

1 **Contrasting water strategies of two Mediterranean shrubs of limited distribution:**
2 **uncertain future under a drier climate**

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12 Running head: Water strategies of species with limited distribution

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20 **Summary**

21 Plants have evolved different strategies to cope with drought, involving alternative
22 ecophysiologicals and different levels of plasticity. These strategies are critical for species
23 of limited distribution, which are especially vulnerable to the current rates of rapid
24 environmental change. The aim of this study was to assess the water strategy of two
25 species with limited distribution, *Cneorum tricoccon* L. and *Rhamnus ludovici-*
26 *salvatoris* R., and evaluate their interpopulation variability along an aridity gradient to
27 estimate their vulnerability to a drier climate. We measured different ecophysiological
28 traits influenced by drought - stomatal conductance, Fv/Fm, carbon isotope ratio and
29 chlorophyll concentration - in two climatically contrasted years, before and during
30 summer drought. Both species were vulnerable to drought at the aridity limit of the
31 gradient, but showed contrasting water strategies: while *C. tricoccon* was consistent in
32 its water conservation strategy across the aridity gradient, *R. ludovici-salvatoris* was
33 not, displaying higher and more variable stomatal conductances and being able to
34 increase water use efficiency at the most xeric sites. Changes in length and intensity of
35 drought events may favor one species' strategy to the detriment of the other: *C.*
36 *tricoccon* is more vulnerable to chronic and prolonged droughts whereas short but acute
37 droughts might have a stronger effect on *R. ludovici-salvatoris*. In those communities
38 where these two species coexist, such different strategies might lead to changes in
39 community structure under climate change scenarios, with unknown cascade effects on
40 ecosystem functioning.

41 **Key words:** limited-range species; climate change; drought; water strategy; stomatal
42 conductance; water use efficiency; phenotypic plasticity; Mediterranean shrubs.

43

44 **Introduction**

45 Species ranges are dynamic (Gaston 2003), with climate playing a major role in limiting
46 terrestrial species' distribution (Parmesan et al. 1999; Wilson et al. 2007). Major shifts
47 in species distribution can be expected considering the climatic changes currently
48 forecast (Christensen 2007). In fact, many animal species have suffered a reduction in
49 their distributional range due to recent climate change events, increasing their risk of
50 extinction in the near future (Ainley et al. 2003; Derocher 2005; Wilson et al. 2005).
51 Evidences for plants are increasing (Lenoir et al. 2008, Lenoir and Svenning 2013) but
52 they are mainly centered on shifts of species ranges along altitudinal gradients (Jump et
53 al. 2009). Endemic and relict plant species are especially vulnerable to environmental
54 changes because they have small population sizes, which are exposed to increased
55 inbreeding, lower individual fitness and lower genetic variability (Ellstrand and Elam
56 1993; Aizen et al. 2002; Angeloni et al. 2011). Furthermore, species of limited
57 distribution have potentially less evolutionary capacity to respond to climate change as
58 they are exposed to only a small range of environmental heterogeneity. Thus, small
59 changes in temperature and precipitation patterns could affect their growth and survival
60 to a larger extent than to their more common and widespread counterparts (Gulías et al.
61 2002, 2003).

62 However, even species with limited range are able to evolve and adapt to
63 changing environments at expanding range margins (Parmesan 2006). These range
64 expansions and changes in resource use have been attributed to a large extent to
65 'phenotypic plasticity' - the ability of a single genotype to alter its phenotype in
66 response to environmental conditions - (Pigliucci 2001). Plasticity may have important
67 adaptative effects, mitigating for instance the negative impacts of a drier climate

68 (Callaway et al. 2003; Valladares et al. 2007; Couso and Fernandez 2012). Despite the
69 plasticity of each individual, populations may not be able to keep pace with the rate of
70 environmental change and therefore might be still vulnerable to extinction (Nussey et al.
71 2005; Bradshaw and Holzapfel 2006).

72 In Mediterranean ecosystems, water is the main environmental constraint for
73 plant growth and reproduction (Blondel et al. 2010) and it is likely to become an even
74 more limiting resource under the increasing aridity expected for this region by most
75 climate models (Christensen 2007). Consequently, ecophysiological traits that
76 determine the ability of species to cope with drought may explain their distribution and
77 fitness in this region, at least partially (Gulías et al. 2002; Lambers et al. 2008).
78 Different ecophysiological strategies to cope with environmental constraints typically
79 incorporate an important tradeoff between carbon assimilation and water loss by
80 evapotranspiration, particularly in arid regions (Joffre et al. 1999; Lambers et al. 2008).
81 Plants with isohydric regulation of water status avoid drought by maintaining wide
82 margins of safety from hydraulic failure via stomata closure. However, they suffer
83 greater vulnerability to carbon starvation (Damesin et al. 1998; Gulías et al. 2002).
84 Conversely, anisohydric plants are relatively drought-tolerant. They are predisposed to
85 hydraulic failure as they operate with narrower hydraulic safety margins during drought,
86 while maintaining high carbon assimilation rates (Gucci et al. 1997; Martinez-Ferri et
87 al. 2000; McDowell et al. 2008). Most plants have been shown to segregate along this
88 axis from iso- to anisohydric strategies (David et al. 2007, Quero et al. 2011), which
89 contributes to explain coexistence under certain conditions but predispose some species
90 more than others to further environmental changes.

91 We have studied two Mediterranean shrubs of limited distribution and for which
92 the mechanisms of water strategy are still poorly known: *Cneorum tricoccon* L.
93 (Cneoraceae) and *Rhamnus ludovici-salvatoris* (Rhamnaceae). The former species is
94 part of a relict flora that evolved during the early Tertiary under tropical conditions
95 (Raven 1973). It has been categorized as vulnerable (IUCN), with human activities and
96 urban sprawl described as the main historical threats for the species. *R. ludovici-*
97 *salvatoris* is an endemic species to the Balearic Islands, whose distribution has also
98 diminished, with recent extinctions of several populations (Gulías et al. 2002; Traveset
99 et al. 2003). *R. ludovici-salvatoris*' decline is mainly due to deforestation, but climatic
100 changes occurring in the region during the Holocene have also been partly responsible
101 (Postigo et al. 2009). Furthermore, many current populations of *C. tricoccon* and *R.*
102 *ludovici-salvatoris* are characterized by a low proportion of seedlings and saplings,
103 suggesting low establishment success (Traveset et al. 2012, 2003). We assessed the
104 interpopulation and ecophysiological variability along an aridity gradient in the water
105 strategy of these two species in order to estimate their vulnerability to an increased
106 aridity such as that expected for the region (Christensen 2007). Sampling was conducted
107 at sites where both species were present along a wide aridity gradient during two years
108 of contrasting precipitations and temperatures.

109 Our specific hypotheses were: 1) Populations of both species at their limit of
110 aridity show worse performance and lower survival than others established on more
111 mesic sites, as suggested by studies on the rear distribution edge of plants in
112 Mediterranean zones (Hampe and Petit 2005; Jump et al. 2006; Linares and Tiscar
113 2010) and by previous works on these species (Gulías et al. 2003, 2004; Traveset et al.
114 2003, Varone et al. 2012); 2) The two co-occurring species differ in their water strategy
115 (Gulías et al. 2009), which may result into a differential vulnerability to ongoing climate

116 change. We argue that isohydric species can cope with acute but not prolonged
117 droughts, while the reverse is true for anisohydric plants, which has implications for
118 their performance in future climatic scenarios. Lastly, we hypothesize that 3)
119 phenotypic plasticity can attenuate the impacts of a drier climate (Callaway et al. 2003;
120 Gianoli and González-Teuber 2005; Valladares et al. 2007) and, therefore, species with
121 a more plastic water strategy across sites and over time should perform better under the
122 driest conditions.

123

124 **Materials and methods**

125 *Study species*

126 *Cneorum tricoccon* L. (Cneoraceae) is a small (usually <1m tall) perennial shrub, which
127 inhabits the western Mediterranean area (Balearic Islands, some localities in the eastern
128 and southern Iberian Peninsula, south-eastern France, Sardinia, Giannutri and Tuscany).
129 *C. tricoccon* represents a relict species of the Tertiary (Raven 1973), currently, it is
130 found usually within the coastal maquis and more rarely within holm oak (*Quercus ilex*)
131 forests. *C. tricoccon* is an andromonoecious, insect-pollinated species (Traveset 1995a).
132 Seed dispersal is mediated by endemic lizards (*Podarcis lilfordi*, *P. pityusensis* and *P.*
133 *siculus*), and by introduced carnivorous mammals (*Martes martes* and *Genetta genetta*)
134 when lizards are extinct (Traveset 1995b; Celedón-Neghme et al. 2013).

135 *Rhamnus ludovici-salvatoris* R. (Rhamnaceae) is an up to 2m high perennial
136 shrub endemic to the Balearic Islands (Mallorca, Menorca and Cabrera). It occurs from
137 sea level up to 1200 m a.s.l., usually in holm oak forests, and more rarely in dry
138 garrigues. *R. ludovici-salvatoris* is a dioecious, insect-pollinated species and seed
139 dispersal is mediated by two frugivorous birds; *Turdus merula* and *Sylvia*
140 *melanocephala* (Traveset et al. 2003; Gullías and Traveset 2012). For both species the
141 period of leaf development and shoot elongation reach typically favorable periods of
142 spring (April to June) and early autumn (Sep and Oct).

143 *Study sites*

144 The study was performed in three locations that cover a pronounced climatic gradient
145 (precipitation and temperature) on the Balearic Islands, West Mediterranean Basin. The
146 three locations ranged from a maximum mean annual precipitation of 1224 mm to a

147 minimum of 391 mm (Table 1, Figure 1). We have calculated Emberger's (1955) index
148 of aridity (Q) as $Q = (2000 \times P)/(T_{\max}^2 - T_{\min}^2)$, where P was mean annual precipitation;
149 T_{\max} , maximum annual temperature and T_{\min} , minimum annual temperature.
150 Following Traveset et al. (2012), values of Emberger's index were logarithmically
151 transformed for statistical analyses because Q increases nonlinearly in more mesic
152 habitats. Selected populations met the following criteria: i) the population was large and
153 well established, with >50 individuals; ii) the site was not managed; iii) the population
154 did not adjoin highly disturbed areas and iv) the population was separated from other
155 sampled population by at least 40 Km.

156 For abiotic and biotic characterization of study sites we measured the slope and
157 visually estimated the percentage of bare soil, cover of rocks, woody plants and herbs
158 on ten randomly selected transects (2 x 10 m) per population (See Table 1 for study sites
159 characterization). With a portable climatic station (SIL451, SILVA, Bromma, Sweden)
160 we measured temperature, PAR, humidity, VPD and wind speed at population level at
161 the time of measurements. We also measured PAR at microhabitat level (0.5 m to every
162 sampled individual) with a portable PAR leaf-clip (FMS2, Hansatech, UK).

163 In the most mesic study sites (Lluc and Banyalbufar), *C. tricoccon* and *R.*
164 *ludovici-salvatoris* occur in the understory of holm oak forests with surrounding
165 vegetation dominated by *Pistacia lentiscus*, *Olea europaea*, *Cistus albidus*, *Daphne*
166 *gnidium* and *Smilax aspera*. In the driest study site (Cabrera) they inhabit coastal
167 maquis dominated by species such as *Pistacia lentiscus*, *Olea europea*, *Rhamnus*
168 *alaternus*, *Cistus monspeliensis* and *Rosmarinus officinalis*. All populations occur in
169 calcareous soils.

170 *Morphological, ecophysiological and fitness surrogate measurements*

171 Maximum height, crown and trunk diameter were measured in 68-108 adult plants of
172 each species in each population (*C. tricocon* n=280 and *R. ludovici-salvatoris* n=247).
173 In order to ensure that sampled individuals were adult we selected reproductive
174 individuals.

175 On July 2006, leaf elevation angles were measured in five fully expanded leaves
176 from each of 15 individuals per population, except in Cabrera where two small
177 population initially considered independently were finally merged and studied together
178 since distance between them was short and environmental conditions almost identical
179 (total n=60 individuals in each species). Leaves were measured with a protractor, from
180 the upper and most exposed part of the crown.

181 Ecophysiological measurements were taken in the same 15 individuals per
182 population (n=60 in each species) in two climatically contrasting years, 2006 and 2010.
183 According to the reports of the Spanish Meteorological Agency, 2006 was an extremely
184 hot year in the Balearic Islands (temperatures exceeded the maximum value for the
185 reference period 1971-2010) and was also very dry (i.e. 2006 was among the 20% driest
186 years of the period) while 2010 was mild (temperatures were around the median) and
187 also very wet (i.e. 2010 was among the 20% wettest years of the period). In 2006, data
188 were obtained before (May) and during the summer drought (July), whereas in 2010
189 measurements were made during summer drought (July). Selected individuals for
190 ecophysiological measurements met the following criteria: i) neighbour plants grew at
191 least 3 m apart to minimize intense resources competition, ii) individuals had similar
192 height and crown diameters (around the mean for each population) to reduce plant size
193 as a confounding factor, iii) sampled individuals grew in habitats with similar
194 irradiances, specifically with photosynthetically active radiation (PAR) during the

195 central hours of the day between 900-1400 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$. In *R. Ludovici-*
196 *salvatoris* both male and female individuals were measured to explore any influence of
197 gender on performance. Four different ecophysiological assessments were taken, i)
198 stomatal conductance (g_s) was measured with a leaf porometer (SC-1, Decagon
199 Devices, Pullman, WA) from 10:00 to 12:00 GTM in four fully expanded leaves per
200 plant from the upper parts of the canopy, ii) maximal photochemical efficiency of
201 photosystem II (Fv/Fm) was measured with a portable pulse-modulated fluorometer
202 (FMS2, Hansatech, UK) from 09:00 to 12:00 GMT in three healthy leaves dark-adapted
203 for 30 min with leaf clips. Minimal (F_o) and maximal (F_m) fluorescence were
204 measured, and these values were used to calculate Fv/Fm as $F_v/F_m = (F_m - F_o)/F_m$
205 (Maxwell and Johnson 2000), iii) chlorophyll concentration (Chl) was measured with a
206 portable SPAD (Minolta, Japan) in five fully expanded leaves per plant and iv) carbon
207 isotope ratio ($\delta^{13}\text{C}$) for which two current-year leaves from the upper and most exposed
208 part of the crown were collected, dried and ground; this material was analyzed with a
209 Finnigan MAT Delta C isotope ratio mass spectrometer at Stable Isotope Facility on
210 University of California, Davis. The carbon isotope ratio ($\delta^{13}\text{C}$) is an index of intrinsic
211 water use efficiency (WUE) integrated over time of plant organ growth (Dawson et al.
212 2002; McCarroll and Loader 2004). All ecophysiological measurements were taken in
213 15 individuals per population except in Cabrera where 30 individuals were sampled (n=
214 60 for each species).

215 Fruit crop was examined on 2006 as a surrogate of fitness in the three
216 populations. For *C. tricocon*, fully developed fruits were observed from January to
217 June, with a peak at the end of May (Traveset 1995a) whereas for *R. ludovici-salvatoris*
218 full-sized fruit occurs about June (Traveset et al. 2003). For this reason, we examined
219 fruit crop of both species on May, including fruits at different stages of development.

220 Due to the phenology of each population we started with the driest populations and
221 ended with the mesic ones in order to cover the range of fruit peak of each population.

222

223 *Statistical analysis*

224 We performed two-way ANOVAs to test for differences among populations (Po, 2 df)
225 and species (Sp, 1 df) in height, crown and trunk diameter and leaf angle. A mixed
226 model ANCOVA was used to test for differences among populations (Po, 2 df), season
227 (Se, 1 df) and species (Sp, 1 df) in g_s , Fv/Fm, Chl and $\delta^{13}C$, considering PAR as a
228 covariate. The model included Po, Se and Sp as main effects and all possible
229 interactions between them. We used a three-way ANOVA to test the effects of
230 population (Po, 2 df), year (Ye, 1 df) and species (Sp, 1 df) in g_s , Fv/Fm. For *R.*
231 *ludovici-salvatoris* we have performed two-way ANOVAs to test for gender, population
232 differences and its interaction. As a post-hoc test, we used Fisher's LDS in all cases.
233 Normality and homogeneity of variance of the dependent variables were tested prior to
234 analyses by means of the Kolmogorov-Smirnov and the Levene's test, respectively. All
235 statistical analyses were performed using Statistica 6.0 (StatSoft Inc., Tulsa, OK, USA).

236 We performed structural equation models (SEM) to test not only the direct
237 influence of abiotic factors on morphological, ecophysiological and fitness variables but
238 also the indirect effects in an aprioristic model where the causal relationships between
239 our variables were explicitly included (Iriando et al. 2003, Shipley 2000, Milla et al.
240 2009). We built independent models for *C. tricoccon* and *R. Ludovici-salvatoris* since
241 we hypothesized that causal relationships among variables change between the two
242 species. Besides, we built another two models considering ecophysiological differences

243 before vs. during summer drought since these seasonal differences could explain
244 interpopulation fitness variability. Since the sample sizes were relatively small (n=95 in
245 the best case) we limited the number of predictors included in the model as much as
246 possible as recommended by Shipley (2000). We selected two variables to characterize
247 the abiotic environment: light, as mean PAR measured at microsite level, and aridity,
248 calculated as the aridity Emberger's index (see above for index details); two
249 ecophysiological variables: WUE and Fv/Fm; one morphological trait: height; and fruit
250 crop as a fitness proxy Standardized path coefficients were estimated using maximum
251 likelihood algorithm (Shipley 2000). The degree of fit between the observed and
252 expected covariance structures was assessed by RMSEA statistics (Steiger 1990).
253 RMSEA values below 0.08 show a good fit, between 0.08 to 0.10 provide a mediocre fit
254 and above 0.10 indicate poor fit (MacCallum et al. 1996). Moreover, model fit to the
255 data was also evaluated by the goodness-of-fit index (GFI) and the Bentler and Bonett's
256 normed-fit index (NFI). Values of GFI and NFI range between 0 and 1, and values > 0.9
257 indicate an acceptable fit of the model to the data (Iriondo et al. 2003). Analyses were
258 performed using AMOS 5.0 (Arbuckle 1994).

259

260 **Results**

261 We found significant differences between species and populations for height, crown and
262 trunk diameters (Table 2). *C. tricoccon* was taller and had larger crowns diameters in
263 more mesic and colder sites. This pattern was not as clear in *R. ludovici-salvatoris* for
264 height and trunk diameters although the xeric population had plants with smaller crowns
265 (Fig. 2 a, b, c). The two species showed larger fruit crops in more mesic and colder
266 populations (Table 2, Fig. 3).

267 Leaf elevation angles were significantly higher for *C. tricocon* than for *R.*
268 *ludovici-salvatoris* (Table 2, Fig. 4). *C. tricocon* showed higher leaf elevation angles in
269 more xeric and warmer populations whereas *R. ludovici-salvatoris* populations did not
270 differ in this parameter (Table 2, Fig. 4).

271 Differences in stomatal conductance (g_s), carbon isotope ratio ($\delta^{13}\text{C}$),
272 photochemical efficiency of PSII (Fv/Fm) and Chlorophyll concentration (Chl) were
273 also found between species, among populations, and between seasons, with significant
274 interactions among these factors (Table 3). Before drought, *C. tricocon* showed higher
275 g_s in the most mesic population whereas it showed very low values of g_s during drought
276 in all populations (Fig. 5 a). By contrast, *R. ludovici-salvatoris* showed higher g_s before
277 drought in the most mesic populations and lower g_s in the most xeric one. Stomatal
278 conductance of *R. ludovici-salvatoris* decreased during drought and the pattern along
279 the mesic to xeric gradient was not found. In all cases, values of this species were
280 always higher than those of *C. tricocon* (Fig. 5 b). For both species, there was a
281 significant interaction between population and season in g_s (Table 3), with a
282 consistently greater decrease before vs during drought in Lluc, the most mesic site (Fig.
283 5 a, b).

284 Carbon isotope ratio ($\delta^{13}\text{C}$) of leaves differed significantly between species,
285 among populations, and depending on the season (Table 3). Before drought, *C.*
286 *tricocon* showed significantly lower $\delta^{13}\text{C}$ values in the most mesic populations (Fig. 5
287 c), suggesting lower WUE in them compared to the xeric population. During drought,
288 $\delta^{13}\text{C}$ increased in all populations and the most xeric population showed significantly
289 higher $\delta^{13}\text{C}$, suggesting higher WUE (Fig. 5 c). *R. ludovici-salvatoris* showed lower
290 $\delta^{13}\text{C}$ values than *C. tricocon*; however, it increased $\delta^{13}\text{C}$ values, i.e. water use

291 efficiency, at the most xeric site. *R. ludovici-salvatoris* showed also significantly lower
292 $\delta^{13}\text{C}$ values before drought in the mesic populations; during drought, $\delta^{13}\text{C}$ increased
293 significantly in mesic populations whereas it remained very similar in the xeric
294 population (Fig. 5 d). For both species, the significant interaction population x season in
295 $\delta^{13}\text{C}$ (Table 3) indicated that the increase in $\delta^{13}\text{C}$ between seasons was different for the
296 three populations of each species (Fig. 5 c, d).

297 Photochemical efficiency of PSII (Fv/Fm) also differed significantly between
298 species, populations, and seasons (Table 3). Before drought, Fv/Fm for *C. tricoccon* was
299 significantly higher in mesic populations than in the most xeric one. During drought,
300 values decreased significantly in all populations, reaching lowest values in the driest
301 population (Fig. 5 e). *R. ludovici-salvatoris* kept high Fv/Fm before drought in all
302 populations, with higher values for mesic populations. During drought, Fv/Fm
303 decreased and the pattern along the mesic to xeric gradient was no longer found; these
304 values were always higher than those of *C. tricoccon* (Fig. 5 f). There was a significant
305 interaction population x season for both species (Table 3), the decrease of Fv/Fm values
306 before vs. during drought was consistently greater in Lluc and Cabrera (Fig. 5 e, f).

307 Chlorophyll concentration (Chl) differed significantly between species and
308 among populations (Table 3). Before drought, Chl for both species was higher in mesic
309 populations than in the xeric one. During drought, Chl decreased in all populations, with
310 again higher values in the mesic than in the xeric ones. Chl values in *R. ludovici-*
311 *salvatoris* were always higher than those of *C. tricoccon*. We found greater differences
312 in Chl among populations in *C. tricoccon* than in *R. ludovici-salvatoris*, indicated by the
313 significant interaction population x species (Table 3).

314 When comparing xeric and mesic years, we found significant differences in
315 stomatal conductance (g_s), and photochemical efficiency of PSII (Fv/Fm) (Table 4),
316 with lower g_s in the dry than in the mesic year for both species (Fig. 6 a, b). There was
317 also a significant population x year interaction, with greater differences between years
318 on mesic populations. The interaction between population x year x species was also
319 significant, as there were higher population-year differences in *R. ludovici-salvatoris*
320 than in *C. tricocon*. Higher values of Fv/Fm were found on the mesic year for both
321 species (Fig. 6 c, d), and a significant interaction population x year also affected Fv/Fm:
322 *C. tricocon* showed greater Fv/Fm differences between years on the mesic populations,
323 while this pattern was not evidenced for *R. ludovici-salvatoris*. Still, there was a
324 significant year x species interaction, with greater differences between years in *C.*
325 *tricocon* than in *R. ludovici-salvatoris*.

326 Non significant effects of gender in g_s and Fv/Fm were found in *R. ludovici-*
327 *salvatoris* (F=0.71, P=0.4; F=1.91, P=0.17, respectively).

328 The four proposed structural equation models (Fig. 7) showed that goodness of
329 fit of the data to the aprioristic model was high as denoted by significant RMSEA
330 statistic (≤ 0.08) and by high NFI and GFI values (> 0.9). Three consistent effects have
331 been found on *C. tricocon* and *R. ludovici-salvatoris* general structural equation
332 models (Fig. 7 b, c). First, light did not have a significant direct nor indirect effect on
333 neither fruit crop nor height; second, aridity had a significant negative effect on fruit
334 crop for both species; and finally, aridity had a significant positive effect on WUE for
335 both species. However the strength of the different hypothesized relationships varied
336 between species. Aridity had a stronger negative effect on fruit crop in *C. tricocon* than
337 in *R. ludovici-salvatoris* (standardized regression weights of -0.45 and -0.39,

338 respectively) and greater positive effect on WUE in *R. ludovici-salvatoris* than in *C.*
339 *tricocon* (0.66 and 0.41, respectively). Moreover, aridity had a negative effect on
340 height for both species, but while the effect was only indirect for *R. ludovici-salvatoris*
341 (through a negative effect of WUE in height), we found direct and indirect effects of
342 aridity for *C. tricocon* (through a negative effect of Fv/Fm in height). Fv/Fm was only
343 affected in *C. tricocon* by aridity but not in *R. ludovici-salvatoris*. Considering
344 seasonal ecophysiological differences (before vs. during summer drought) in *C.*
345 *tricocon* and *R. ludovici-salvatoris* structural equation models have confirmed first,
346 that light did not have a significant effect on fruit crop neither on height, and second,
347 that aridity have greater negative effect on fruit crop and on height in *C. tricocon* than
348 in *R. ludovici-salvatoris*. There was a negative relationship between aridity and WUE
349 increase in *C. tricocon* (-0.46) while there was not in *R. ludovici-salvatoris*. Increases
350 in WUE had a negative effect on fruit crop in *C. tricocon* (-0.30) while we found a
351 positive relationship with height in *R. ludovici-salvatoris* (0.38). Aridity had a negative
352 effect on Fv/Fm decrease in *R. ludovici-salvatoris*, i.e. populations at the limit of aridity
353 showed lower Fv/Fm decrease. In *C. tricocon*, Fv/Fm decrease had a negative effect on
354 height (-0.29), but not in *R. ludovici-salvatoris*. In *C. tricocon*, height had a positive
355 effect on fruit crop, while in *R. ludovici-salvatoris* did not, hence any factor affecting
356 height had a negative effect on fitness in *C. tricocon* populations.

357 **Discussion**

358 The two study species presented contrasting responses to drought and showed different
359 interpopulation variability in functional traits related to drought resistance. These
360 findings reveal different potential vulnerabilities to the expected aridity increase.
361 *Cneorum tricocon* displayed low variability in its water strategy with a consistent

362 behavior across the aridity gradient. Its water conservation strategy was characterized
363 by a wide safety margin against extreme drought events with a significantly negative
364 correlation between stomatal conductance and aridity of the site. Moreover, all *C.*
365 *tricocon* populations showed a drastic stomatal control and high water use efficiency
366 (WUE) under drought conditions, a pattern observed both seasonally and across years
367 and sites. In contrast, *Rhamnus ludovici-salvatoris* showed greater variability in its
368 water strategy. In general, this species displayed narrow safety margins in the face of
369 drought and maintained higher stomatal conductances and lower WUE than *C.*
370 *tricocon*. However, *R. ludovici-salvatoris* was able to reduce water loss in the most
371 xeric site, with relatively high WUE values. This behavior, shifting from water spending
372 to water saving at the driest site, is an adaptive mechanism observed in other woody
373 plants species (Zhang et al. 1995) that could result from local adaptation. Many
374 anatomical and physiological features involved in drought tolerance, like vulnerability
375 to xylem cavitation (Kavanagh et al. 1999), hydraulic conductance (Comstock 2000)
376 and stomata size and density (Mitton et al. 1998) have been shown to result from
377 intraspecific genetic variability and population differentiation.

378 The most xeric population of *C. tricocon* and *R. ludovici-salvatoris* showed
379 lower plant size, worse ecophysiological condition (as shown by lower levels of
380 stomatal conductance and photochemical efficiencies of PSII) and poorer fitness
381 (estimated by a lower fruit crop), which agrees with studies of other Mediterranean
382 woody species (Castro et al. 2004; Jump et al. 2006; Linares and Tiscar 2010). The
383 population at the driest site seems to be at their limit of aridity tolerance and is thus
384 threatened by the increase in aridity expected in the most likely climate change
385 scenarios. This situation, explored in a number of woody species across the

386 Mediterranean basin (e.g. Macías et al. 2006; Carrer et al. 2010) could be counteracted
387 by high levels of phenotypic plasticity and a rapid evolution leading to drought adapted
388 ecotypes (Aspelmeier and Leuschner 2004; Gianoli and Gonzalez-Teuber 2005;
389 Matesanz et al. 2010). The two species studied differed in their potential for
390 accommodating to local and current conditions, with *R. ludovici-salvatoris* displaying a
391 more plastic response to aridity than *C. tricocon*. While the former was able to regulate
392 water consumption according to the water available at each site and time, *C. tricocon*
393 maintained a similar water conservation strategy across sites and over time. However,
394 despite the plastic water strategy of *R. ludovici-salvatoris*, it was not able to counteract
395 the adversity of the most xeric site as shown by the lower estimated fitness of this
396 population. SEM analyses confirmed these trends. First, aridity had a significant
397 negative effect on fitness, as it decreased fruit crop in both species. However, the effect
398 was stronger for *C. tricocon* than for *R. ludovici-salvatoris*. Second, aridity enhanced
399 WUE in both species; however the effect was greater for *R. ludovici-salvatoris* than for
400 *C. tricocon*. Summer drought in the Mediterranean is associated with high irradiance
401 and temperature, further increasing plant stress over this critical period. Species from
402 Mediterranean habitats exhibit different ecophysiological mechanisms to cope with light
403 stress and overheating, which are essential for their survival (Faria et al. 1998;
404 Martinez-Ferri et al. 2000). *Cneorum tricocon* reduced interception of maximum
405 irradiances by displaying greater leaf elevation angles than *R. ludovici-salvatoris*. In
406 fact, there was a positive correlation between leaf elevation angles and population
407 aridity in *C. tricocon*. Increased leaf elevation angles allow to minimize light and
408 thermal stresses but also to reduce water loss through evapotranspiration (Valladares
409 and Pearcy 1997; Valladares et al. 2000; Granado-Yela et al. 2011). However, despite
410 their higher elevation angles, *C. tricocon* was more prone to photoinhibition than *R.*

411 *ludovici-salvatoris* as revealed by the marked decreases of the photochemical efficiency
412 of PSII and chlorophyll concentrations observed in *C. tricocon* during drought and
413 SEM analyses. These findings match well with a water conservation strategy (*sensu*
414 McDowell 2008). In contrast, the lower sensitivity to photoinhibition of *R. ludovici-*
415 *salvatoris* could allow higher carbon gain under moderate drought as seen in other
416 species (Werner et al. 1999, 2001, 2002).

417 *C. tricocon* avoided drought through stomatal closure, thus averting hydraulic
418 failure under water deficit conditions at the expense of carbon assimilation (McDowell
419 2008). Conversely, *R. ludovici-salvatoris* kept open stomata during dry periods
420 maintaining high assimilation rates at the expense of a high risk of cavitation
421 (McDowell 2008). These contrasted strategies allow them to exploit resources
422 differentially, but rendering similar results in terms of growth and survival, which can
423 partially explain the current co-existence of both species (Chesson 2000, Götzenberger
424 et al. 2012). However, future changes in the length and intensity of drought events could
425 favor one species to the detriment of the other. This becomes particularly important in
426 Mediterranean ecosystems, where global circulation models forecast not only an
427 increase in aridity (Christensen 2007), but also an increase in climatic variability with
428 changes in the frequency and intensity of extreme weather and climate events such as
429 heat waves, droughts and floods (Easterling et al. 2000; Lloret et al. 2012). In this
430 sense, a chronic, prolonged drought could have a stronger effect on *C. tricocon* than on
431 *R. ludovici-salvatoris* since under long droughts and a curtailed photosynthesis, carbon
432 starvation becomes a serious threat. Conversely, short but very intense droughts could
433 have a greater effect on *R. ludovici-salvatoris* than on *C. tricocon* due to the risk of

434 mortality by hydraulic failure in the former species, which keeps stomata more open
435 over a wide range of conditions.

436 Most likely climatic scenarios for the Mediterranean region involve an increase
437 in climatic extreme events, where chronic droughts are interrupted by sporadic torrential
438 precipitation events, with subsequent surface runoff (Beniston et al. 2007; Giorgi and
439 Lionello 2008). This situation directly affects the viability of those individuals without
440 access to the water table (Archaux and Wolters 2006). Under this scenario, *C. tricoccon*
441 will be more vulnerable than *R. ludovici-salvatoris*. However, high rates of water
442 consumption by the latter species are only possible if the water table can be reached,
443 which might not be the case in many rocky outcrops where the species is confined.
444 Either a high phenotypic plasticity or a rapid population evolution and adaptation could
445 attenuate the growing climatic threat over these two species. Our results suggest that
446 despite its apparently higher plasticity and potential for local adaptation, *R. ludovici-*
447 *salvatoris* might not be able to phenotypically and evolutionarily track the expected
448 climate change with decreasing soil moisture posing a significant threat to its water
449 spending strategy.

450 In short, *C. tricoccon* and *R. ludovici-salvatoris* are both vulnerable to the
451 current rates of rapid environmental changes, and their responses to further aridity
452 increases will be influenced by the specific future climatic scenarios, primarily duration
453 vs. intensity of the drought. Anticipating these responses requires interdisciplinary
454 research, integrating demographic and genetic work with community and evolutionary
455 ecology.

456

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473

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- 679

680 **Table 1.** Descriptive variables of the study sites. Standard deviations are shown in
681 brackets. *P*mean, mean annual precipitation; *T*max, maximum annual temperature;
682 *T*min, minimum annual temperature; Emberger's index of aridity logarithmically
683 transformed (see text for index details); *T*, temperature; *PAR*, photosynthetic active
684 radiation; *VPD*, vapour pressure density. Climatic data were obtained from the closest
685 weather station of the Spanish Meteorological Agency (AEMET), series 1980-2010.

686

	LLUC	BANY	CABR
Latitude	39.7887	39.6782	39.1511
Longitude	2.8649	2.5437	2.9363
Altitude (m a.s.l.)	600	250	55
Meteorologic station	Monast. Lluc	Banyalbufar	Ses Salines
<i>P</i> mean (mm)	1224.5	499.8	391.7
<i>T</i> max (°C)	35.27	35.63	34.37
<i>T</i> min (°C)	-3.77	-0.01	2.12
Emberguer aridity index	-7.6	-6.67	-6.5
Slope (%)	65.9 (6.01)	9.8 (2.48)	19.00 (10.93)
Bare soil mean (%)	9.00 (5.16)	10 (3.33)	19.5 (8.56)
Cover rocks mean (%)	12.00 (7.74)	20.00 (3.33)	25.00 (12.35)
Cover woody plants mean (%)	55.00 (4.08)	55.00 (5.77)	43.75 (8.09)
Cover herbs mean (%)	24.00 (5.16)	15.00 (5.77)	11.75 (11.03)
<u><i>At the time of measurement:</i></u>			
<i>T</i> (°C)	25.68 (1.55)	25.13 (1.39)	29.55 (1.28)
<i>PAR</i> (μmol m⁻² s⁻¹)	1694.9 (394.5)	1286.7 (439.1)	1588.5 (283.5)
Humidity (%)	40.58 (3.34)	38.23 (5.99)	57.91 (2.87)
<i>VPD</i> (hPa)	19.76 (2.73)	19.88 (3.18)	17.52 (2.44)
Wind speed (Km/h)	0.52 (0.91)	0.09 (0.39)	3.79 (1.78)

687

688

689 **Table 2.** Effects of population (Po), species (Sp) and their interaction (Po x Sp) on
 690 height, crown and trunk diameters, fruit crop and leaf angles. Significant effects ($p <$
 691 0.05) are indicated in bold.

		Two-way ANOVA		
		DF	<i>F</i>	<i>p</i>
Height	Po	2	19.86	< 0.001
	Sp	1	209.73	< 0.001
	Po x Sp	2	5.48	0.004
Crown diameters	Po	2	32.36	< 0.001
	Sp	1	105.77	< 0.001
	Po x Sp	2	10.33	< 0.001
Trunk diameters	Po	2	65.32	< 0.001
	Sp	1	960.46	< 0.001
	Po x Sp	2	57.24	< 0.001
Fruit Crop	Po	2	4.82	0.011
	Sp	1	9.51	0.003
	Po x Sp	2	4.88	0.010
Leaf angles	Po	2	2.52	0.082
	Sp	1	9.88	0.002
	Po x Sp	2	1.00	0.369

692

693

694 **Table 3.** Effects of population (Po), season (Se), species (Sp) and their interactions on
695 ecophysiological traits. Abbreviations are: g_s , stomatal conductance; $\delta^{13}\text{C}$, carbon
696 isotope ratio; Fv/Fm, photochemical efficiency of PSII and Chl, chlorophyll
697 concentration. PAR was included as a covariate. Significant effects ($p < 0.05$) are
698 indicated in bold.

	g_s		$\delta^{13}\text{C}$		Fv/Fm		Chl	
	F	p	F	p	F	p	F	p
Po	21.74	<0.001	183.03	<0.001	47.49	<0.001	76.19	<0.001
Se	377.14	<0.001	44.66	<0.001	266.37	<0.001	14.43	<0.001
Sp	186.38	<0.001	147.51	<0.001	29.75	<0.001	66.14	<0.001
Po x Se	29.80	<0.001	6.21	<0.001	7.32	<0.001	9.22	<0.001
Po x Sp	16.99	<0.001	32.77	<0.001	5.21	0.01	41.84	<0.001
Ti x Sp	30.69	<0.001	0.50	0.48	43.66	<0.001	8.89	<0.001
Po x Se x Sp	0.25	0.78	0.86	0.42	0.65	0.52	3.58	0.03
PAR	0.43	0.51	0.97	0.33	0.42	0.52	3.92	0.05

699

700

701 **Table 4.** Effects of population (Po), year (Ye), species (Sp) and their interactions on
702 stomatal conductance (g_s) and photochemical efficiency of PSII (Fv/Fm). Significant
703 effects ($p < 0.05$) are indicated in bold.

704

	g_s		Fv/Fm	
	F	p	F	p
Po	31.51	<0.001	65.27	<0.001
Ye	151.63	<0.001	158.67	<0.001
Sp	137.03	<0.001	36.87	<0.001
Po x Ye	23.17	<0.001	4.55	0.011
Po x Sp	10.89	<0.001	9.01	<0.001
Ye x Sp	27.13	<0.001	17.26	<0.001
Po x Ye x Sp	5.90	0.003	2.68	0.070

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708 **Figure captions**

709 **Figure 1.** Mean climatic features of the three study sites. Column and lines represent
710 monthly mean precipitation and temperature, respectively, for the period 1980-2010 and
711 in the two study years (2006 and 2010). Data were obtained from the closest weather
712 station of the Spanish Meteorological Agency (AEMET).

713 **Figure 2.** Differences in height (a), crown diameter (b) and trunk diameter (c) between
714 populations of *Cneorum tricoccon* (black columns) and *Rhamnus ludovici-salvatoris*
715 (grey columns) along an aridity gradient. Values are mean \pm SE. Different letters
716 indicate significant differences after Fisher's LDS test. N=68-108 for each species and
717 population. Abbreviations are: M, mesic; D, dry; study sites codes in Table 1.

718 **Figure 3.** Differences in fruit crop among populations of *C. tricoccon* (left) and *R.*
719 *ludovici-salvatoris* (right). Values given as mean \pm SE. Different letters indicate
720 significant differences after Fisher's LDS test. N=15-30 for each species and
721 population. Abbreviations are: M, mesic; D, dry; study sites codes in Table 1.

722 **Figure 4.** Leaf angle differences between populations of *C. tricoccon* (black columns)
723 and *R. ludovici-salvatoris* (grey columns). Values are mean \pm SE. Different letters
724 indicate significant differences after Fisher's LDS test. N=15-30 for each species and
725 population. Abbreviations are: M, mesic; D, dry; study sites codes in Table 1.

726 **Figure 5.** Mean values of stomatal conductance (g_s), carbon isotope ratio ($\delta^{13}C$)
727 photochemical efficiency of PSII (Fv/Fm) and chlorophyll concentration (Chl) before
728 (black columns) and during summer drought (grey columns) in populations of *C.*
729 *tricoccon* (left) and *R. ludovici-salvatoris* (right). Values are mean \pm SE. Different

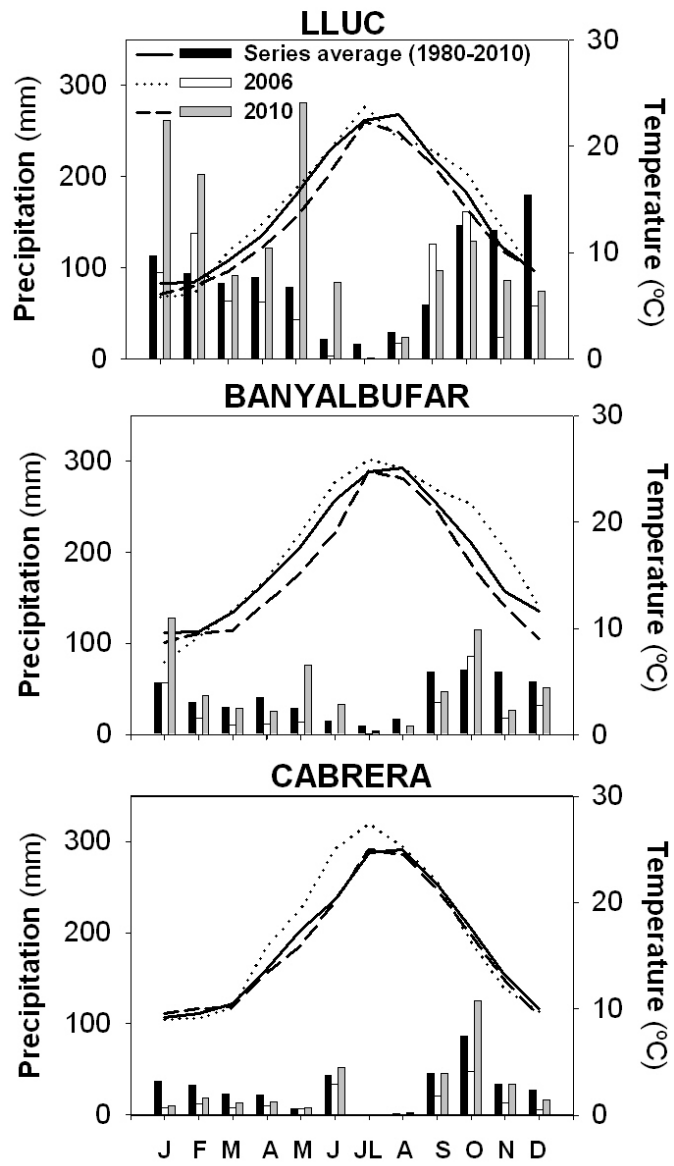
730 letters indicate significant differences after Fisher's LSD test. N=15-30 for each species
731 and population. Abbreviations are: M, mesic; D, dry; study sites codes in Table 1.

732 **Figure 6.** Mean values of stomatal conductance (g_s) and photochemical efficiency of
733 PSII (Fv/Fm) in two climatically contrasted years: mesic (black columns) and dry year
734 (grey columns) in populations of *C. tricocon* (left) and *R. ludovici-salvatoris* (right).
735 Values are mean \pm SE. Different letters indicate significant differences after Fisher's
736 LSD test. N=15-30 for each species and population. Abbreviations are: M, mesic; D,
737 dry; study sites codes in Table 1.

738 **Figure 7.** General structural equation model representing hypothesized casual
739 relationships among abiotic predictors, ecophysiological, morphological and fitness
740 variables (a). Fitted General model in *C. tricocon* and *R. ludovici-salvatoris* (b and c,
741 respectively) and considering seasonal ecophysiological differences (before vs. during
742 summer drought) in *C. tricocon* and *R. ludovici-salvatoris* (d and e, respectively).
743 Solid and dashed arrows indicate positive and negative effects, respectively, with
744 standardized regression weights indicated. Arrow widths are proportional to p - values.
745 Paths with coefficients non-significantly different from 0 ($p < 0.1$) are omitted. Fit
746 statistics of the model (RMSEA, GFI and NFI) and sample size (N) are given for each
747 proposed model.

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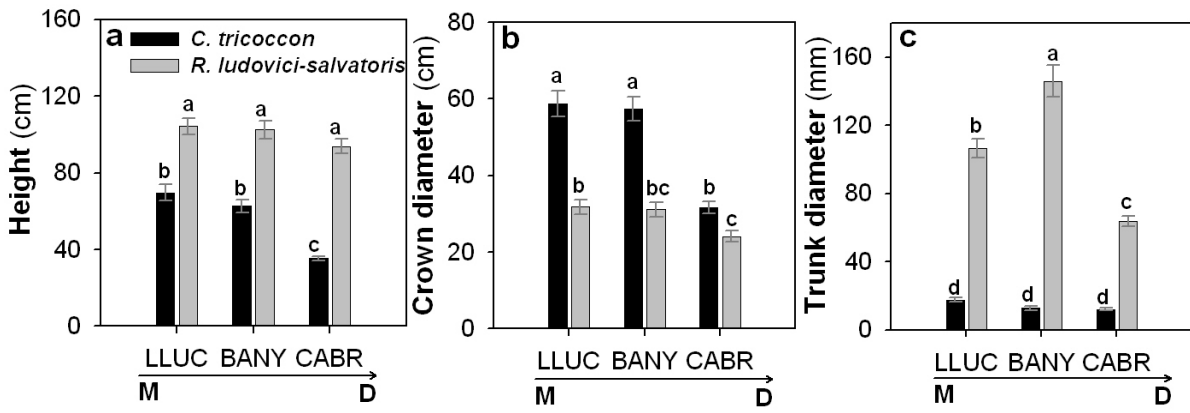
749 **Figure 1.**



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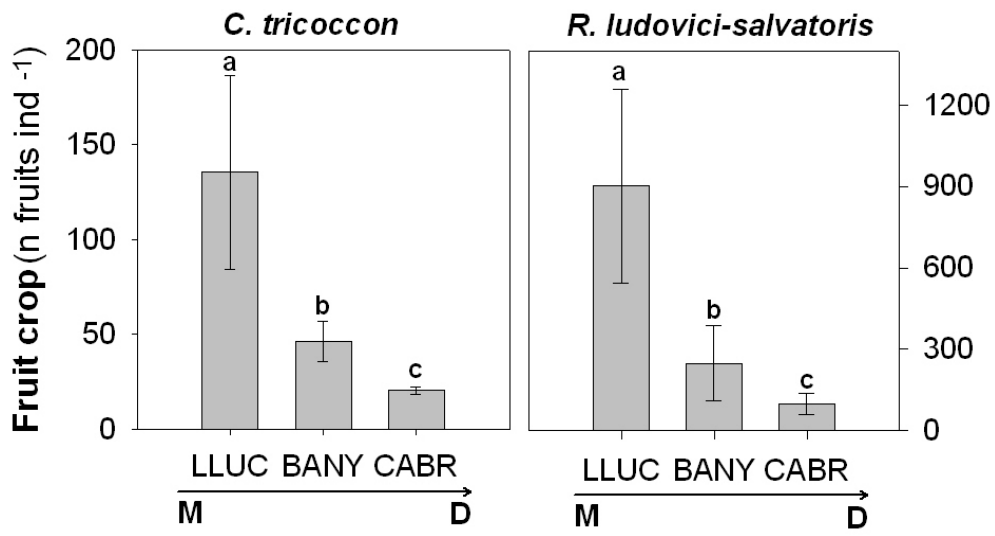
752 **Figure 2.**



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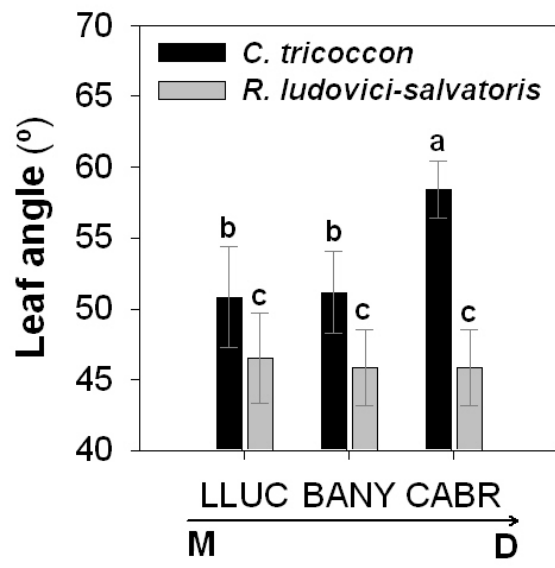
755 **Figure 3.**

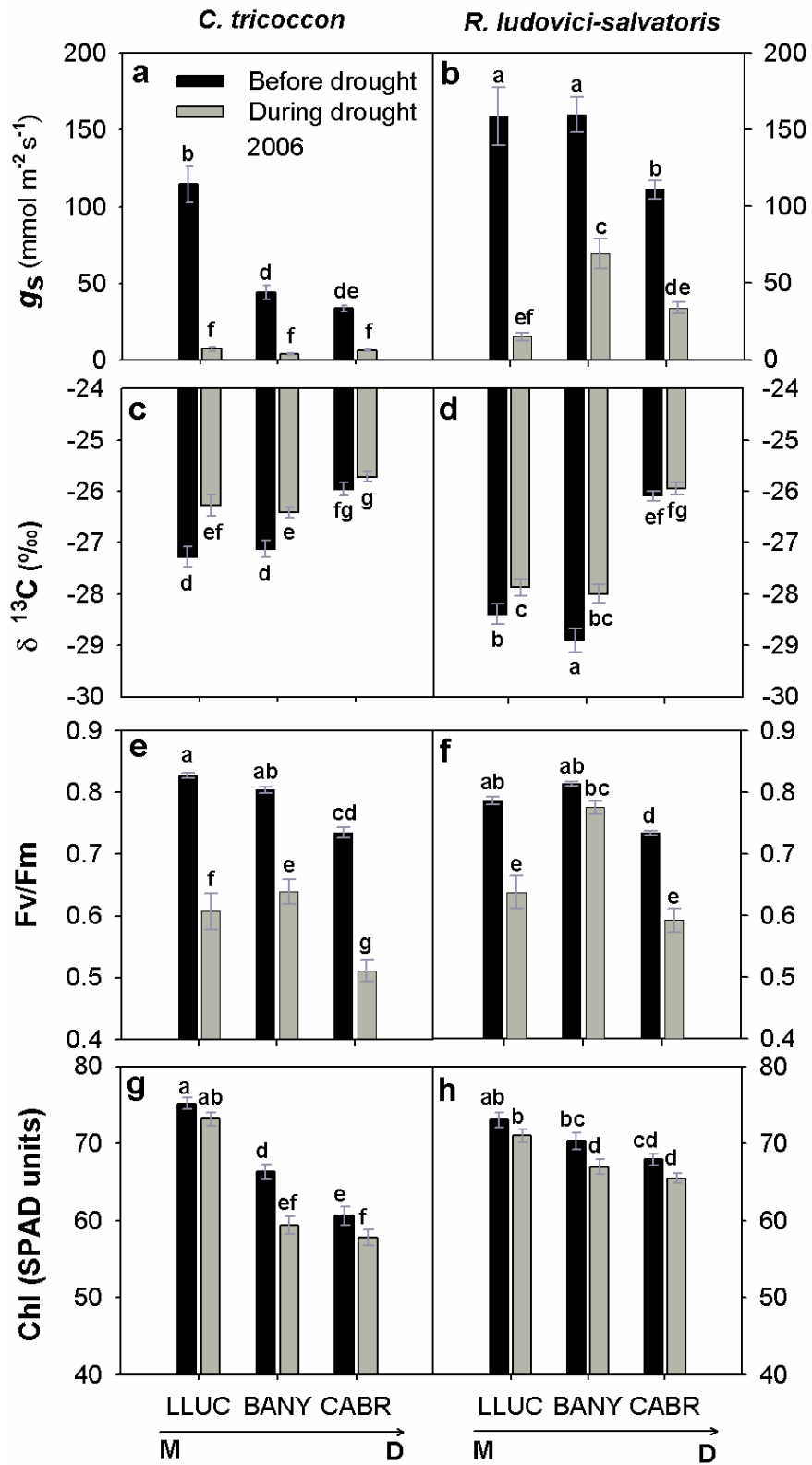


758 **Figure 4.**

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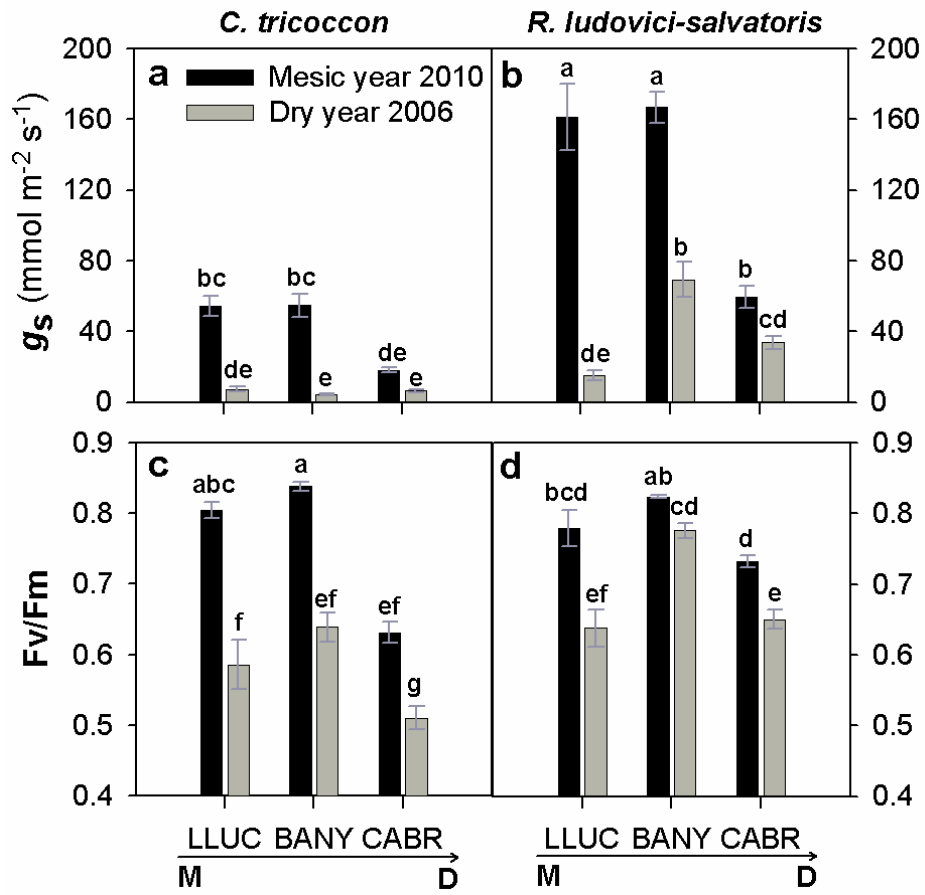
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763 **Figure 6.**

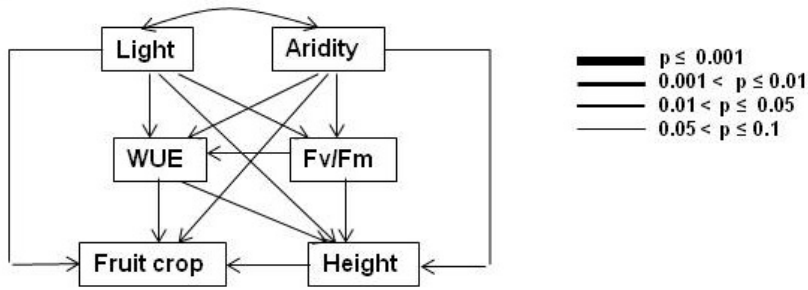
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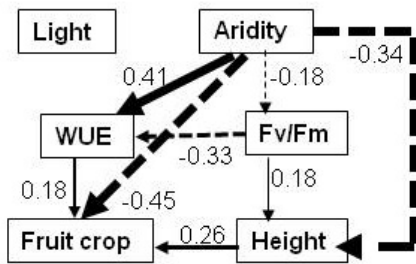
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a) General model path

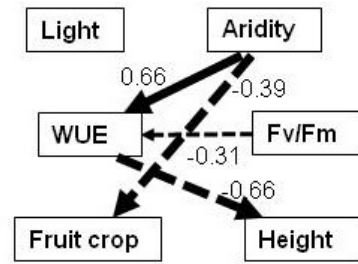


b) *C. tricocon*. General model



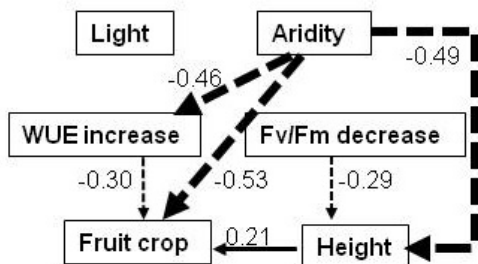
RMSEA < 0.01; NFI = 0.99; GFI = 1.00; N = 95

c) *R. ludovici-salvatoris*. General model



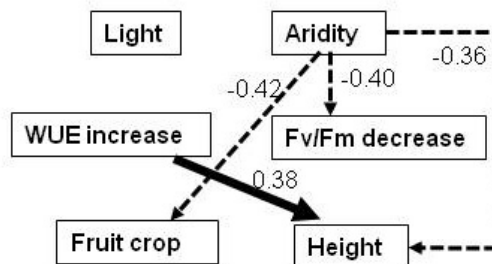
RMSEA = 0.067; NFI = 0.98; GFI = 0.99; N = 95

d) *C. tricocon*. Seasonal differences



RMSEA = 0.047; NFI = 0.98; GFI = 0.99; N = 48

e) *R. ludovici-salvatoris*. Seasonal differences



RMSEA = 0.074; NFI = 0.93; GFI = 0.98; N = 48