, 2). One
284 explanation for this may be that infection led to increased acidification of the rhizosphere
285 which would increase the mobility of Al and Fe ions for uptake by roots (Haynes 1990). This
286 increased acidification of the rhizosphere may have occurred in response to the negative
287 effect of infection on plant potassium concentrations across all sites (Tables 1, 2). Decreases
288 in foliar potassium are known to lead to increased potassium uptake by roots, which then
289 results in increased extrusion of protons to maintain charge balance in root cells. The
290 increased release of protons causes acidification of the rhizosphere (Houmani *et al.* 2015).
291 This response is thought to be the first line of defence against K deficiency (Houmani *et al.*
292 2015), however it is unknown whether foliar K levels in the infected plants in our study were
293 low enough to trigger this response. The decrease in potassium of infected plants is likely to
294 be a consequence of its removal by the parasite. Indeed, parasitic plants are well known to
295 accumulate potassium (Pate 1995), and in our study *C. pubescens* had around double the K
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
298 consequences for host plant performance. For example, plant exposure to micromolar
299 concentrations of Al (for less than 60 min) can be toxic and impair root growth (Delhaize and
300 Ryan 1995).

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

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

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

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

358 **Conclusion**

359 We acknowledge that there is a possibility that some variables measured in this study might
360 not be independent. However the low *P* values for all but one of the host parameters
361 discussed minimise the possibility of basing our conclusion on spurious significant effects.
362 Also, while non-manipulative field studies such as ours cannot conclusively demonstrate
363 cause and effect, the combination of the field data reported here and the controlled
364 experimental evidence (e.g. Cirocco *et al.* 2016a; Cirocco *et al.* 2016b; 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
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, this would 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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505 **Table 1. Two-way ANOVA results (*P*-values) for the effect of infection with *Cassytha***
 506 ***pubescens* (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 ($\delta^{13}C$), nitrogen (N), aluminium (Al),**
 509 **iron (Fe), potassium (K) and sodium (Na) concentration of *Ulex europaeus* spines.**

510 **Significant effects are in bold**

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

512 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

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527 **Table 2. Concentrations of nitrogen (N, %), aluminium (Al, mg/kg), iron (Fe, mg/kg),**
 528 **potassium (K, mg/kg) and sodium (Na, mg/kg) in *Ulex europaeus* spines when either**
 529 **uninfected (–) or infected (+) with *Cassutha pubescens* at three field sites (Engelbrook:**
 530 **E; Bradbury: B; Crafers: C) in the Mt. Lofty Ranges of South Australia**

531 Data are means (\pm s.e.), different letters indicate significant differences for independent
 532 infection (I) effect on N, Al, Fe and K (uninfected $n = 25$; infected $n = 22$) and independent
 533 site (S) effect on N, Al, K and Na (E, $n = 17$; B, $n = 10$; C, $n = 20$). There were no I x S
 534 interactions detected; $n = 10$ (except at Bradbury $n = 5$, and $n = 7$ for infected plants at
 535 Engelbrook)

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

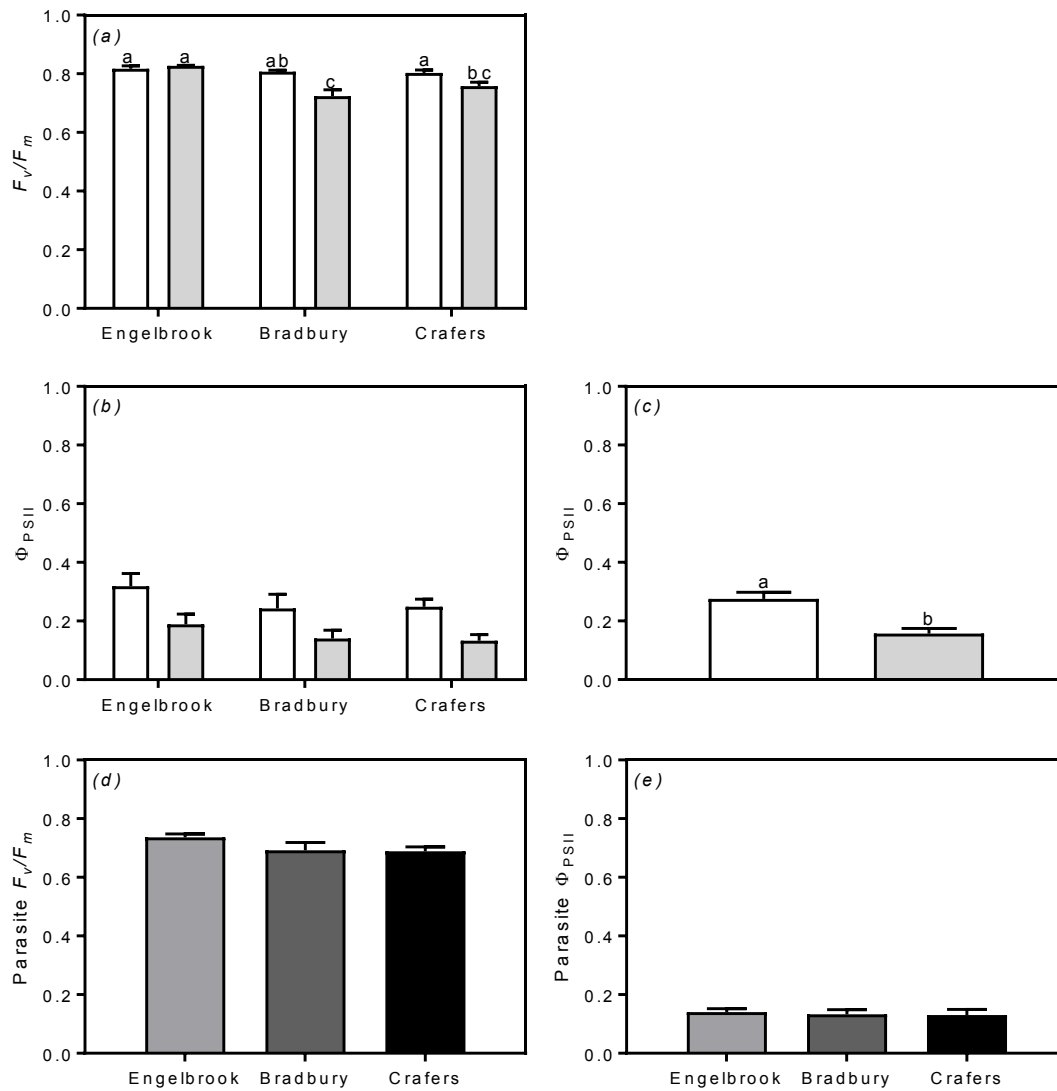
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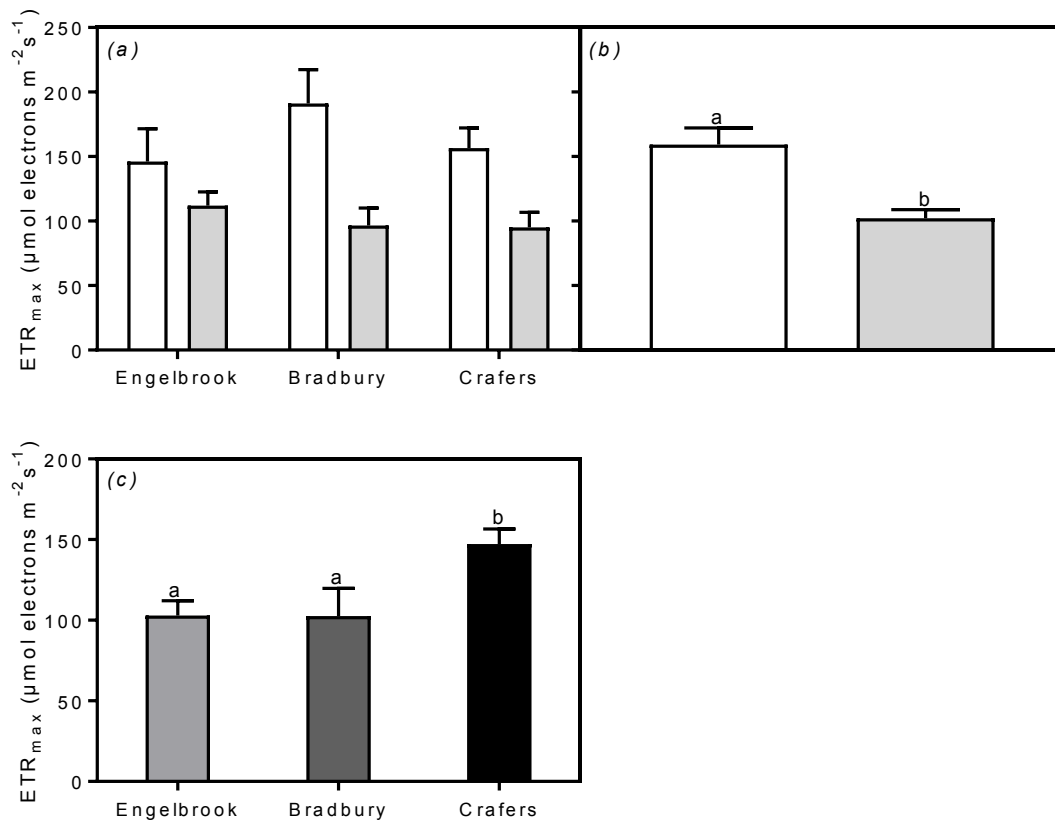
542 **Fig. 1.** (a) Pre-dawn (F_v/F_m) and (b) midday (Φ_{PSII}) PSII efficiency of *Ulex europaeus* either
 543 uninfected (open bars) or infected (light grey bars) with *Cassythia pubescens* at three field
 544 sites in the Mt. Lofty Ranges of South Australia. (c) Independent infection effect on host
 545 Φ_{PSII} . (d) F_v/F_m and (e) Φ_{PSII} of *C. pubescens* infecting *U. europaeus* at the three sites. Data
 546 are means (\pm s.e.), different letters indicate significant differences and $n = 10$ (a, b, d, e)
 547 (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 (\pm 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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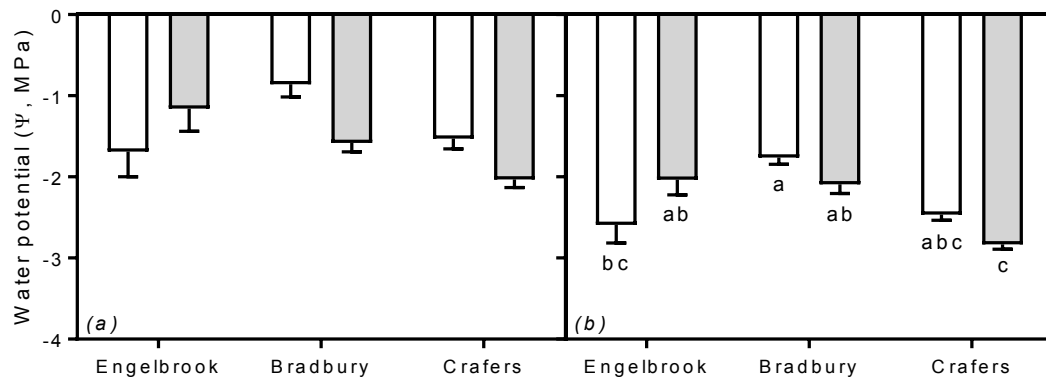
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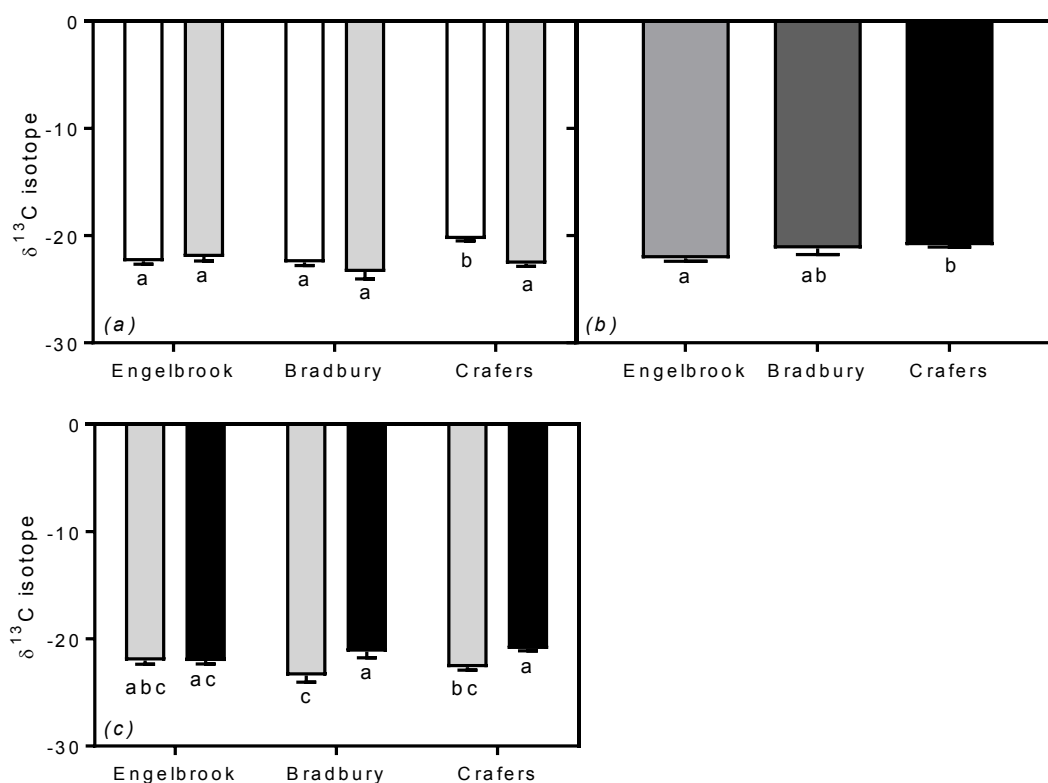
567 **Fig. 3.** (a) Pre-dawn and (b) midday shoot water potentials of *Ulex europaeus* either
 568 uninfected (open bars) or infected (light grey bars) with *Cassytha pubescens* at three field
 569 sites in the Mt. Lofty Ranges of South Australia. Data are means (\pm s.e.), different letters
 570 indicate significant differences and $n = 10$ (a, b) (except at Bradbury, $n = 5$).

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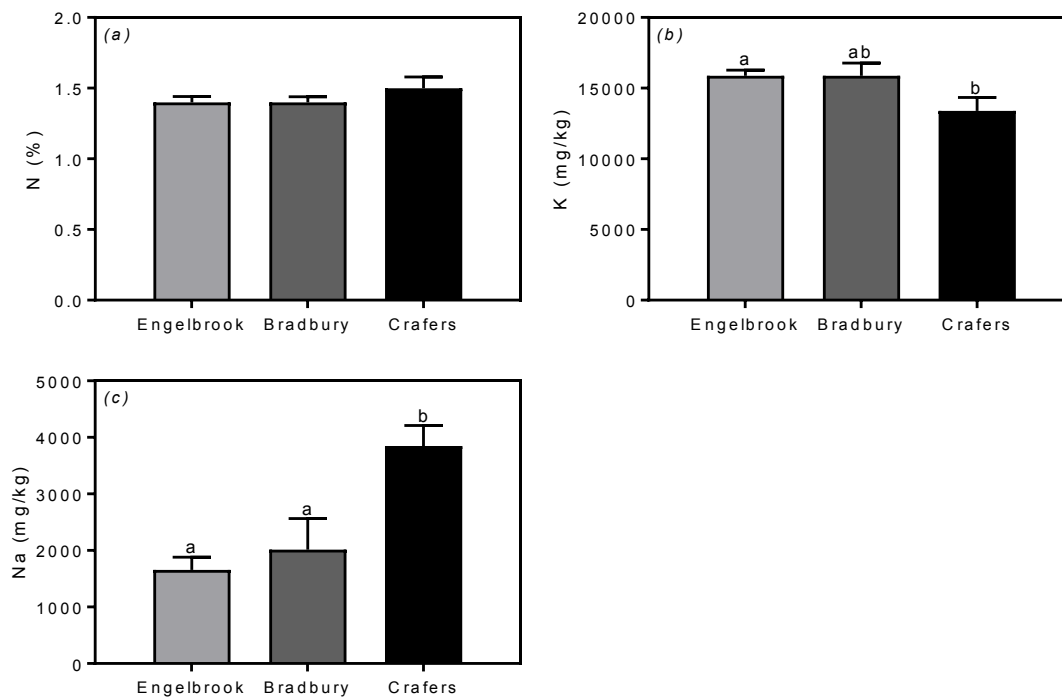
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 576 **Fig. 4.** (a) Spine $\delta^{13}\text{C}$ (‰) of *Ulex europaeus* when either uninfected (open bars) or infected
 577 (light grey bars) with *Cassutha pubescens* at three field sites in the Mt. Lofty Ranges of South
 578 Australia. (b) $\delta^{13}\text{C}$ of *C. pubescens* stems at the three sites. (c) $\delta^{13}\text{C}$ 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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590 **Fig. 5.** (a) Nitrogen, (b) potassium and (c) sodium concentration of *Cassytha pubescens*
 591 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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