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5 ***Gender dimorphism in Corema album across its biogeographical area***
6 ***and implications under a scenario of extreme drought events***

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18

19 **Abstract**

20 In dioecious species, traits may have evolved because of significantly different resource
21 demands associated with male versus female sexual reproduction. It is generally
22 assumed that females have higher reproductive costs, thereafter in long-lived species,
23 males often exceed females in vigour and suffer lower physiological limitations under
24 stress. However, large-scale studies of gender dimorphism and the link between survival
25 and physiological responses and compensation mechanisms are still lacking. In this
26 study, we compared canopy performance, photochemical efficiency, leaf water
27 potential, $\Delta^{13}C$, leaf nitrogen content and $\delta^{15}N$ in both genders of the dioecious species
28 *Corema album* (Ericaceae) across all the species' biogeographical distribution. This

29 area corresponds to a wide climatic gradient, from temperate and humid to

30 Mediterranean, across the Atlantic coast of the Iberian Peninsula.

31 Our results evidenced that the species responds to the bioclimatic gradient, and most

32 variables were significantly correlated with annual rainfall and bioclimatic indices,

33 although gender dimorphism was only manifested in $\Delta^{13}C$ and in leaf water potential at

34 the peak of the drought season. Female plants have significantly higher values of $\Delta^{13}C$,

35 which could be attributed to lower water use efficiency; however, male plants presented

36 more negative leaf water potentials than females, especially in the populations with the

37 warmest and most arid climate.

38 We concluded that *C. album* populations adjust to the climatic gradient at different

39 hierarchical scales, from physiological instantaneous to time-integrated traits, with

40 gender dimorphism only occurring in sub-optimal environments The absence of

41 morphological differences between genders and the maintenance of a less negative

42 water potential in females in the dry period in southern more arid populations, suggests

43 the existence of compensatory mechanisms at underground level.

44

45 ***Keywords:*** chlorophyll fluorescence; climatic gradient; dioecious scrub; leaf area index

46 (LAI); reproductive costs; water stress

47

48   **1. Introduction**

49 In dioecious plant species gender-specific ecological and morphological traits may have

50 evolved because of significantly different resource demands associated with male versus

51 female sexual reproduction (Dawson and Bliss, 1989; Dawson and Ehleringer, 1993). If

52 resources are limited, reproduction competes directly with vegetative growth and

53 investment in defence mechanisms; so that an increase in the energy allocated to one-

function results in a decrease in the energy allocated to the others (Juvany and Munné-Bosch, 2015; Obeso, 2002). In the majority of cases, it has been postulated that females of dioecious species have greater reproductive allocation than males in order to produce flowers and fruits, which may cause a loss of growth or an increase in mortality especially in stressful environments. On the other hand, in order to produce pollen, male plants should need more nitrogen supply than females, which may lead to different resource demands (Barrett and Hough, 2013); these differences should be more important in wind-pollinated plants during the flowering period, generating a trade-off in the investment of growth and reproduction (Obeso, 2002; Zunzunegui et al., 2006). In relation to these ideas, in the literature there are many studies on gender distribution, morphology, growth and survival in dioecious plants (Allen and Antos, 1993; Álvarez-Cansino et al., 2010a; Diaz-Barradas and Correia, 1999; Herrera, 1988; Morales et al., 2016; Nicotra, 1999; Obeso, 1997; Zunzunegui et al., 2006). Yet, studies on gender-specific physiology in dioecious species are limited (Álvarez-Cansino et al., 2012; Álvarez-Cansino et al., 2010b; Correia and Díaz Barradas, 2000; Dawson et al., 2004; Dawson and Ehleringer, 1993; Juvany et al., 2014; Juvany and Munné-Bosch, 2015; Sánchez-Vilas and Retuerto, 2009). In particular, there is little knowledge on how differential physiological performance among genders is linked to gender-related differences in performance as growth and survival. Available studies are mostly at local scale, supporting the evidence that genders might exhibit differential competitive abilities derived from the different resource allocation to reproduction, which imply a certain degree of niche divergence (Eppley, 2006). These may lead to gender differential survival and spatial segregation of the sexes (SSS) in a spatially heterogeneous habitat (Bierzychudek and Eckhart, 1988; Sánchez-Vilas and Retuerto, 2009).

79   Several studies suggest that female plants present compensatory mechanisms to

80   maintain their greater reproductive demands (Álvarez-Cansino et al., 2010b; Case and

81   Barrett, 2004; Dawson et al., 2004). For example, higher CO_2 assimilation, associated

82   with higher reproductive allocation rates, has been found in females plants in relation to

83   males in several species (Rakocevic et al., 2009). This may explain the lack of gender-

84   related differences in growth, mortality or SSS.

85   It is widely known that species adjust their physiological responses to

86   environmental gradients (Ashcroft et al., 2016; Case and Barrett, 2004), and phenotypic

87   plasticity is considered the major means by which plants cope with environmental

88   heterogeneity (Valladares et al., 2007). Yet, how physiological responses, compensation

89   mechanisms and their link to survival differ among sexes is not known at a

90   representative species distribution scale. There is thus a need for a wide assessment of

91   physiological differences among genders representative of species distribution areas,

92   vegetative performance and its relationship with sex ratio and plant distribution. In this

93   study we provide data on sexual dimorphism and morphological and physiological

94   variations of the woody dioecious species *Corema album* (L.) D. Don (Ericaceae) over a

95   wide environmental gradient corresponding to the entire biogeographical area. To our

96   knowledge, this is the first study covering the distribution area of a dioecious species

97   that analyses morpho-physiological gender dimorphism.

98   *Corema album* is a dioecious shrub endemic to the West coast of the Iberian

99   Peninsula (Valdés et al., 1987). The species occurs along a wide bioclimatic gradient

100   from Galicia in the Northwest with an Atlantic climate, to Gibraltar strait in the South-

101   West with a typical Mediterranean climate, ranging along all the Atlantic coast of the

102   Iberian Peninsula. Rainfall oscillates from 1500 in the North to 500 mm in the South

103   along the species' biogeographical area (Álvarez-Cansino et al. 2013). Previous studies,

104 including our own, show that *C. album* exhibits gender-related differences in

105 reproductive allocation; female shrubs show greater reproductive effort than males, due

106 to fruit production (Álvarez-Cansino et al., 2010a; Guitián et al., 1997; Zunzunegui et

107 al., 2006). Demographic differences between sexes reflected in sex ratio deviations or

108 the occurrence of spatial segregation were found to be unrelated to any climatic variable

109 across the complete biogeographical area, suggesting the existence of compensatory

110 mechanisms that may counterbalance the higher reproductive effort of female plants

111 (Álvarez-Cansino et al., 2012; Martins et al., 2017).

112   In a previous study on physiological traits on male and female plants of this

113 species in three selected points of its biogeographical area (north, centre and south),

114 results have shown that plants were physiologically stressed in the southernmost

115 population during the summer drought, exacerbating gender-related differences

116 (Álvarez-Cansino et al., 2012). Yet, this study was at the local scale and limited to one

117 study year. In this study, we improved the limited existing data by extending our

118 research to the complete biogeographical area of the species to test for the first time

119 how physiological instantaneous and time-integrated variables change over the

120 complete area of the species. We hypothesized that the absence of SSS found in *Corema*

121 *album* is explained by female's compensatory mechanisms and that genders won't

122 present any differences in performance in physiological and canopy structure (LAI).

123   Climate changes in the Mediterranean region are predicted to occur as a result of

124 global warming (Christensen et al., 2007; IPCC, 2013), including a rise in mean

125 temperature (2–4 ºC) and a decrease in the quantity and frequency of rainfall. In

126 Mediterranean-type ecosystems, where water availability is one of the main

127 environmental constraints limiting plant species regeneration (Castro et al., 2004;

128 Gómez-Aparicio et al., 2008), a climatic scenario of increased drought is expected to

129 drive changes in community composition and geographic distribution of species. Under

130 this scenario, plant performance and gender dimorphism in *C. album* will be affected,

131 especially in the southern-most arid limit of its distribution. If climate is impacting

132 genders survival differently, with the expected climate change gender dimorphism,

133 compensatory mechanisms and sex ratio might be affected (Hultine et al., 2018; Munné-

134 Bosch, 2015). In this study, we completed the existing data set by repeating

135 measurements over several years, to determine gender related differences that may be

136 explained by inter annual variation. We provide extensive new data on the physiological

137 ecology and performance of the dioecious shrub *Corema album* along the complete

138 climatic gradient included in its distribution area.

139   We assumed that intraspecific morphological and physiological differences could

140 be found along the studied bioclimatic gradient, but we were interested in knowing if

141 significant gender differences in resource use (water and nitrogen), physiology and

142 vegetative performance could be identified and if these could account for differences in

143 habitat-specific conditions.

144   We hypothesised that gender-physiological and morphological dimorphism will

145 directly respond to rainfall availability and aridity intensity, and that they will increase

146 under extreme drought events. Due to their higher reproductive allocation, we expected

147 females to respond more negatively than males to harsher conditions during dryer and

148 warmer years.

149   We specifically addressed the following questions: (i) is vegetative performance

150 measured through LAI higher in males than in females and do these differences increase

151 with drought stress (across the gradient)? (ii) do males and females differ in water

152 economy and photochemical efficiency, and do these differences increase with drought

153 stress? (iii) do males and females differ in nitrogen economy? We approached these

154   questions studying eight different populations with large climatic differences in

155   temperature and rainfall, during the spring and summer seasons, across the entire

156   environmental gradient comprised in *C. album*'s biogeographical range.

157   **2.   Materials and methods**

158   **2.1. Study species**

159   The genus *Corema* D. Don (*Ericaceae)* only comprises two species occupying opposite

160   sites on the North Atlantic coasts: *Corema album* (L.) D. Don is an endemic species of

161   the Western and South-Western coasts of the Iberian Peninsula (*C. album* subsp.

162   a*lbum*), presenting a subspecies in Azores Islands (*C. album* subsp. *azoricum* Pinto da

163   Silva), while *C. conradii* Torr. grows on the Eastern coasts of North America.

164   *C. album* is a dioecious shrub, densely branched, with alternate folded leaves, up

165   to 1 m high, presenting small flowers and wind pollination. Male flowers have 3 red

166   stamens (5 mm size) and female flowers are smaller with 3 red stigmas. The fruit is a

167   more or less spherical berry (diameter 5-10 mm), white or pinkish, with 3 seeds (Valdés

168   et al., 1987). The berries are eaten by a number of animals like rabbits, foxes, seagulls,

169   magpies and other birds (Calviño-Cancela, 2002). Although the species has been

170   described as strictly dioecious, some cases of subdioecy (Sakai and Weller, 1999) have

171   been found in Huelva and Algarve coasts (Zunzunegui et al., 2006).

172   *C. album* subsp. *album* is an important shrub species of coastal dunes, growing on

173   more or less mobile dunes and on sands over coastal cliffs and it can also be found in

174   later successional stages on coastal habitats (Álvarez-Cansino et al., 2013; Clavijo et al.,

175   2002; Díaz-Barradas et al., 2000). Its distribution area extends from the NW border of

176   the Iberian Peninsula (Finisterre) to the straits of Gibraltar (Álvarez-Cansino et al.,

177   2013; Valdés et al., 1987), although a population of this species has been described in

178   the Mediterranean coasts of Spain (López-Dóriga., 2018).

179

2.2. Study sites

181   Eight coastal sites, from the North West to the South West of the Iberian Peninsula,

182   extending over all the biogeographical area of the species, were sampled in this study.

183   At all these sites, with sandy soil, Camariñas, São Jacinto, Osso da Baleia, Caparica,

184   Troia, Santo André, Monte Gordo and El Asperillo, important natural populations of *C.*
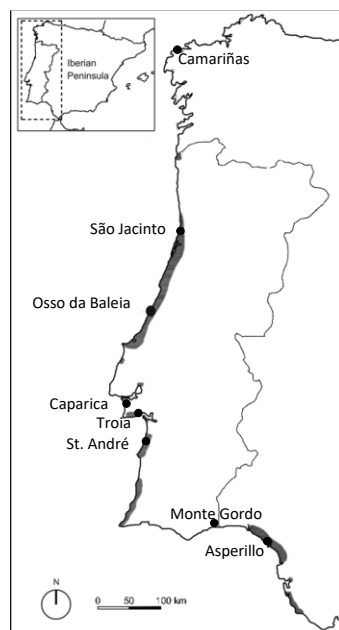
185   *album* were found (Fig. 1).

186

Fig. 1. Distribution map of *Corema album* across the Atlantic coast of the Iberian Peninsula,
denoting the 8 study sites.

197

198   All study sites present climatically different conditions since the objective of the

199   work is to know how gender-physiological and morphological dimorphism responds

200   along the complete species' biogeographic gradient. The chosen populations varied

201   markedly in climatic variables, the difference in mean annual temperature reaches 3.5

202   ºC between the coolest and warmest populations, and annual accumulated precipitation

203   can differ by over 800 mm between the Northernmost (the wettest) and Southernmost

204   (the driest) populations. Light conditions were also different among the study sites, the

205   radiation registered in Camariñas (400-500 μmol m^{-2}s^{-1}) was three times lower than in

206   the remaining areas (1200-1500 μmol m^{-2}s^{-1}).

207   Values of climatic variables were obtained over the past 30 years (1974–2004)

208   from the nearest meteorological stations, from Instituto Nacional de Meteorología

209   (INM, Spain), Xunta de Galicia (Martínez Cortizas et al. 2000), and Sistema Nacional

210   de Informação de Recursos Hídricos (SNIRH, Portugal). To compare climatic

211   conditions among populations and the aridity level along the study gradient two climatic

212   indices were calculated (Table 1):

213   Emberger's aridity index: $Q = (2000 \times MAP) / (T_{max}^2 - T_{min}^2)$.

214   Where: MAP is mean annual precipitation (mm) and T_{max} and T_{min} are the mean

215   maximum temperature of the hottest month (ºK) and the mean minimum temperature of

216   the coldest month (ºK). The thresholds for Q index are the following: <15 Saharian; 15-

217   30 Arid; 30-65 Semi-arid; 65-100 Sub-humid; 100-170 Humid; >170 Per-humid (Daget,

218   1977).

219   Lang's thermopluviometric index: it = MAP/T

220   Where T is annual mean temperature (ºC) and MAP mean annual precipitation (mm), as

221   an indicator (it>40 wet climate, it<40 dry climate) (Tormo Molina et al., 1992).

222

223   Table 1. Climatic variables of the study sites. Mean annual temperature (T), mean maximum

224   (Tmax) and minimum temperatures (Tmin, ºC), mean annual accumulated precipitation (MAP,

225   mm), mean summer precipitation (July, August, September), mean spring precipitation (April,

226   May and June), Emberger's aridity index (Q) and Lang's thermopluviometric index (it index).

Site	T (ºC)	Tmax (ºC)	Tmin (ºC)	MAP (mm)	Psummer (mm)	Pspring (mm)	Q Emberger	it index
Camariñas	13.7	24.0	6.5	1217	144	244	241.43	88.8
São Jacinto	14.2	22.1	6.3	961	64	155	211.56	67.68
Osso Baleia	14.3	24.2	4.8	917	75.7	219.5	164.24	64.13
Caparica	16.8	27.9	8.2	751	36.2	123.8	130.85	44.70
Troia	16	29.0	5.6	746	34	113	109.71	46.63

St. André	15.2	28.0	4.8	651	32.1	100	96.86	42.83
Monte Gordo	17.5	31.9	7.7	483	20	319	68.16	27.6
Asperillo	16.8	32.3	4.6	543	21.3	82.7	67.19	32.32

227

2.3. Leaf Area Index (LAI)

229 In each study population (except for Osso da Baleia), a 200 m belt transect was laid,

230 consisting on ten (10x10 m^2) plots, each separated by 10 meters. In each plot, the

231 number of all male, female and non-reproductive canopies of *C. album* was recorded.

232 Measurements were made during the year 2002 (Álvarez-Cansino et al., 2013).

233 In each transect, a set of 20 reproductive individuals, 10 on each gender, were

234 randomly selected for morphological, physiological and reproductive measurements.

235 All selected plants were of similar size (60-70 cm canopy diameter) and they were

236 sufficiently separated in space (the basal trunk at least 1.5 m between individuals of the

237 same gender), to be clearly identified as different individuals. Old individuals of *C.*

238 *album* with big and open canopy were avoided.

239 In each selected plant, of seven study sites, LAI was estimated using the LAI-

240 2000 Plant Canopy Analyser (LI-COR USA), that uses a fisheye light sensor that

241 measures the attenuation of diffuse radiation simultaneously in five zenith angles

242 comparing radiation measurements above and below the canopy (Welles, 1990). LAI

243 measurements were made in June, which corresponds to the maximum vegetative

244 growth of the species and before leaf fall during the summer period.

245

2.4. Physiological measurements

247 In the same selected plants of seven sites, measurements of midday water potential and

248 photochemical efficiency were made in the middle of May and the beginning of

249 September. The first corresponds to the spring-time when water is available in all the

250 study areas and the temperature is optimum for photosynthetic activity. September

251 represents the end of the summer season when the maximum water stress is manifested

252 especially for the Southern populations. Physiological measurements were made on

253 consecutive sunny days both in May and September; with the exception of Camariñas,

254 in the north, in which cloudy days are the most common meteorological condition.

255   Leaf water potential of 5 terminal shoots per plant was measured at midday using

256 a pressure chamber of Scholander-type (Scholander et al., 1965), the mean value was

257 used for each plant. Midday water potential represents the maximum water deficit

258 experienced by the leaves and the xylem (Ackerly, 2004) and allowed a better

259 comparison between genders and populations. Measurements were performed

260 immediately after collecting the samples.

261   Midday photochemical efficiency of photosystem II (PSII), or maximum quantum

262 yield of PSII (F_v/F_m) was measured in the field with a portable pulse-modulated

263 fluorometer (Mini-Pam Photosynthesis Yield Analyzer; Walz, Effeltrich, Germany).

264 Samples were dark-adapted with a leaf clip holder during 20 minutes, which was found

265 to be sufficient to allow complete reoxidation of the PSII reaction centres and to ensure

266 that all energy-dependent quenching was relaxed. Maximum (F_m) and minimal (F_o)

267 fluorescence were measured to calculate maximum photochemical efficiency of PSII

268 ((F_m-F_o)/F_m which is described as F_v/F_m). Steady-state fluorescence (F) under the

269 prevailing ambient light and maximum fluorescence (F'_m) during saturation pulse were

270 also determined, to estimate midday effective quantum yield of PSII as (F'_m-F)/F'_m

271 (Φ_{PSII}), or photochemical efficiency of PSII in light-adapted state, according to Genty et

272 al. (1989). Five measurements on terminal twigs were carried out in each selected plant,

273 and the mean value per plant was used for statistical analysis. Light experienced by

274 plants during the measurements oscillated between 1200 and 1500 µmol m^{-2}s^{-1}, from

275   São Jacinto to El Asperillo; however in Camariñas the light intensity ranged between

276   400 and 500 µmol m^{-2}s^{-1} due to cloudy conditions.

277

278   **2.5.Stable isotope analysis of leaves**

279   A sample of new leaves was collected in September in all study individuals.

280   Approximately 15-20 leaves per plant were dried at 60ºC, for 24h and dry leaf samples

281   were then milled to a fine powder and passed through a 40-mesh for isotopic analysis.

282   The $\delta^{13}C$ determinations for each plant were made on three 1-2 mg subsamples by a

283   Stable Isotope Mass Spectrometer (IRMS, Isoprime, GV, Manchester, UK) at the

284   University of Lisbon (Portugal). The instrument error (twice the standard deviation)

285   associated with each observation was 0.01‰, and the sample preparation and analysis

286   error between repeated analyses of the same ground tissue was less than 0.22‰. Sample

287   $\delta^{13}C$ was calculated as:

288        $\delta^{13}C_{sample}$ (‰) = ($R_{sample}/R_{standard}-1$) x 1000(‰),

289

290   where R_{sample} and $R_{standard}$ refer to the $^{13}C/^{12}C$ ratios of the plant sample and Pee Dee

291   Belemnite carbonate standard, respectively. The stable carbon isotope ratio ($\delta^{13}C$) of a

292   leaf tissue can serve as a proxy for integrated water-use efficiency (WUE) (Dawson et

293   al., 2002; Farquhar et al., 1989).

294   According to the classic two-step discrimination model (Farquhar et al., 1982),

295   $^{13}C/^{12}C$ discrimination ($\Delta^{13}C$) was calculated as: $\Delta^{13}C$ (‰) = ($\delta^{13}C_{air}$ - $\delta^{13}C_{sample}$) /(1+

296   $\delta^{13}C_{sample}$). Since these plants were studied in natural conditions, we assumed a $\delta^{13}C$

297   value for atmospheric CO_2 of -7.8‰ (Farquhar et al., 1989).

298   Leaves from 5 populations were also used for nitrogen content (%) and isotope

299   analysis. $\delta^{15}N$ was measured against the ammonium sulphate standard (IAEA, N$_2$). N

300   isotope ratios are reported per mil (‰) relative to atmospheric N_2 (Shearer and Kohl

301   1993) as:

302          $\delta^{15}N$ (‰) = $(R_{sample}/R_{atmos}-1)$ x 1000(‰),

303   where R_{sample} represents the isotope ratio ($^{15}N/^{14}N$) in sample foliage, and R_{atmos} is

304    $^{15}N/^{14}N$ for atmosphere N_2 as standard. The standard deviation of 10 repeated samples

305   was <0.2% for $\delta^{15}N$.

306

307   **2.6. Comparison among years**

308   As leaf water potential revealed to be the most significant variable for sexual

309   dimorphism in El Asperillo (see results in this manuscript), we repeated the

310   measurements of midday leaf water potential, in ten individuals of each gender, in this

311   site at the peak of the dry season (September) in three contrasted years: 2002 (with an

312   average annual rainfall of 560.6 mm and average maximum summer temperatures of

313   36.5ºC for July and August), 2003 (rainfall 549.5 mm, but extremely elevated summer

314   temperatures, 41.5ºC) and 2005 (annual rainfall lower than 50% of the average values,

315   173 mm and elevated summer temperatures, 39.5ºC). This comparison, made in the

316   southern limit of species' biogeographical area, could function to forecast the behaviour

317   of this species under a scenario of extreme drought events.

318

319   **2.7. Data analysis**

320   Physiological and morphological variables (LAI, leaf water potential, Fv/Fm, ΦPSII,

321    $\delta^{13}C$, $\delta^{15}N$ and N content), were analysed using a two-way ANOVA to compare sites

322   and gender-related differences within sites (both considered as fixed factors). The

323   Tukey test was used for comparison among sites for each season.

324   Regression analyses were applied to evaluate possible relationships among plant

325 variables with rainfall and bioclimatic indices.

326   The assumption of normality was tested with the Kolmogorov-Smirnov test. All

327 statistical analyses were performed with the SPSS 24 software package (Chicago, IL,

328 USA).

329

330 **3. Results**

331 **3.1. Leaf Area Index (LAI)**

332 LAI clearly followed the bioclimatic gradient. The Northern populations showed higher

333 values than the Southern ones, with a clear biogeographical pattern, but differences

334 between genders were not found (Fig. 2a). In the two-way ANOVA, there were

335 significant differences between sites, but no differences were found between gendersand

336 in the interaction between site and gender either (Table 2). Comparisons among sites,

337 using Tukey test, showed that LAI of plants growing in Camariñas was significantly

338 higher than all other sites (p<0.05) and there was a progressively decrease associated to

339 the increase in aridity, with the lowest values in Monte Gordo and El Asperillo sites

340 (Fig. 2a). For both genders LAI values were significantly correlated with MAP

341 (R^2_{males}=0.92; $R^2_{females}$=0.86), Emberger (R^2_{males}=0.92; $R^2_{females}$=0.87) and Lang's

342 thermopluviometric index (R^2_{males}=0.91; $R^2_{females}$=0.87), (P<0.05 in all cases), (Fig. 2b).

343

344 Table 2. Two-way ANOVA of effects of site and gender on leaf area index of *Corema album*
345 (TypeIII SS: Sum of Squares; df: degrees of freedom).

346

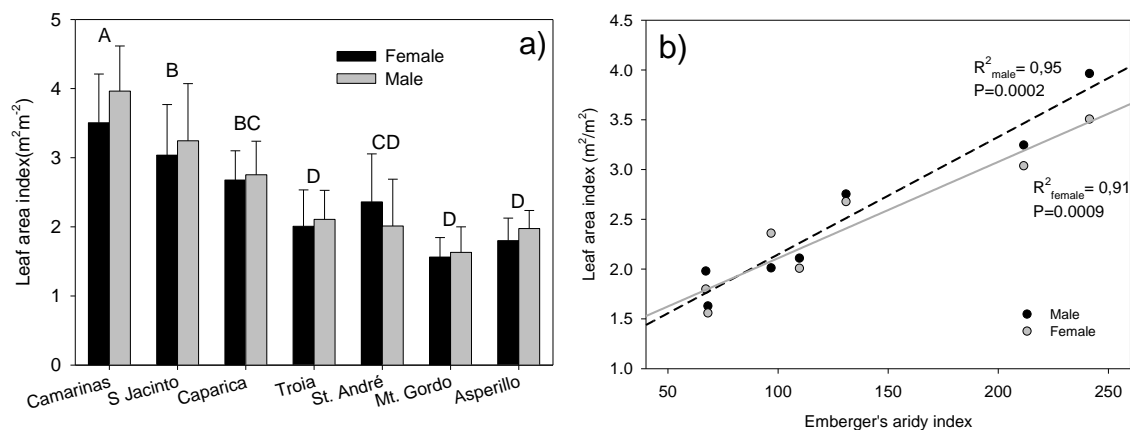|	**TypeIII SS**	**df**	**F**	***P***
Site	60.19	6	29.80	**<0.001**
Gender	0.33	1	0.99	0.322
SitexGender	1.73	6	0.86	0.529

347

348

349

356 Fig. 2. a) LAI of male and female plants in the different study populations (Data represent mean

357 ± SD of 10 individuals of each gender). Letters indicate population groups following Tukey test.

358 b) Average LAI values of 10 male and 10 female plants versus Emberger's aridity index of each

359 location. The regression line for each gender, R^2 and P are also indicated in the plot.

360

361 **3.2. Physiological measurements**

362 Water potential of male and female plants revealed significant differences between sites

363 in May and significant differences between sites, genders, and the interaction between

364 site and gender in September (Table 3).

365

366 Table 3. Two-way ANOVA of effects of site and gender on leaf water potential, F_v/F_m

367 (maximum quantum yield) and effective quantum yield in May and September (SS: Sum of

368 Squares; df: degrees of freedom).

			MAY			SEPTEMBER		
		df	SS	F	*P*	SS	F	*P*
Leaf Water Potential	Site	6	837.32	35.04	**<0.001**	4736.49	34.27	**<0.001**
	Gender	1	9.046	2.27	0.134	367.76	15.96	**<0.001**
	Site x gender	6	28.60	1.19	0.313	602.79	4.36	**0.001**
F_v/F_m	Site	6	0.06	15.74	**<0.001**	0.31	35.54	**<0.001**
	Gender	1	<0.01	0.60	0.439	<0.01	0.04	0.843
	Site x gender	6	0.02	3.91	**0.001**	<0.01	0.56	0.754

Effective Quantum Yield	Site	6	3.093	132.58	**<0.001**	1.093	16.29	**<0.001**
	Gender	1	0.002	0.54	0.462	0.010	0.93	0.335
	Site x gender	6	0.018	0.78	0.585	0.064	0.95	0.456

369

370   Regarding the sites, the Northern populations Camariñas, São Jacinto and

371   Caparica exhibited higher water potential values than those from the Southern

372   populations (p<0.05) in May; while in September the differences amplified, the

373   population of Camariñas exhibited the highest leaf water potential values, although

374   significant differences only existed among northern and southern populations (p<0.05),

375   (Fig. 3a).

376   In September, water potential was significantly correlated with MAP

377   ($R^2_{males}=0.86$; $R^2_{females}=0.67$), Emberger's aridity index ($R^2_{males}=0.87$; $R^2_{females}=0.69$) and

378   Lang's index ($R^2_{males}=0.84$; $R^2_{females}=0.65$), (p<0.05 in all cases), (Fig. 3b).

379   While in May female plants tended to exhibit lower water potential than males, in

380   September the opposite pattern was found, with male plants showing more negative

381   water potential values than females. However, significant differences between genders

382   were only found in September in El Asperillo.
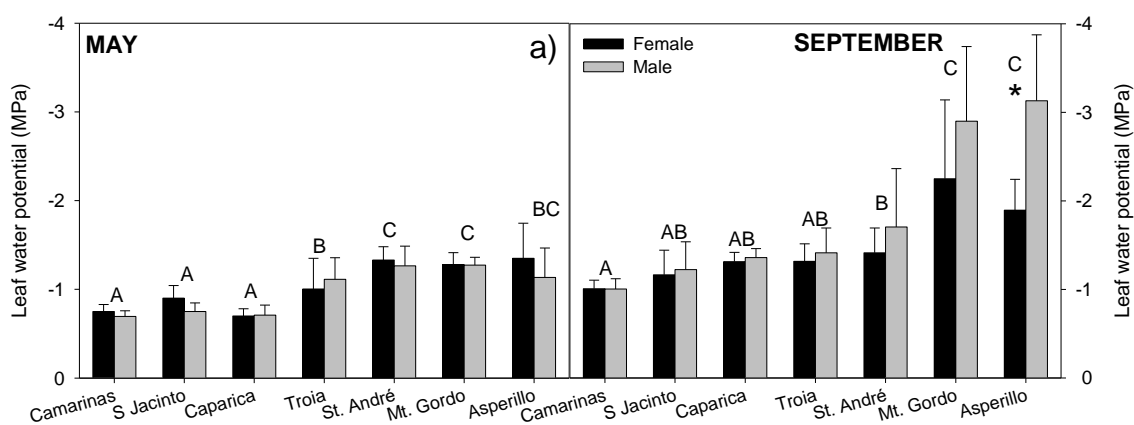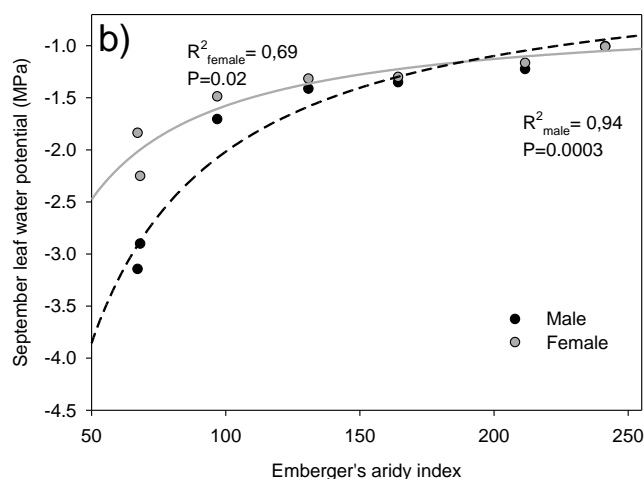
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396 Fig. 3. a) Leaf water potential of male and female plants in the different study populations

397 (spring and summer). Data represent mean ± SD of 10 individuals of each gender. Letters

398 indicate population groups following Tukey test and * the existence of gender significant

399 differences. b) September average leaf water potential values of 10 male and 10 female plants

400 versus Emberger's aridity index of each location. The regression line for each gender, R^2 and P

401 are also indicated in the plot.

402

403 Male-plants' leaf water potential values were progressively lower (more negative)

404 than female values as the mean summer rainfall diminished, as it is shown in the

405 ANOVA results (Fig. 3a and Table 3). It is remarkable to observe that gender

406 differences in water potential were associated with the decrease in water availability in

407 summer, and were progressively accentuated, as the site aridity is more extreme. Gender

408 dimorphism (measured as the difference between male and female leaf water potential

409 in September) was significantly correlated with summer rainfall (R^2=0.69, P=0.02) (Fig.
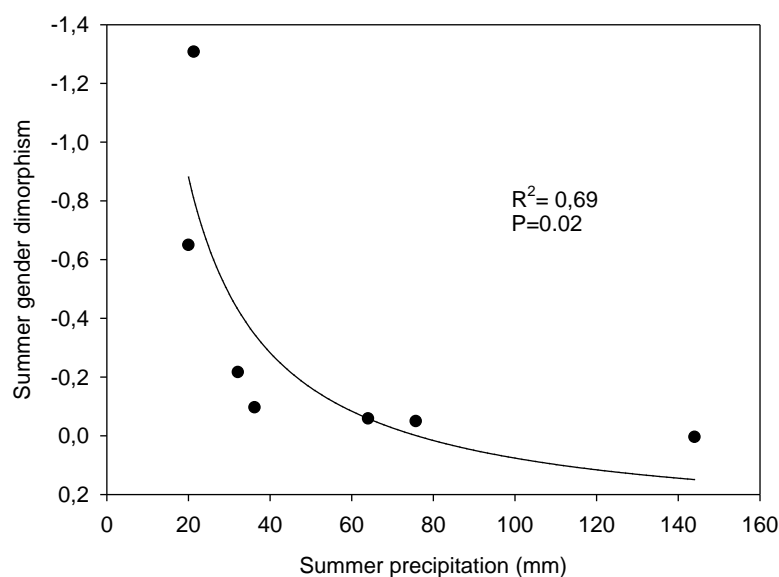
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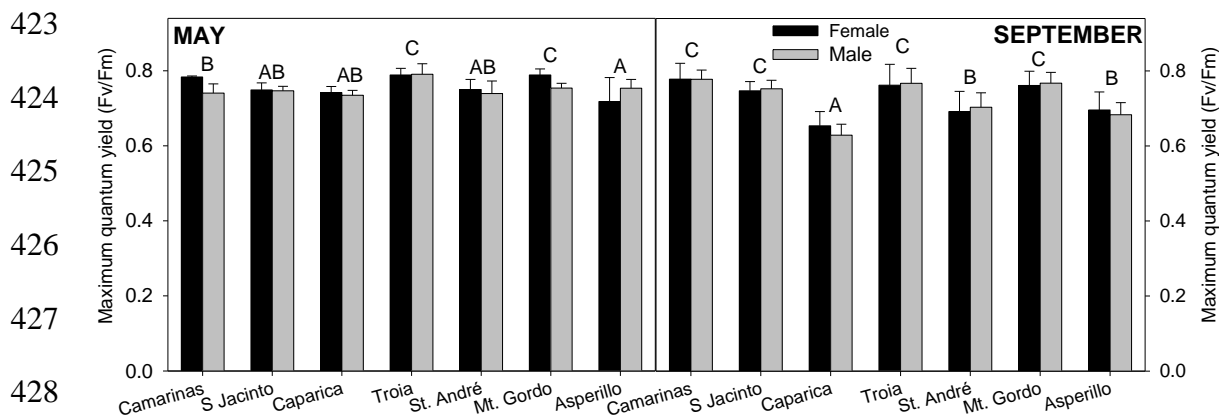411

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419   Fig. 4. Gender dimorphism in each location, measured as the average of 10 male minus 10

420   female leaf water potential in September versus summer rainfall (July, August and September).

421   The regression line, R^2 and *P* are also indicated in the plot.

422

423

428

429   Fig. 5. Maximum quantum yield (F_v/F_m) of male and female plants in the different study

430   populations (spring and summer). Data represent mean ± SD of 10 individuals of each gender.

431   Letters indicate population groups following Tukey test.

432

433   Maximum quantum yield (F_v/F_m) showed significant differences between northern

434   and southern populations either in May and September (Fig. 5). In both months there

435   were significant differences among sites and no differences between genders. The

436   interaction between site and gender was only significant in May (Table 3). The same

437   statistical results were obtained by eliminating from the analysis the northernmost

438   population (Camariñas, with considerably lower radiation conditions); there were

439   significant differences among sites in both months (*P*<0.001) and no differences

440   between genders (*P$_{May}$=0.555 and P$_{September}$=0,724*. Maximum quantum yield in

441   September approximately matched summer rainfall, but the relationship was not

442   significant (*P*=0.08 for males and 0.18 for females).

443   Effective quantum yield always exhibited lower values than optimal quantum

444   yield, showing a thermal dissipation of energy (Table S1). There were no significant

relations with any climatic variables reflecting the biogeographical gradient. In both

sampling periods, May and September, there were significant differences between sites,

but there were no differences between genders and the interaction site x gender was not

significant either (Table 3). In this case, also the same statistical results were obtained

when eliminating the population of Camariñas (significant differences among sites,

$P<0.001$, and no differences between genders, $P_{May}=0.696$ and $P_{September}=0,498$).

3.3. Elemental and stable isotope analysis

The values of $\Delta^{13}C$ were significantly higher in the northern populations although the

data did not follow the bioclimatic gradient (Fig. 6a). There were significant differences

among sites and genders; female values of $\Delta^{13}C$ were higher than male values, although

the interaction site x gender was not significant (Table 4). There was a significant

relationship ($R^2=0.644$, p=0.02) for female $\Delta^{13}C$ with rainfall of the growing season

(winter and spring), while this relationship did not occur in males (Fig. 6b).

Table 4. Two-way ANOVA of effects of site and gender on $\Delta^{13}C$, $\delta^{15}N$ and N content. (SS: Sum
of Squares; df: degrees of freedom;

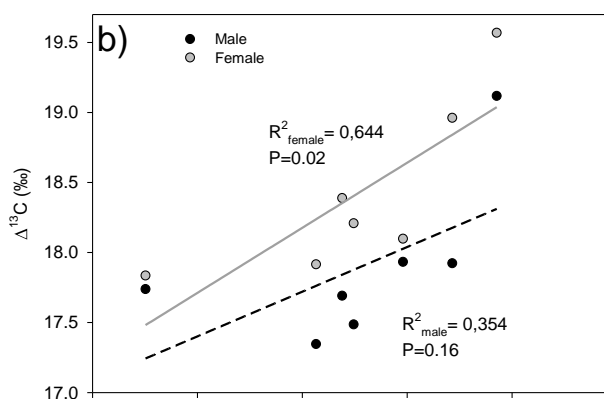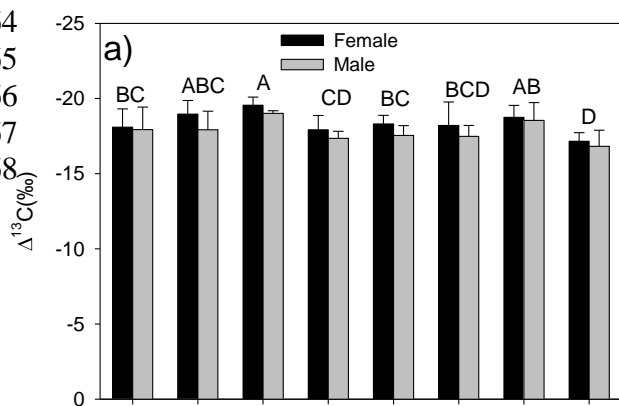|		df	SS	F	P
$\Delta^{13}C$	Site	7	67.513	10.46	**<0.001**
	Gender	1	11.718	12.72	**<0.001**
	SitexGender	7	3.175	0.49	0.839
$\delta^{15}N$	Site	4	323.28	30.55	**<0.001**
	Gender	1	0.625	0.23	0.628
	SitexGender	4	11.615	1.09	0.363
N content	Site	4	0.601	12.93	**<0.001**
	Gender	1	0.005	0.20	0.823
	SitexGender	4	0.027	0.57	0.682

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477 Fig. 6. a) Δ^{13}C values of male and female plants in the different study populations. Data

478 represent mean ± SD of ten individuals of each gender. Letters indicate population groups

479 following Tukey test. b) Average Δ^{13}C of 10 male and 10 female plants versus winter-spring

480 rainfall (January, February and March) of each location. The regression line for each gender, R^2

481 and *P* are also indicated in the plot.

482

483   Values of δ^{15}N were extremely negative in the arid populations (south) and

484 increase progressively to the north, with the exception of Monte Gordo population

485 (Table S1). There were significant differences among sites, but not between genders and

486 neither in the interaction between site and gender (Table 4). In relation to elemental

487 values, El Asperillo exhibited the highest values of N content in leaves (Table S1), there

488 were significant differences among sites but not for genders and the interaction between

489 site and gender was not significant either (Table 4).

490

491 **3.4. Responses to extreme drought events**

492 Comparing the values of leaf water potential in El Asperillo population, in the same

493 season among different years, we can observe the plant response to the inter-annual

494 variability of rainfall (Fig. 7).

495   There were no significant differences between 2002 and 2003, however, the

496 values of the dry year (2005) were significantly lower (*P*<0.05). Gender dimorphism

497 continued to maintain the same observed pattern, male plants presented significantly

498 more negative values than female plants for the three study years. Our results evidenced

499 that gender dimorphism did not increase with the drier conditions.
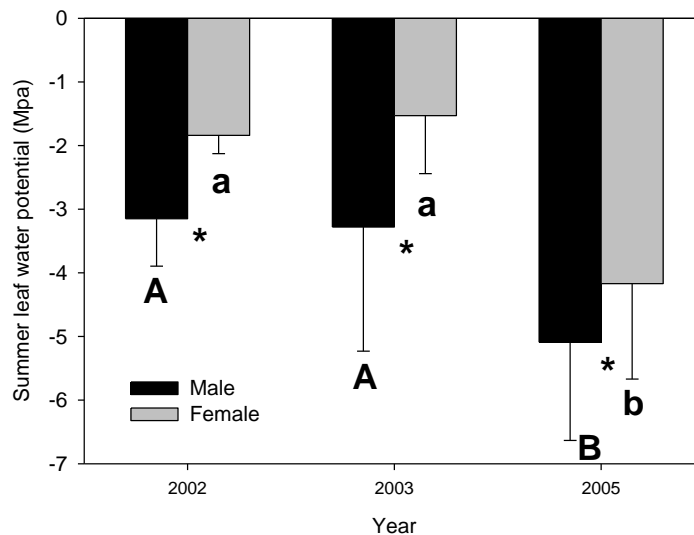
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501 Fig. 7. Comparison of mean values ± standard deviation leaf water potential of 10 male and 10

502 female plants among different years in El Asperillo. Data of September leaf water potential of

503 male and female plants in 2002 (rainfall 560.6 mm, summer maximum temperatures 36.5ºC),

504 2003 (549.5 mm, 41.5 ºC) and 2005 (173.0 mm, 39.5 ºC). Significant differences among

505 genders are indicated with an * ($P<0.05$) and among years with letters ($P<0.05$).

506

507 **4. Discussion**

508 Our results evidenced that populations of *Corema album* exhibited significant

509 differences in morphological and physiological attributes across the species'

510 biogeographical area, which are clearly correlated to climatic variables such as annual

511 rainfall and bioclimatic indices. Gender dimorphism was observed in $\Delta^{13}C$ and clearly

512 manifested in leaf water potential in summer in the most stressful end of the species

513 distribution area. In this study, we have shown how gender dimorphism on

514 physiological instantaneous and time-integrated variables changes over the complete

515   biogeographical area of a dioecious species in response to sub-optimal environmental

516   conditions.

517

4.1.Leaf area index (LAI)

519   In the literature, there are many studies in which male plants of dioecious species

520   usually exhibit higher performance in their aerial structure. For instance, in a review

521   article of sexual dimorphism in flowering plants, Barrett and Hough (2013) established

522   that in long-lived species, males often exceed females in vigour, shoot size, and in their

523   capacity for clonal propagation, although exceptions could occur (Sakai and Burris,

524   1985). In different species of *Leucadendron* the degree of serotiny (accumulating cones

525   for a longer period) was strongly associated with the degree of branching: females in

526   species with well-developed serotiny showed less ramification degree than males

527   (Harris and Pannell, 2010).

528   Accordingly with the results of this study, *C. album* plants did not show any

529   gender dimorphism in the aerial canopy, measured as LAI. This time-integrated index

530   only responded to the bioclimatic gradient, with no differences between male and

531   female plants. Previous results in *C. album* evidenced the existence of a sexual

532   segregation in branch elongation timing, associated to gender reproductive efforts

533   (Zunzunegui et al., 2006), but at the end of the growing season, the accumulated

534   elongation was the same in male and female plants.

535

4.2.Physiological responses

537   Reproductive costs result in physiological trade-offs in resource distributions, and these

538   can influence future vegetative growth and reproduction (Barrett and Hough, 2013). In

539   *C. album*, leaf water potential during the growth season (May) exhibited significant

540   differences among Northern and Southern populations as expected across a large

541   bioclimatic gradient. In some study sites, female plants tended to have slightly more

542   negative values than males, attending to trade-offs for fruit production, but differences

543   were not significant. Similar results were locally found by Álvarez-Cansino et al.

544   (2010b) and Zunzunegui et al. (2006) in a population in the South of Spain. However,

545   during the peak of the dry season in September, gender dimorphism increased from the

546   Northern to the Southern populations, in association to the summer rainfall decrease. In

547   contrast with our expectations of males performing better under drier conditions, due to

548   their relatively lower investment in reproduction (Obeso, 2002), females of *C. album*

549   showed significantly higher water potentials values than males at the extreme end of the

550   biogeographical area during the drought season, which might be associated to a better

551   hydrological status. This dimorphism is clearly manifested by the good correlation

552   found in this study between gender leaf water potential differences and summer rainfall

553   (Fig. 4).

554   In May all populations exhibited a fairly good physiological performance,

555   although there were significant differences for F_v/F_m for some populations. However, in

556   September the situation was different and Southern populations suffered a remarkable

557   physiological stress. In several populations, summer F_v/F_m showed values below the

558   theoretical optimum of 0.83 measured for most plant species (Maxwell and Johnson,

559   2000), indicating signs of photoinhibition of PSII (Correia and Ascensão, 2017).

560   Additionally, all studied populations of *C. album* presented values of Φ_{PSII} that

561   indicated stress to PSII via heat energy dissipation (Maxwell and Johnson, 2000), in

562   response to the midday increased radiation and VPD.

563   These results can be explained by a response to high solar radiation and

564   temperatures coupled with low water availability, amplified by sandy soils' properties

565   (similar results, analysing three populations, were found by Álvarez-Cansino et al.,

566   2012). The importance of local conditions was manifested through the significant

567   differences in F_v/F_m among populations. Yet, we found no signs of gender dimorphism

568   in the photosynthetic response. Other studies have found signs of higher photoinhibition

569   in female plants of dioecious species, associated to the reproductive period and drought

570   stress, as it has been reported in the dwarf palm *Chamaerops humilis* (Morales et al.,

571   2016) and in the subdioecious species *Honckenya peploides* (Sánchez-Vilas and

572   Retuerto, 2011). Or what is more, when comparing reproductive and non-reproductive

573   shoots of female *Pistacia lentiscus*, higher photoinhibition was observed in reproductive

574   shoots (Juvany et al., 2014).

575   In relation to water economy, females tended to show higher values of $\Delta^{13}C$,

576   which could be attributed to lower integrated WUE. Since females allocate a greater

577   investment to reproduction in order to produce an enormous amount of fleshy fruits

578   during the summer season (Díaz-Barradas et al., 2016; Zunzunegui et al., 2006), they

579   should have a less conservative water use response than males. Similar results have

580   been reported in other dioecious species such as *Acer negundo* (Dawson and Ehleringer,

581   1993) and *Ilex aquifolium* (Retuerto et al., 2000). Moreover, populations' $\Delta^{13}C$ values

582   were positively correlated to winter-spring rainfall in females when most of the leaf

583   biomass is produced. This result can be interpreted as a sign of plant response to water

584   availability during the period of leaf biomass production.

585   Although *Corema album* is a wind-pollinated plant, with extremely large pollen

586   production during the flowering period, which requires a high investment in nitrogen-

587   rich pollen (Delph et al., 1993; Harris and Pannell, 2008), we were not able to find any

588   gender dimorphism in nitrogen content in leaves and nitrogen sources, measured

589   through $\delta^{15}N$. However, $\delta^{15}N$ values decreased from the southern to the northern

590   populations, which indicate shifts in nitrogen sources associated with the precipitation

591   regime.

592   The results of this study evidence that females seem to present lower water use

593   efficiency in leaves, and on the other hand better water status during summer at the drier

594   end of the species' biogeographical area. The explanation for this apparent paradox of

595   females presenting a better physiological status than males during the summer season

596   could be explained by the implementation of finely integrated morphological or

597   physiological compensatory mechanisms, as it has been described in dioecious plants by

598   other authors (Álvarez-Cansino et al., 2010b; Obeso, 2002; Tozawa et al., 2009).

599   In a previous local study, through the analysis of water stable isotopes, it was

600   found that females of *C. album* should have deeper roots than males conferring them

601   potential higher water availability during the drought period (Álvarez-Cansino et al.,

602   2010b). On the other hand, this result has been supported by a reproductive effort

603   experiment, in which female plants continued to exhibit higher vegetative growth

604   during the second year after reproductive effort had been removed, which evidenced the

605   idea of higher female-storing capacity in underground structures (Álvarez-Cansino et

606   al., 2010a). Compensatory mechanisms favouring water capture in female individuals is

607   a logical explanation for the enhanced water status found in females in relation to males

608   during the dry period (Álvarez-Cansino et al., 2010b) and could ultimately explain the

609   lack of biased sex ratio among the species distribution area, despite a 3-fold higher

610   reproductive effort in females due to fruit production (Álvarez-Cansino et al., 2013;

611   Martins et al., 2017; Zunzunegui et al., 2006). Having a larger root system, females

612   would increase both nutrient and water uptake and as a consequence, they might

613   improve C acquisition. Gender-specific differences in biomass allocation have been

614   found in the subdioecious plant *Honckenya peploides*, in which females had greater

belowground biomass than males (Sánchez-Vilas et al., 2012). In Northern populations of *C. album*, where water and nutrients don't represent a limited resource, plants probably do not present such extreme compensatory mechanisms and sexual dimorphism was not so evident, as occurred in other dioecious species that only exhibit gender dimorphism under harsher conditions (Kohorn, 1994; Wallace and Rundel, 1979).

In extremely dry years, plants suffer a dramatic water stress and a higher variability among individuals within a single population, which has been manifested by our results in the extreme southern end of plant biogeographical area. If this situation continued over a longer period, it would probably affect population survival and limit its distribution area, as it has been proposed in different models of climate change (Hultine et al. 2018). In dioecious species such as *C. album* one gender may be differentially affected under sub-optimal environmental conditions of its biogeographical area. For slow-growing, long-lived species as *C. album*, global change represents an array of opposing selective pressures which at long-time scales may affect the ecological limits of phenotypic plasticity, decoupling climate from local adaptations and leading to an increase in vulnerability and a higher risk of mortality, which can be differential between genders under this new climatic scenarios (Munné-Bosch, 2015; Valladares et al., 2007).

Since dioecious plants, as all plants, present hierarchical organization, and allocation to reproduction is affected by modularity, it is important to consider several levels of the modular hierarchy for the analysis of the cost of reproduction under different environmental conditions (Juvany and Munné-Bosch, 2015; Obeso, 1997).In our study, we analysed different levels of plant organization, from instantaneous photochemical efficiency, to time-integrated water-use efficiency through $\Delta^{13}C$ and

640 plant canopy performance as LAI. Our results evidenced the existence of sexual

641 dimorphism in leaf water potential, and are supported by other previous data (Álvarez-

642 Cansino et al., 2012; Álvarez-Cansino et al., 2010a), that point out to the existence of

643 sexual dimorphism located at underground level.

644   In the literature, Spatial Sexual Segregation (SSS) has been reported in >30

645 dioecious species from 20 families, and in the vast majority of cases male-biased sex

646 ratios are reported in more stressful sites (Bierzychudek and Eckhart, 1988; Mercer and

647 Eppley, 2010). The general model of dioecious plants proposes that SSS occurs by

648 females living in better microsites; in *C*. SSS was not found in any location of the

649 species' biogeographical area (Álvarez-Cansino et al., 2013; Martins et al., 2017). The

650 results of this study, together with previous evidence (Álvarez-Cansino et al., 2010;

651 Sánchez-Vilas et al., 2012, Hultine et al., 2018) suggest that SSS might exist at the

652 underground level, where females can exploit deeper soil profiles, especially in the

653 extreme end of the species' biogeographical area. With this mechanism, they could

654 maintain the same canopy performance than males, lower integrated WUE and an

655 extraordinary production of fleshy fruits during the summer period that can reach more

656 than 7000 berries m^{-2} plant (Díaz-Barradas et al., 2016). Our results are therefore

657 consistent with a higher efficiency in resource use in females than in males of *C. album*

658 (Álvarez-Cansino et al., 2012; Álvarez-Cansino et al., 2010a).

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665

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841   Supplementary content

842   Table S1. Average and ± standard deviation of the experimental data in all study populations of
843   *Corema album* for: leaf area index (LAI, $m^2 m^{-2}$), maximum quantum yield (F_v/F_m), effective
844   quantum yield (Φ_{PSII}) and leaf water potential (Ψ, MPa), in May and September. Leaf carbon
845   isotope discrimination ($\Delta^{13}C$, ‰), leaf nitrogen isotope ratio ($\delta^{15}N$, ‰) leaf nitrogen content
846   (%).

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		LAI	Fv/Fm May	Φ_{PSII} May	Fv/Fm Sept	Φ_{PSII} Sept	Ψ Pa May	Ψ MPa Sept	$\Delta^{13}C$‰	$\delta^{15}N$‰	N %
Camariñas	M	3.96	0.74	0.55	0.78	0.44	-0.70	-1.00	17.93	-5.51	0.59
		±0.65	±0.025	±0.059	±0.024	±0.119	±0.064	±0.120	±1.50	±1.93	±0.12
	F	3.51	0.78	0.60	0.78	0.46	-0.75	-1.01	18.10	-4.16	0.59
		±0.70	±0.003	±0.092	±0.042	±0.15	±0.077	±0.097	±1.21	±2.11	±0.18
S. Jacinto	M	3.25	0.75	0.62	0.75	0.26	-0.75	-1.22	17.92	-7.32	0.62
		±0.83	±0.011	±0.072	±0.023	±0.083	±0.097	±0.314	±1.24	±1.00	±0.08
	F	3.04	0.75	0.64	0.75	0.21	-0.90	-1.17	18.96	-7.69	0.59
		±0.73	±0.019	±0.060	±0.024	±0.067	±0.142	±0.277	±0.908	±1.24	±0.12
Osso Baleia	M		0.79	0.55	0.77	0.32		-1.36	19.01		
			±0.013	±0.085	±0.029	±0.18		±0.101	±0.177		
	F		0.79	0.55	0.76	0.25		-1.31	19.56		
			±0.016	±0.048	±0.038	±0.11		±0.104	±0.54		
Caparica	M	2.75	0.74	0.31	0.63	0.16	-0.71	-1.41	17.35	-9.42	0.56
		±0.49	±0.028	±0.07	±0.040	±0.051	±0.112	±0.281	±0.470	±0.79	±0.06
	F	2.68	0.74	0.28	0.65	0.17	-0.70	-1.32	17.92	-9.07	0.62
		±0.42	±0.018	±0.051	±0.056	±0032	±0.081	±0.198	±0.960	±1.16	0.09
Troia	M	2.11					-1.11		17.55		
		±0.42					±0.24		±0.646		
	F	2.01					-1.10		18.31		
		±0.53					±0.35		±0.575		
S. André	M	2.01	0.74	0.27	0.70	0.21	-1.27	-1.70	17.49		
		±0.68	±0.034	±0.051	±0.038	±0.069	±0.222	±0.660	±0.719		
	F	2.36	0.75	0.28	0.69	0.21	-1.33	-1.41	18.21		
		±0.69	±0.027	±0.077	±0.054	±0.042	±0.151	±0.281	±1.56		
Monte Gordo	M	1.63	0.79	0.55	0.77	0.55	-1.27	-2.90	18.55	-5.51	0.71
		±0.37	±0.013	±0.048	±0.029	±0.048	±0.088	±0.842	±1.18	±2.07	±0.06
	F	1.56	0.79	0.55	0.76	0.55	-1.28	-2.25	18.74	-4.95	0.68
		±0.28	±0.016	±0.047	±0.038	±0.045	±0.133	±0.890	±0.803	±2.17	±0.12
Asperillo	M	1.97	0.75	0.26	0.68	0.18	-1.14	-3.13	16.82	-9.17	0.84
		±0.26	±0.024	±0.042	±0.033	±0.040	±0.331	±0.745	±1.070	±1.60	±0.09
	F	1.80	0.72	0.26	0.70	0.22	-1.114	-1.90	17.15	-9.12	0.83
		±0.33	±0.064	±0.041	±0.048	±0.056	±0.394	±0.350	±0.573	±1.23	±0.06

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850 **Highlights**

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852   1. *Corema album* populations respond to the climatic gradient of the species'
853   biogeographical area.

854   2. Gender dimorphism is only recognized in $\Delta^{13}C$ and in leaf water potential in the
855   dry season.

856   3. Female plants have lower integrated water use efficiencies to maintain fruit
857   production.

858   4. Leaf water potential is more negative in male plants (September in southern
859   populations).

860   5. Females might have deeper roots than males as a compensatory mechanism,
861   presenting underground SSS.

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