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## Research paper

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

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

**Keywords:** climate change, drought, limited-range species, phenotypic plasticity, stomatal conductance, water-use efficiency.

## Introduction

Species ranges are dynamic (Gaston 2003), with climate playing a major role in limiting terrestrial species' distribution (Parmesan et al. 1999, Wilson et al. 2007). Major shifts in species distribution can be expected considering the climatic changes currently forecast (Christensen 2007). In fact, many animal species have suffered a reduction in their distributional range due to recent climate change events, increasing their risk of extinction in the near future (Ainley et al. 2003, Derocher 2005, Wilson et al. 2005). Evidence for plants is increasing

(Lenoir et al. 2008, Lenoir and Svenning 2013) but is mainly centered on shifts of species ranges along altitudinal gradients (Jump et al. 2009). Endemic and relict plant species are especially vulnerable to environmental changes, because they have small population sizes, which are exposed to increased inbreeding, lower individual fitness and lower genetic variability (Ellstrand and Elam 1993, Aizen et al. 2002, Angeloni et al. 2011). Furthermore, species of limited distribution have potentially less evolutionary capacity to respond to climate change as they are exposed to only a small range of

environmental heterogeneity. Thus, small changes in temperature and precipitation patterns could affect their growth and survival to a larger extent than those of their more common and widespread counterparts (Gulías et al. 2002, 2003).

However, even species with limited range are able to evolve and adapt to changing environments at expanding range margins (Parmesan 2006). These range expansions and changes in resource use have been attributed to a large extent to 'phenotypic plasticity', the ability of a single genotype to alter its phenotype in response to environmental conditions (Pigliucci 2001). Plasticity may have important adaptative effects, mitigating for instance the negative impacts of a drier climate (Callaway et al. 2003, Valladares et al. 2007, Couso and Fernandez 2012). Despite the plasticity of each individual, populations may not be able to keep pace with the rate of environmental change and therefore might still be vulnerable to extinction (Nussey et al. 2005, Bradshaw and Holzapfel 2006).

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

We have studied two Mediterranean shrubs of limited distribution and for which the mechanisms of water strategy are still poorly known: *Cneorum tricoccon* L. (Cneoraceae) and *Rhamnus ludovici-salvatoris* Chodat. (Rhamnaceae). The former species is part of a relict flora that evolved during the early Tertiary under tropical conditions (Raven 1973). It has been categorized as vulnerable (International Union for Conservation of Nature), with human activities and urban sprawl described as the main

historical threats for the species. *Rhamnus ludovici-salvatoris* is an endemic species to the Balearic Islands, whose distribution has also diminished, with recent extinctions of several populations (Gulías et al. 2002, Traveset et al. 2003). *Rhamnus ludovici-salvatoris*' decline is mainly due to deforestation, but climatic changes occurring in the region during the Holocene have also been partly responsible (Postigo et al. 2009). Furthermore, many current populations of *C. tricoccon* and *R. ludovici-salvatoris* are characterized by a low proportion of seedlings and saplings, suggesting low establishment success (Traveset et al. 2003, 2012). We assessed the interpopulation and ecophysiological variability along an aridity gradient in the water strategy of these two species in order to estimate their vulnerability to increased aridity such as that expected for the region (Christensen 2007). Sampling was conducted at sites where both species were present along a wide aridity gradient during 2 years of contrasting precipitation levels and temperatures.

Our specific hypotheses were the following. (i) Populations of both species at their limit of aridity show worse performance and lower survival than others established on more mesic sites, as suggested by studies on the rear distribution edge of plants in Mediterranean zones (Hampe and Petit 2005, Jump et al. 2006, Linares and Tiscar 2010) and by previous works on these species (Gulías et al. 2003, 2004, Traveset et al. 2003, Varone et al. 2012). (ii) The two co-occurring species differ in their water strategy (Gulías et al. 2009), which may result in a differential vulnerability to ongoing climate change. We argue that isohydric species can cope with acute but not prolonged droughts, while the reverse is true for anisohydric plants, which has implications for their performance in future climatic scenarios. (iii) Lastly, we hypothesize that phenotypic plasticity can attenuate the impacts of a drier climate (Callaway et al. 2003, Gianoli and Gonzalez-Teuber 2005, Valladares et al. 2007) and, therefore, species with a more plastic water strategy across sites and over time should perform better under the driest conditions.

## Materials and methods

### Study species

*Cneorum tricoccon* L. (Cneoraceae) is a small (usually <1 m tall) perennial shrub, which inhabits the western Mediterranean area (Balearic Islands, some localities in the eastern and southern Iberian Peninsula, south-eastern France, Sardinia, Giannutri and Tuscany). *Cneorum tricoccon* represents a relict species of the Tertiary (Raven 1973); currently, it is found usually within the coastal maquis and more rarely within holm oak (*Quercus ilex*) forests. *Cneorum tricoccon* is an andromonoecious, insect-pollinated species (Traveset 1995a). Seed dispersal is mediated by endemic lizards (*Podarcis lilfordi*, *Podarcis pityusensis* and *Podarcis siculus*), and by introduced carnivorous mammals

(*Martes martes* and *Genetta genetta*) when lizards are extinct (Traveset 1995b, Celedón-Neghme et al. 2013).

*Rhamnus ludovici-salvatoris* Chodat. (Rhamnaceae) is an up to 2 m high perennial shrub endemic to the Balearic Islands (Mallorca, Menorca and Cabrera). It occurs from sea level up to 1200 m above sea level (a.s.l.), usually in holm oak forests, and more rarely in dry garrigues. *Rhamnus ludovici-salvatoris* is a dioecious, insect-pollinated species and seed dispersal is mediated by two frugivorous birds: *Turdus merula* and *Sylvia melanocephala* (Traveset et al. 2003, Gúñas and Traveset 2012). For both species the period of leaf development and shoot elongation occurs in typically favourable periods of spring (April–June) and early autumn (September and October).

### Study sites

The study was performed in three locations that cover a pronounced climatic gradient (precipitation and temperature) on the Balearic Islands, West Mediterranean Basin. The three locations ranged from a maximum mean annual precipitation of 1224 mm to a minimum of 391 mm (Table 1, Figure 1). We have calculated Emberger's (1955) index of aridity ( $Q$ ) as  $Q = (2000 \times P) / (T_{\max}^2 - T_{\min}^2)$ , where  $P$  is the mean annual precipitation,  $T_{\max}$  is the maximum annual temperature and  $T_{\min}$  is the minimum annual temperature. Following Traveset et al. (2012), values of Emberger's index were logarithmically transformed for statistical analyses, because  $Q$  increases nonlinearly in more mesic habitats. Selected populations met the following criteria: (i) the population was large and well established, with >50 individuals; (ii) the site was not managed; (iii) the population did

not adjoin highly disturbed areas; and (iv) the population was separated from other sampled populations by at least 40 km.

For abiotic and biotic characterization of study sites we measured the slope and visually estimated the percentage of bare soil, cover of rocks, woody plants and herbs on 10 randomly selected transects (2 × 10 m) per population (see Table 1 for study sites characterization). With a portable climatic station (SIL451, SILVA, Bromma, Sweden) we measured temperature, photosynthetically active radiation (PAR), humidity, vapour pressure density and wind speed at population level at the time of measurements. We also measured PAR at the microhabitat level (0.5 m from every sampled individual) with a portable PAR leaf-clip (FMS2, Hansatech, Norfolk, UK).

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

### Morphological, ecophysiological and fitness surrogate measurements

Maximum height, crown and trunk diameter were measured in 68–108 adult plants of each species in each population (*C. tricocon*  $n = 280$  and *R. ludovici-salvatoris*  $n = 247$ ). To ensure sampled individuals were adult, we selected reproductive individuals.

Table 1. Descriptive variables of the study sites.

	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
$P_{\text{mean}}$ (mm)	1224.5	499.8	391.7
$T_{\text{max}}$ (°C)	35.27	35.63	34.37
$T_{\text{min}}$ (°C)	−3.77	−0.01	2.12
Emberger 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)
At the time of measurement			
$T$ (°C)	25.68 (1.55)	25.13 (1.39)	29.55 (1.28)
PAR ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	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)
VPD (hPa)	19.76 (2.73)	19.88 (3.18)	17.52 (2.44)
Wind speed ( $\text{km h}^{-1}$ )	0.52 (0.91)	0.09 (0.39)	3.79 (1.78)

Standard deviations are shown in brackets. LLUC, Lluc; BANY, Banyalbufar; CABR, Cabrera;  $P_{\text{mean}}$ , mean annual precipitation;  $T_{\text{max}}$ , maximum annual temperature;  $T_{\text{min}}$ , minimum annual temperature; Emberger's index of aridity logarithmically transformed (see text for index details);  $T$ , temperature; PAR, photosynthetically active radiation; VPD, vapour pressure density. Climatic data were obtained from the closest weather station of the Spanish Meteorological Agency (AEMET), series 1980–2010.

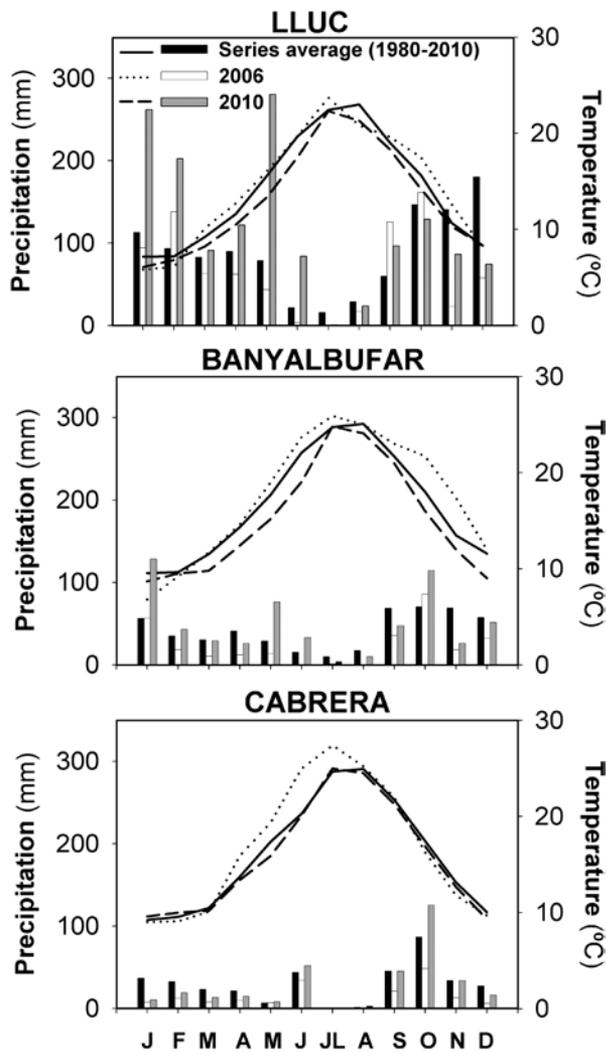


Figure 1. Mean climatic features of the three study sites. Columns and lines represent monthly mean precipitation and temperature, respectively, for the period 1980–2010 and in the two study years (2006 and 2010). Data were obtained from the closest weather station of the Spanish Meteorological Agency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

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

Ecophysiological measurements were taken in the same 15 individuals per population ( $n = 60$  in each species) in two climatically contrasting years, 2006 and 2010. According to the reports of the Spanish Meteorological Agency, 2006 was an extremely hot year in the Balearic Islands (temperatures exceeded the maximum value for the reference period 1971–2010) and was also very dry (i.e., 2006 was among the 20% driest years of the period) while 2010 was mild (temperatures

were around the median) and also very wet (i.e., 2010 was among the 20% wettest years of the period). In 2006, data were obtained before (May) and during the summer drought (July), whereas in 2010 measurements were made during summer drought (July). Selected individuals for ecophysiological measurements met the following criteria: (i) neighbour plants grew at least 3 m apart to minimize intense competition for resources; (ii) individuals had similar height and crown diameters (around the mean for each population) to reduce plant size as a confounding factor; (iii) sampled individuals grew in habitats with similar irradiances, specifically with PAR during the central hours of the day in the range of 900–1400  $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ . In *R. ludovici-salvatoris* both male and female individuals were measured to explore any influence of gender on performance. Four different ecophysiological assessments were taken: (i) stomatal conductance ( $g_s$ ) was measured with a leaf porometer (SC-1, Decagon Devices, Pullman, WA, USA) from 10:00 to 12:00 GMT in four fully expanded leaves per plant from the upper parts of the canopy; (ii) maximal photochemical efficiency of photosystem II ( $F_v/F_m$ ) was measured with a portable pulse-modulated fluorometer (FMS2, Hansatech, Norfolk, UK) from 09:00 to 12:00 GMT in three healthy leaves dark-adapted for 30 min with leaf clips (minimal ( $F_o$ ) and maximal ( $F_m$ ) fluorescence were measured, and these values were used to calculate  $F_v/F_m$  as  $F_v/F_m M(F_m - F_o)/F_m$  (Maxwell and Johnson 2000)); (iii) chlorophyll concentration (Chl) was measured with a portable SPAD (Minolta, Japan) in five fully expanded leaves per plant; and (iv) carbon isotope ratio ( $\delta^{13}\text{C}$ ), for which two current-year leaves from the upper and most exposed part of the crown were collected, dried and ground; this material was analyzed with a Finnigan MAT Delta C isotope ratio mass spectrometer at Stable Isotope Facility on University of California, Davis. The  $\delta^{13}\text{C}$  is an index of intrinsic water-use efficiency (WUE) integrated over time of plant organ growth (Dawson et al. 2002, McCarroll and Loader 2004). All ecophysiological measurements were taken in 15 individuals per population, except in Cabrera, where 30 individuals were sampled ( $n = 60$  for each species).

The fruit crop was examined in 2006 as a surrogate of fitness in the three populations. For *C. tricocon*, fully developed fruits were observed from January to June, with a peak at the end of May (Traveset 1995a), whereas for *R. ludovici-salvatoris*, full-sized fruit occurs about June (Traveset et al. 2003). For this reason, we examined the fruit crop of both species in May, including fruits at different stages of development. Owing to the phenology of each population we started with the driest populations and ended with the mesic ones in order to cover the range of fruit peak of each population.

### Statistical analysis

We performed two-way ANOVAs to test for differences among populations (Po, 2 df) and species (Sp, 1 df) in height, crown and trunk diameter, and leaf angle. A mixed-model ANCOVA

was used to test for differences among populations (Po, 2 df), seasons (Se, 1 df) and species (Sp, 1 df) in  $g_s$ , Fv/Fm, Chl and  $\delta^{13}\text{C}$ , considering PAR as a covariate. The model included Po, Se and Sp as main effects and all possible interactions between them. We used a three-way ANOVA to test the effects of population (Po, 2 df), year (Ye, 1 df) and species (Sp, 1 df) in  $g_s$  and Fv/Fm. For *R. ludovici-salvatoris* we performed two-way ANOVAs to test for gender, population differences and their interaction. As a post hoc test, we used Fisher's least significant difference (LSD) in all cases. Normality and homogeneity of variance of the dependent variables were tested prior to analyses by means of the Kolmogorov–Smirnov and the Levene's test, respectively. All statistical analyses were performed using Statistica 6.0 (StatSoft Inc., Tulsa, OK, USA).

We performed structural equation models (SEMs) to test not only the direct influence of abiotic factors on morphological, ecophysiological and fitness variables, but also the indirect effects in an aprioristic model where the causal relationships between our variables were explicitly included (Shiple 2002, Iriondo et al. 2003, Milla et al. 2009). We built independent models for *C. tricocon* and *R. ludovici-salvatoris*, since we hypothesized that causal relationships among variables differ between the two species. In addition, we built another two models considering ecophysiological differences before vs during summer drought, since these seasonal differences could explain interpopulation fitness variability. Since the sample sizes were relatively small ( $n = 95$  in the best case), we limited the number of predictors included in the model as much as possible as recommended by Shiple (2002). We selected two variables to characterize the abiotic environment: light, as the mean PAR measured at the microsite level, and aridity, calculated as the aridity Emberger's index (see above for index details); two ecophysiological variables, WUE and Fv/Fm; one morphological trait, height; and fruit crop as a fitness proxy. Standardized path coefficients were estimated using the maximum likelihood algorithm (Shiple 2002). The degree of fit between the observed and expected covariance structures was assessed by root mean square error of approximation (RMSEA) statistics (Steiger 1990). RMSEA values  $< 0.08$  show a good fit, between 0.08 to 0.10 provide a mediocre fit and above 0.10 indicate a poor fit (MacCallum et al. 1996). Moreover, model fit to the data was also evaluated by the goodness-of-fit index (GFI) and the Bentler and Bonett's normed-fit index (NFI). Values of GFI and NFI range between 0 and 1, and values  $> 0.9$  indicate an acceptable fit of the model to the data (Iriondo et al. 2003). Analyses were performed using AMOS 5.0 (Arbuckle 1994).

## Results

We found significant differences between species and populations for height, crown and trunk diameters (Table 2). *Cneorum*

Table 2. Effects of population (Po), species (Sp) and their interaction (Po  $\times$  Sp) on height, crown and trunk diameters, fruit crop and leaf angles.

	Two-way ANOVA		
	DF	F	P
Height			
Po	2	19.86	<b>&lt;0.001</b>
Sp	1	209.73	<b>&lt;0.001</b>
Po $\times$ Sp	2	5.48	<b>0.004</b>
Crown diameters			
Po	2	32.36	<b>&lt;0.001</b>
Sp	1	105.77	<b>&lt;0.001</b>
Po $\times$ Sp	2	10.33	<b>&lt;0.001</b>
Trunk diameters			
Po	2	65.32	<b>&lt;0.001</b>
Sp	1	960.46	<b>&lt;0.001</b>
Po $\times$ Sp	2	57.24	<b>&lt;0.001</b>
Fruit crop			
Po	2	4.82	<b>0.011</b>
Sp	1	9.51	<b>0.003</b>
Po $\times$ Sp	2	4.88	<b>0.010</b>
Leaf angles			
Po	2	2.52	0.082
Sp	1	9.88	<b>0.002</b>
Po $\times$ Sp	2	1.00	0.369

Significant effects ( $P < 0.05$ ) are indicated in bold.

*tricocon* was taller and had larger crown diameters in more mesic and colder sites. This pattern was not as clear in *R. ludovici-salvatoris* for height and trunk diameters, although the xeric population had plants with smaller crowns (Figure 2). The two species showed larger fruit crops in more mesic and colder populations (Table 2, Figure 3).

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

Differences in  $g_s$ ,  $\delta^{13}\text{C}$ , Fv/Fm and Chl were also found between species, among populations and between seasons, with significant interactions among these factors (Table 3). Before drought, *C. tricocon* showed higher  $g_s$  in the most mesic population, whereas it showed very low values of  $g_s$  during drought in all populations (Figure 5a). In contrast, *R. ludovici-salvatoris* showed higher  $g_s$  before drought in the most mesic populations and lower  $g_s$  in the most xeric one. Stomatal conductance of *R. ludovici-salvatoris* decreased during drought and the pattern along the mesic to xeric gradient was not found. In all cases, values for this species were always higher than those for *C. tricocon* (Figure 5b). For both species, there was a significant interaction between population and season in  $g_s$  (Table 3), with a consistently greater decrease before vs during drought in Lluc, the most mesic site (Figure 5a and b).

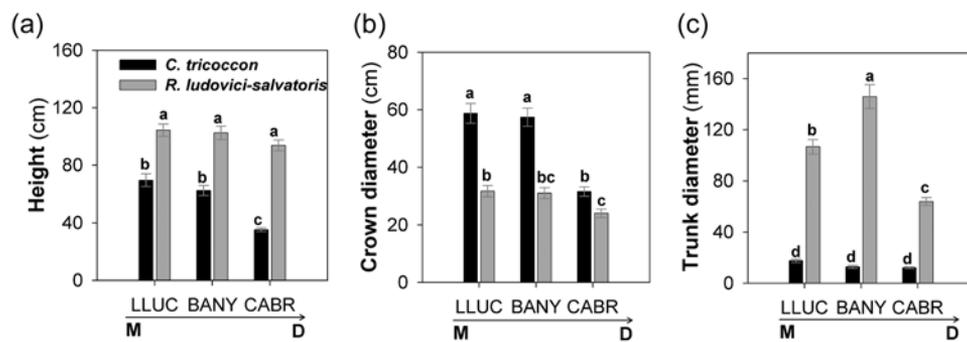


Figure 2. Differences in height (a), crown diameter (b) and trunk diameter (c) between populations of *C. tricocon* and *R. ludovici-salvatoris* along an aridity gradient. Values are mean  $\pm$  SE. Different letters indicate significant differences after Fisher's LSD test.  $N = 68$ – $108$  for each species and population. M, mesic; D, dry; study sites' codes are given in Table 1.

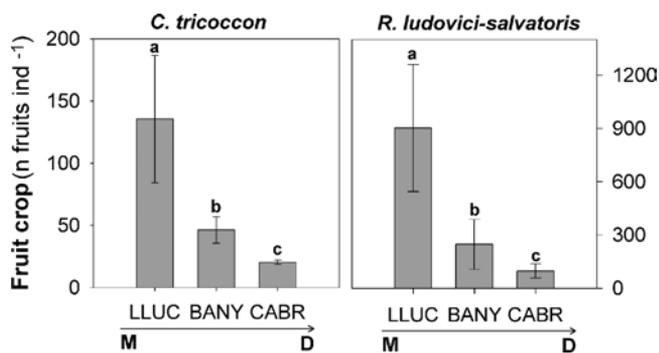


Figure 3. Differences in fruit crop among populations of *C. tricocon* and *R. ludovici-salvatoris*. Values are given as mean  $\pm$  SE. Different letters indicate significant differences after Fisher's LSD test.  $N = 15$ – $30$  for each species and population. M, mesic; D, dry; study sites' codes are given in Table 1.

The  $\delta^{13}\text{C}$  of leaves differed significantly between species, among populations and depending on the season (Table 3). Before drought, *C. tricocon* showed significantly lower  $\delta^{13}\text{C}$  values in the most mesic populations (Figure 5c), suggesting lower WUE in them compared with the xeric population. During drought,  $\delta^{13}\text{C}$  increased in all populations and the most xeric population showed significantly higher  $\delta^{13}\text{C}$ , suggesting higher WUE (Figure 5c). *Rhamnus ludovici-salvatoris* showed lower  $\delta^{13}\text{C}$  values than *C. tricocon*; however, it showed increased  $\delta^{13}\text{C}$  values, i.e., WUE, at the most xeric site. *Rhamnus ludovici-salvatoris* showed also significantly lower  $\delta^{13}\text{C}$  values before drought in the mesic populations; during drought,  $\delta^{13}\text{C}$  increased significantly in mesic populations, whereas it remained very similar in the xeric population (Figure 5d). For both species, the significant population  $\times$  season interaction in  $\delta^{13}\text{C}$  (Table 3) indicated that the increase in  $\delta^{13}\text{C}$  between seasons was different for the three populations of each species (Figure 5c and d).

The Fv/Fm also differed significantly between species, populations and seasons (Table 3). Before drought, Fv/Fm for *C. tricocon* was significantly higher in mesic populations than in the most xeric one. During drought, values decreased significantly

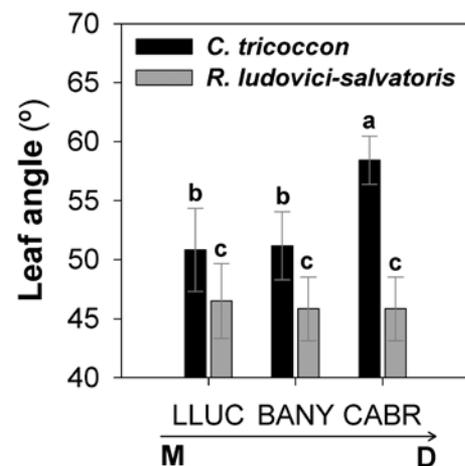


Figure 4. Leaf angle differences between populations of *C. tricocon* and *R. ludovici-salvatoris*. Values are mean  $\pm$  SE. Different letters indicate significant differences after Fisher's LSD test.  $N = 15$ – $30$  for each species and population. M, mesic; D, dry; study sites' codes are given in Table 1.

in all populations, reaching lowest values in the driest population (Figure 5e). *Rhamnus ludovici-salvatoris* kept high Fv/Fm before drought in all populations, with higher values for mesic populations. During drought, Fv/Fm decreased and the pattern along the mesic to xeric gradient was no longer found; these values were always higher than those of *C. tricocon* (Figure 5f). There was a significant population  $\times$  season interaction for both species (Table 3); the decrease of Fv/Fm values before vs during drought was consistently greater in Lluc and Cabrera (Figure 5e and f).

Chlorophyll concentration differed significantly between species and among populations (Table 3). Before drought, Chl for both species was higher in mesic populations than in the xeric one. During drought, Chl decreased in all populations, with again higher values in the mesic than in the xeric ones. Chl values in *R. ludovici-salvatoris* were always higher than those of *C. tricocon*. We found greater differences in Chl among populations in *C. tricocon* than in *R. ludovici-salvatoris*,

Table 3. Effects of population (Po), season (Se) and species (Sp) and their interactions on ecophysiological traits.

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

PAR was included as a covariate. Significant effects ( $P < 0.05$ ) are indicated in bold.

indicated by the significant population × species interaction (Table 3).

When comparing xeric and mesic years, we found significant differences in  $g_s$  and Fv/Fm (Table 4), with lower  $g_s$  in the dry than in the mesic years for both species (Figure 6a and b). There was also a significant population × year interaction, with greater differences between years on mesic populations. The population × year × species interaction was also significant, as there were higher population-year differences in *R. ludovici-salvatoris* than in *C. tricocon*. Higher values of Fv/Fm were found on the mesic year for both species (Figure 6c and d), and a significant population × year interaction also affected Fv/Fm: *C. tricocon* showed greater Fv/Fm differences between years on the mesic populations, while this pattern was not evidenced for *R. ludovici-salvatoris*. Still, there was a significant year × species interaction, with greater differences between years in *C. tricocon* than in *R. ludovici-salvatoris*.

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

The four proposed SEMs (Figure 7) showed that GFI of the data to the aprioristic model was high as denoted by a significant RMSEA statistic ( $\leq 0.08$ ) and by high NFI and GFI values ( $> 0.9$ ). Three consistent effects have been found on *C. tricocon* and *R. ludovici-salvatoris* general SEMs (Figure 7b and c). First, light did not have a significant direct or indirect effect on either fruit crop or height; second, aridity had a significant negative effect on fruit crop for both species; and finally, aridity had a significant positive effect on WUE for both species. However, the strength of the different hypothesized relationships varied between species. Aridity had a stronger negative effect on fruit crop in *C. tricocon* than in *R. ludovici-salvatoris* (standardized regression weights of  $-0.45$  and  $-0.39$ , respectively) and a greater positive effect on WUE in *R. ludovici-salvatoris* than in *C. tricocon* ( $0.66$  and  $0.41$ , respectively). Moreover, aridity had a negative effect on height for both species, but while the effect was only indirect for *R. ludovici-salvatoris* (through a negative effect of WUE on height), we found direct and indirect effects of aridity for *C. tricocon* (through a negative effect of Fv/Fm on

height). Fv/Fm was affected by aridity in *C. tricocon*, but not in *R. ludovici-salvatoris*. Considering seasonal ecophysiological differences (before vs during summer drought) in *C. tricocon* and *R. ludovici-salvatoris*, SEMs have confirmed, first, that light did not have a significant effect on fruit crop or on height and, second, that aridity has a greater negative effect on fruit crop and on height in *C. tricocon* than in *R. ludovici-salvatoris*. There was a negative relationship between aridity and WUE increase in *C. tricocon* ( $-0.46$ ), but not in *R. ludovici-salvatoris*. Increases in WUE had a negative effect on fruit crop in *C. tricocon* ( $-0.30$ ), while we found a positive relationship with height in *R. ludovici-salvatoris* ( $0.38$ ). Aridity had a negative effect on Fv/Fm decrease in *R. ludovici-salvatoris*, i.e., populations at the limit of aridity showed lower Fv/Fm decrease. In *C. tricocon*, Fv/Fm decrease had a negative effect on height ( $-0.29$ ), but not in *R. ludovici-salvatoris*. In *C. tricocon*, height had a positive effect on fruit crop, while in *R. ludovici-salvatoris* it did not; hence any factor affecting height had a negative effect on fitness in *C. tricocon* populations.

## Discussion

The two study species presented contrasting responses to drought and showed different interpopulation variability in functional traits related to drought resistance. These findings reveal different potential vulnerabilities to the expected aridity increase. *Cneorum tricocon* displayed low variability in its water strategy, with a consistent behaviour across the aridity gradient. Its water conservation strategy was characterized by a wide safety margin against extreme drought events with a significantly negative correlation between  $g_s$  and aridity of the site. Moreover, all *C. tricocon* populations showed a drastic stomatal control and high WUE under drought conditions, a pattern observed both seasonally and across years and sites. In contrast, *R. ludovici-salvatoris* showed greater variability in its water strategy. In general, this species displayed narrow safety margins in the face of drought and maintained higher  $g_s$  and lower WUE than *C. tricocon*. However, *R. ludovici-salvatoris* was able to reduce water loss in the most xeric site,

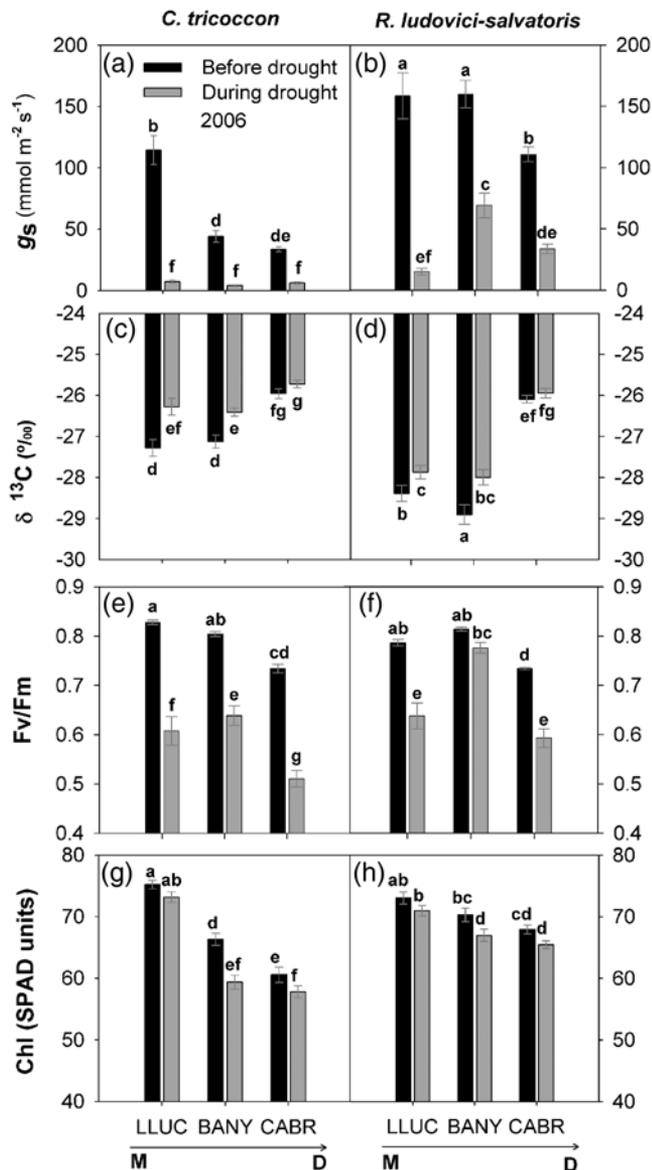


Figure 5. Mean values of  $g_s$ ,  $\delta^{13}\text{C}$ , Fv/Fm and Chl before and during summer drought in populations of *C. tricocon* and *R. ludovici-salvatoris*. Values are mean  $\pm$  SE. Different letters indicate significant differences after Fisher's LSD test.  $N = 15\text{--}30$  for each species and population. M, mesic; D, dry; study sites' codes are given in Table 1.

with relatively high WUE values. This behaviour, shifting from water spending to water saving at the driest site, is an adaptive mechanism observed in other woody plants species (Zhang et al. 1995) that could result from local adaptation. Many anatomical and physiological features involved in drought tolerance, such as vulnerability to xylem cavitation (Kavanagh et al. 1999), hydraulic conductance (Comstock 2000) and stomata size and density (Mitton et al. 1998) have been shown to result from intraspecific genetic variability and population differentiation.

The most xeric population of *C. tricocon* and *R. ludovici-salvatoris* showed lower plant size, worse ecophysiological

Table 4. Effects of population (Po), year (Ye), species (Sp) and their interactions on  $g_s$  and Fv/Fm.

	$g_s$		Fv/Fm	
	F	P	F	P
Po	31.51	<b>&lt;0.001</b>	65.27	<b>&lt;0.001</b>
Ye	151.63	<b>&lt;0.001</b>	158.67	<b>&lt;0.001</b>
Sp	137.03	<b>&lt;0.001</b>	36.87	<b>&lt;0.001</b>
Po $\times$ Ye	23.17	<b>&lt;0.001</b>	4.55	<b>0.011</b>
Po $\times$ Sp	10.89	<b>&lt;0.001</b>	9.01	<b>&lt;0.001</b>
Ye $\times$ Sp	27.13	<b>&lt;0.001</b>	17.26	<b>&lt;0.001</b>
Po $\times$ Ye $\times$ Sp	5.90	<b>0.003</b>	2.68	0.070

Significant effects ( $P < 0.05$ ) are indicated in bold.

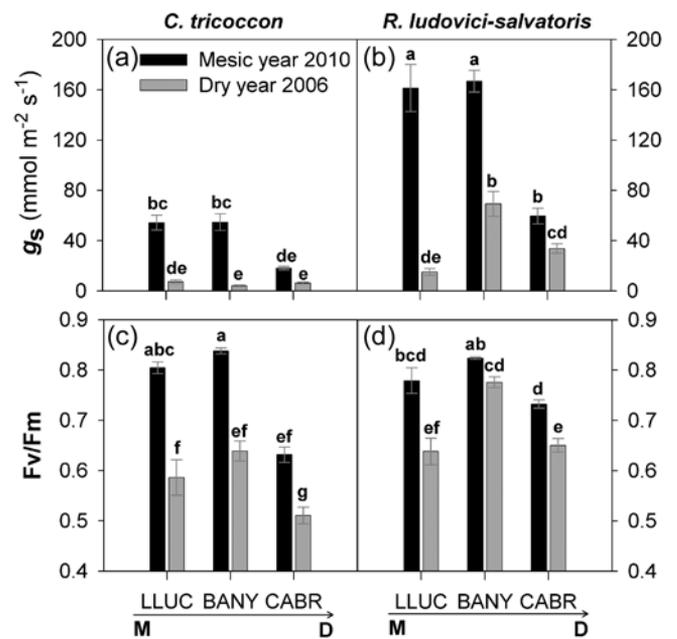


Figure 6. Mean values of  $g_s$  and Fv/Fm in two climatically contrasting years, mesic and dry, in populations of *C. tricocon* and *R. ludovici-salvatoris*. Values are mean  $\pm$  SE. Different letters indicate significant differences after Fisher's LSD test.  $N = 15\text{--}30$  for each species and population. M, mesic; D, dry; study sites' codes are given in Table 1.

condition (as shown by lower levels of  $g_s$  and Fv/Fm) and poorer fitness (estimated by a lower fruit crop), which agrees with studies of other Mediterranean woody species (Castro et al. 2004, Jump et al. 2006, Linares and Tiscar 2010). The population at the driest site seems to be at its limit of aridity tolerance and is thus threatened by the increase in aridity expected in the most likely climate change scenarios. This situation, explored in a number of woody species across the Mediterranean basin (e.g., Macías et al. 2006, Carrer et al. 2010), could be counteracted by high levels of phenotypic plasticity and a rapid evolution leading to drought-adapted ecotypes (Aspelmeier and Leuschner 2004, Gianoli and Gonzalez-Teuber 2005, Matesanz et al. 2010). The two species studied differed in their potential for accommodating to local and current conditions, with *R. ludovici-salvatoris*

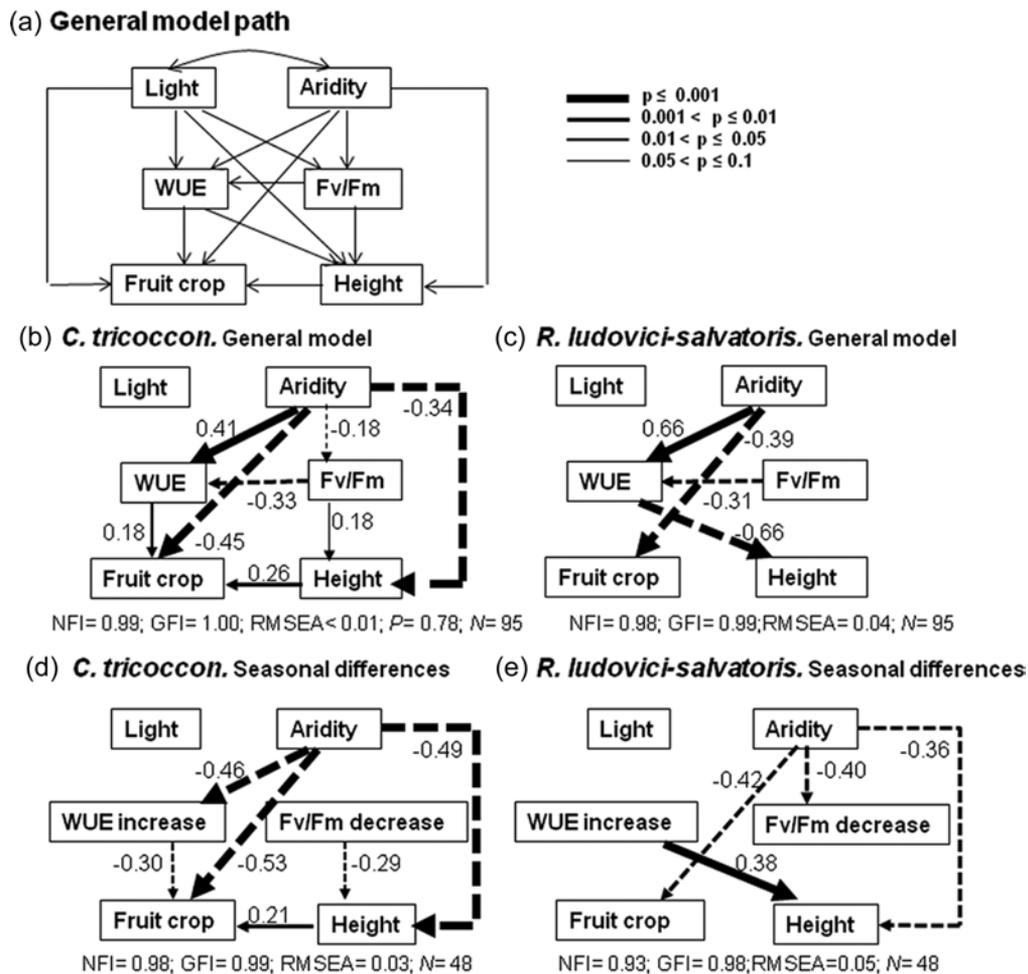


Figure 7. General SEM representing hypothesized casual relationships among abiotic predictors, ecophysiological, morphological and fitness variables (a). A fitted general model in *C. tricocon* and *R. ludovici-salvatoris* (b and c, respectively) and considering seasonal ecophysiological differences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

displaying a more plastic response to aridity than *C. tricocon*. While the former was able to regulate water consumption according to the water available at each site and time, *C. tricocon* maintained a similar water conservation strategy across sites and over time. However, despite the plastic water strategy of *R. ludovici-salvatoris*, it was not able to counteract the adversity of the most xeric site as shown by the lower estimated fitness of this population. Structural equation model analyses confirmed these trends. First, aridity had a significant negative effect on fitness, as it decreased fruit crop in both species. However, the effect was stronger for *C. tricocon* than for *R. ludovici-salvatoris*. Second, aridity enhanced WUE in both species; however, the effect was greater for *R. ludovici-salvatoris* than for *C. tricocon*. Summer drought in the Mediterranean is associated with high irradiance and temperature, further increasing plant stress over this critical period. Species from Mediterranean habitats exhibit different ecophysiological

mechanisms to cope with light stress and overheating, which are essential for their survival (Faria et al. 1998, Martínez-Ferri et al. 2000). *Cneorum tricocon* reduced interception of maximum irradiances by displaying greater leaf elevation angles than *R. ludovici-salvatoris*. In fact, there was a positive correlation between leaf elevation angles and population aridity in *C. tricocon*. Increased leaf elevation angles allow these plants to minimize light and thermal stresses but also to reduce water loss through evapotranspiration (Valladares and Pearcy 1997, Valladares et al. 2000, Granado-Yela et al. 2011). However, despite their higher elevation angles, *C. tricocon* was more prone to photoinhibition than *R. ludovici-salvatoris* as revealed by the marked decreases of the Fv/Fm and Chl observed in *C. tricocon* during drought and SEM analyses. These findings match well with a water conservation strategy (sensu McDowell et al. 2008). In contrast, the lower sensitivity to photoinhibition of *R. ludovici-salvatoris* could allow higher

carbon gain under moderate drought, as seen in other species (Werner et al. 1999, 2001, 2002).

*Cneorum tricoccon* avoided drought through stomatal closure, thus averting hydraulic failure under water deficit conditions at the expense of carbon assimilation (McDowell et al. 2008). Conversely, *R. ludovici-salvatoris* kept their stomata open during dry periods, maintaining high assimilation rates at the expense of a high risk of cavitation (McDowell et al. 2008). These contrasting strategies allow them to exploit resources differentially, but rendering similar results in terms of growth and survival, which can partially explain the current coexistence of both species (Chesson 2000, Götzenberger et al. 2012). However, future changes in the length and intensity of drought events could favour one species to the detriment of the other. This becomes particularly important in Mediterranean ecosystems, where global circulation models forecast not only an increase in aridity (Christensen 2007), but also an increase in climatic variability with changes in the frequency and intensity of extreme weather and climate events such as heat waves, droughts and floods (Easterling et al. 2000, Lloret et al. 2012). In this sense, a chronic, prolonged drought could have a stronger effect on *C. tricoccon* than on *R. ludovici-salvatoris* since under long droughts and curtailed photosynthesis, carbon starvation becomes a serious threat. Conversely, short but very intense droughts could have a greater effect on *R. ludovici-salvatoris* than on *C. tricoccon* due to the risk of mortality by hydraulic failure in the former species, which keeps stomata more open over a wide range of conditions.

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

In short, *C. tricoccon* and *R. ludovici-salvatoris* are both vulnerable to the current rates of rapid environmental changes, and their responses to further aridity increases will be influenced by the specific future climatic scenarios, primarily duration vs intensity of the drought. Anticipating these responses

requires interdisciplinary research, integrating demographic and genetic work with community and evolutionary ecology.

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## Conflict of interest

None declared.

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