

1 This is the peer-reviewed version of the article accepted for publication in *PLANT PHYSIOLOGY AND*
2 *BIOCHEMISTRY* Volume 154, 219-228. 2020, which has been published in final form at
3 <https://doi.org/10.1016/j.plaphy.2020.06.001>

4 **Plant response to water stress of native and non-native *Oenothera drummondii* populations**

5 Díaz-Barradas, MC¹; Gallego-Fernández, JB¹; Zunzunegui, M¹

6 ¹Departamento de Biología Vegetal y Ecología, Universidad de Sevilla, Apartado 1095, 41080 Sevilla,
7 SPAIN

8 Corresponding author: Mari Cruz Díaz-Barradas, email: diaz@us.es, phone number: 0034954557062

9 **Abstract**

10 Invasive plants can spread over climatically diverse areas. We explore the effects of drought on gas
11 exchange and water relations on the invasive dune species *Oenothera drummondii*, using seeds from four
12 populations with different rainfall and temperature regimes.

13 Plant material was obtained from germinating plants from one native and three non-native populations in a
14 greenhouse. Drought stress was induced by withholding water. Responses to drought-stressed plants were
15 then compared to well-watered controls. Measurements of gas exchange, chlorophyll fluorescence and leaf
16 traits were taken initially and every 10 days after water was withheld until day 36 when plants were re-
17 watered and recovery capacity was measured.

18 The effect of water stress was more evident in F_v/F_m and gas exchange variables. The results suggest that
19 this species possesses a mechanism of thermal dissipation of energy. Leaf relative water content was
20 significantly lower in drought-stressed than control plants. At the end of withholding water period, stressed
21 plants are separated from control plants along the axis I of the ordination analysis evidencing differences
22 in functional traits. All plants recovered well after re-watering.

23 Our results provide evidence for permanent differences in morphological traits and functional responses to
24 drought stress among native and invasive populations of *O. drummondii*. Although we have only studied
25 four populations, these results may provide evidence for the role of plasticity in contributing to the invasion
26 success of this species'.

27

28 **Keywords:** coastal dunes; drought resistance; functional traits; geographical origin; invasive species; trait
29 variability; water use efficiency

30

31 1. Introduction

32 Exotic plant invasions are one of the most important causes of global change, contributing to the
33 decline of biodiversity (Brook et al., 2008; Pyšek and Richardson, 2010; Vitousek et al., 1997). Invasive
34 species may substantially alter ecosystem functions and processes, often by increasing nutrient input,
35 altering nutrient cycling and/or water balances, and by modifying diversity, community composition and
36 vegetation structure (Castro-Díez et al., 2014; Ehrenfeld, 2010; Liao et al., 2008; Pyšek et al., 2012), leading
37 in some cases to the extinction of native species with huge ecological and economic impacts (Mozdzer and
38 Zieman, 2010; Novoa et al., 2013; Paine et al., 2016).

39 In general, the success of exotic invasive species is a multifactorial process related to (i) invasive
40 traits that allow for success in the new environment, as the existence of active dispersion vectors and
41 reproductive mechanisms, the production of allelopathic compounds, the changes in soil nutrient and water
42 balances among others (Antunes et al., 2018; Díaz-Barradas et al., 2015; Rascher et al., 2011; Stanisci
43 et al., 2010) and (ii) habitat invasibility or vulnerability to invasion (Gallego-Fernández et al., 2019; Santoro
44 et al., 2012).

45 In several studies, it has been reported that under conditions of low resource ability, invasive species
46 might be more efficient than native species using some limited resources, as occurs with some species of
47 *Acacia* (Crous et al., 2012; Funk and Vitousek, 2007; Morais and Freitas, 2012). This capacity is probably
48 achieved owing to having higher phenotypic plasticity than native species, which allows them to acclimate
49 to a variety of environmental characteristics (Davidson et al., 2011). Shifts in a number of biological
50 features have been documented in invasive plant populations relative to their native locations, however,
51 differences in physiological traits between alien-invasive and native populations of a single plant species
52 have not been fully investigated (Brodersen et al., 2008).

53 Plant dune species have to live in a harsh and unstable environment and they exhibit a set of specific
54 adaptations in order to resist drought, lack of nutrients, wind, burial and sand movement: as prostrate growth
55 form, horizontal branches, succulent or hairy leaves, slow growth rate among other traits (García-Mora et
56 al., 1999; Hesp, 1991). Coastal dune communities are especially sensitive to biological invasions due to the
57 frequent disturbances, low vegetation cover and the existence of open patches free of competitive
58 interactions (Jørgensen and Kollmann, 2009; Pardini et al., 2015). Several authors have emphasized that
59 coastal dunes are among the ecosystems and habitats that have been most affected by invasive species, and

60 sometimes, can be considered and “extreme case of species invasion” (Castillo and Moreno-Casasola, 1996;
61 Gallego-Fernández et al., 2019).

62 *Oenothera drummondii* subsp. *drummondii* Hook. (Onagraceae) is a short-lived perennial species,
63 with hairy leaves, yellow flowers and dry fruits. This species is native of coastal dunes from the Gulf of
64 Mexico and SE of USA, all these regions share a tropical and subtropical climate and water supply all over
65 the year. Nowadays it has spread over many coastal areas of different continents and it is considered as
66 invasive in Spain, Israel, China and Australia and naturalized in many places of the world, shifting to
67 regions with different climatic characteristics. In some coastal areas of the SW of Spain *O. drummondii* has
68 significantly reduced the abundance of native dune species as *Ammophila arenaria* and other have become
69 locally extinct as *Achillea maritima*, *Eryngium maritimum*, *Medicago marina* and *Elymus farctus*, although
70 the impact varies across the gradient from the beach to the inland dunes (Gallego-Fernández et al., 2019).

71 In order to invade these new habitats, *O. drummondii* should have high levels of physiological
72 tolerance and plasticity, or it must undergo genetic differentiation to achieve required levels of fitness to
73 the diversity of environmental conditions outside their biogeographic area (Richardson and Pyšek, 2006).
74 Field studies have shown that *O. drummondii* is well acclimated to Mediterranean coastal dunes, exhibiting
75 higher photosynthetic rate and better water performance than the native species *A. maritima* (Zunzunegui
76 et al., 2020). These authors conclude that the underlying mechanism that confers this species its competitive
77 capacity might be its ability for water uptake.

78 As it has been mentioned before, all the native populations of *O. drummondii* grow on coastal dunes
79 with tropical climate and field studies have revealed a good capacity of water uptake in Mediterranean
80 climate (Zunzunegui et al., 2020). Therefore, we hypothesized that the responses of this species to drought
81 could be different between the populations from different environmental conditions, in particular, that the
82 plants growing in drier climate regions should face water deficit more effectively than those from milder
83 climates. To test this hypothesis, we subjected *O. drummondii* plants from different locations (one
84 representative native and three non-native populations) to a drought treatment, in order to test how
85 photosynthetic characteristics, as well as morphological traits, vary among native and invasive populations
86 from regions with different water availability.

87 We consider that monitoring changes in functional traits as light responses and water-relation
88 variables under drought stress may provide clues to the distribution and spread of *O. drummondii* (Naidoo
89 and Naidoo, 2018; Vilagrosa et al., 2013) and might be valuable for the control of the invasion.

90

91 2. Material and methods

92 2.1. Study areas

93 Our plant material was originated from four *O. drummondii* natural populations located in different
94 areas of the world with contrasted climatic conditions: México (El Laurel beach), Israel (Rishon Lezion
95 beach) and two locations in Spain (Zarautz and Dique) Table 1.

96

97 Table 1. Geographic location and climatic data (mean of annual temperature, total annual precipitation, and
98 climatic classification) of native and non-native populations of *Oenothera drummondii* studied. Climatic
99 data were obtained in the Climate-data.org website. 1 Mean annual temperature. 2 Total annual
100 precipitation.

101

	Population	Country	Latitude	Longitude	T (°C) ¹	P (mm) ²	Köppen Climate
Native	El Laurel	Mexico	20° 29'N	97° 01'W	24.0	1497	Am
East-Mediterranean	Rishon-Lezion	Israel	31° 59'N	34° 43'W	19.5	571	Csa
West-Mediterranean	Dique	Spain	37° 09'N	06° 54'W	17.8	467	Csa
Atlantic	Zarautz	Spain	43° 17'N	02° 09'W	14.6	1255	Cfb

102

103 El Laurel (México) corresponds to a native population, growing under a tropical type climate (Am)
104 with annual rainfall distributed all over the year (Figure 1). Hereby Native Population.

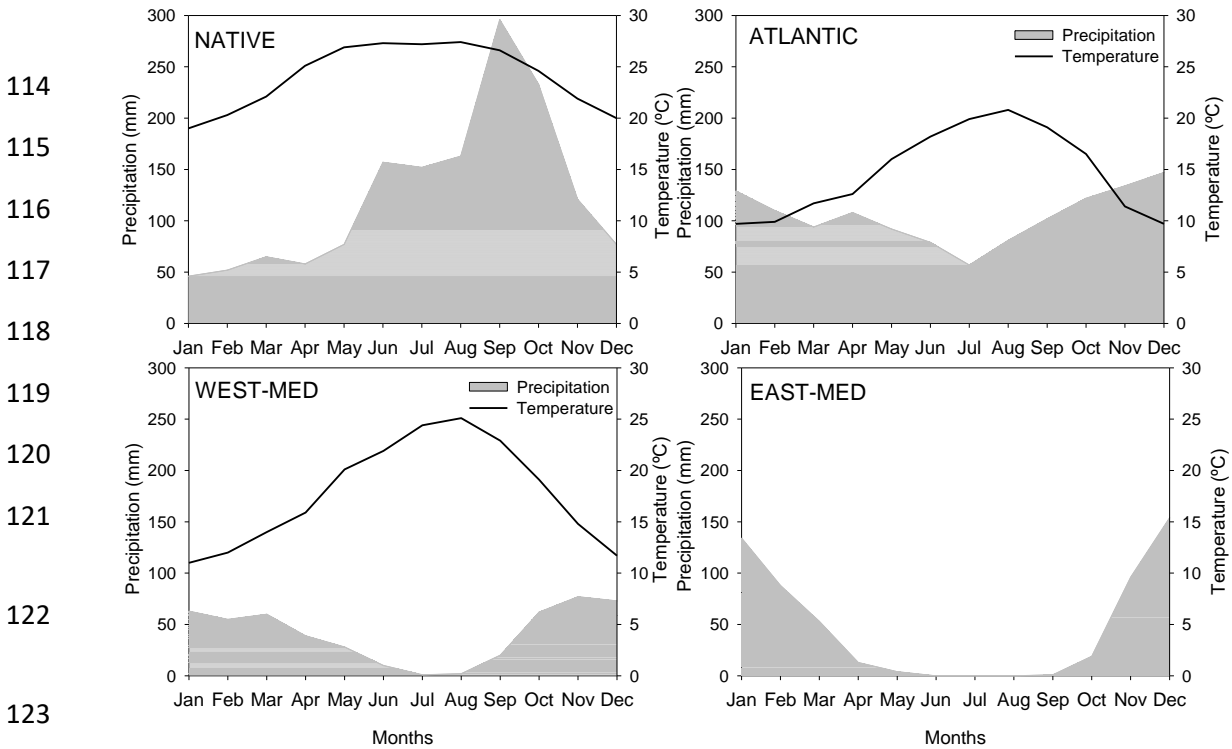
105 Rishon Lezion Beach (Israel) presents dunes with low vegetation cover. In this site, *O. drummondii*
106 is an alien species, growing under Mediterranean climate (Csa), with a marked drought season (Figure 1).
107 Hereby East-Mediterranean Population (East-Med).

108 The population of Zarautz is located in Northern Spain. The climate is temperate and humid (Cfb)
109 and annual rainfall is distributed all over the year (Figure 1). Hereby Atlantic Population.

110 Dique is located in Huelva province in the SW Spain. The climate is Mediterranean (Csa) with a
111 marked drought season (Figure 1). Hereby West-Mediterranean Population (West-Med).

112

113



114
115
116
117
118
119
120
121
122
123
124 **Fig. 1.** Climatic diagrams corresponding to the original sites from which the seeds of the four study
125 populations have been collected. NATIVE: El Laurel (Mexico), ATLANTIC: Zarautz (North Spain),
126 WEST-MED: Dique (SW Spain) and EAST-MED: Rishon-Lezion (Israel).

127

128 **2.2. Seed collection and seedling growing**

129 In each population, fruits of at least 30 randomly individuals were collected. Seeds were extracted
130 from fruits and stored in paper bags, under laboratory conditions (room temperature) before being used.

131 In December of 2015, seed lots of each population were germinated in pots of 2.5 l with a substrate
132 composed of 70% sand and 30% perlite under greenhouse conditions. Temperature ranged between 15-
133 20°C, relative humidity between 40-60% and natural light was supplemented with artificial light to maintain
134 a 14h photoperiod. Pots were fertilized (universal fertilizer, Flower, NPK, 6-4-6) in April, May and
135 September 2016. For each population, a solution of 5 liters of water with 25 ml of liquid fertilizer was
136 prepared, so each pot was enriched with 250 ml of fertilized water (1.25 ml of fertilizer per pot). In
137 November 2016, we selected 20 plants from each population in order to develop our experimental design
138 (80 pots in total).

139

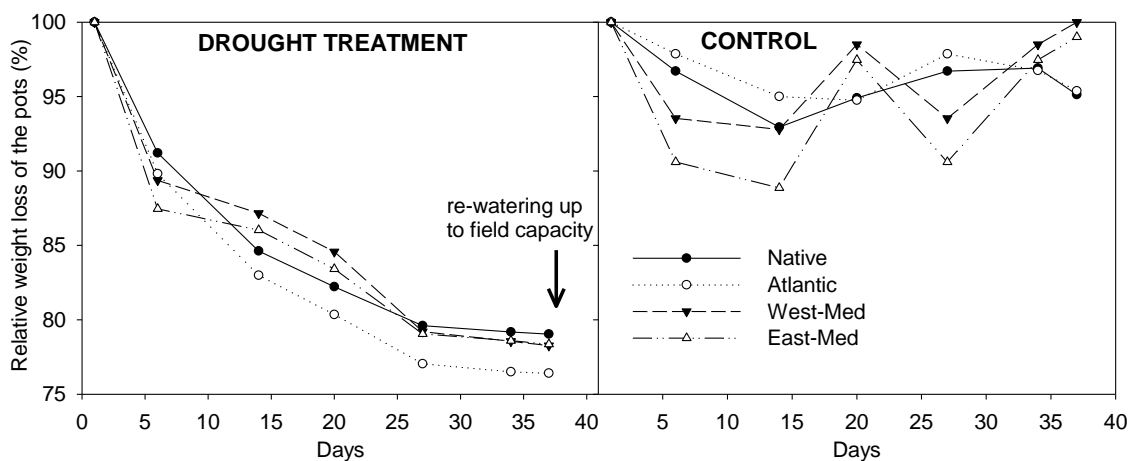
140 **2.3. Experimental design**

141 Regular watering was maintained until the 11th November of 2016, when all the pots were watered,
142 all plants were measured and afterwards the drought treatment started.

143 From this date we sorted the 20 pots of each population in two groups, 10 were maintained under
144 field capacity (control plants) and the other 10 were subjected to a severe drought treatment by withholding
145 water (stressed plants). In order to maintain the plants alive, pots of stressed plants were weekly weighted
146 and water supply was equivalent of 10% of the total weight loss. At the end of the drought treatment the
147 weight of the pots of stressed plants was approximately 75-80% of the original weight (Figure 2), while
148 control plants were maintained at 100%. After 36 days since water was withheld, all the plants were re-
149 watered until field capacity, and after 9 days we recorded plant recovery capacity. For measurements, we
150 randomly selected 5 pots per population and treatment, in order to perform all the physiological
151 measurements into a comparable period of time.

152 Measurements were carried out the following days: 11th (first day of experiment of water deficit),
153 18th and 28th November and 7th, 16th December (day 36 of the water deficit experiment and beginning of
154 re-watering) and 27th December (day 45, end the experiment).

155 During the experiment, we measured different morphological and physiological traits in 5 pots per
156 treatment and population (5 plants x 2 treatments x 4 populations= 40 pots), every 10 days. Stomatal density
157 was only measured before drought stress.



158
159 **Fig. 2.** Relative weight loss of the different experimental pots subjected to water withholding in relation to
160 the initial weight (100%) and oscillation of pot weight in well-watered plants (control) during the period of
161 the drought experiment. The arrow indicates the day of re-watering until field capacity.

162

163 **2.4. Morphological Traits**

164 Stomatal density was measured using an electronic microscope (Phenom Pro), of the Research
165 Facilities of the University of Seville (S.G.I. Celestino Mutis). We randomly selected 10 individuals per
166 population, one leaf per individual and 3 microscope fields per leaf (on the abaxial surface of the leaf, on
167 which stomatal were more abundant). We used 950 microscope magnifications, which corresponded to a
168 field diameter of 285 μm . We registered the number of stomata per field and calculated density as stomatal
169 number per mm^2 .

170 A subsample of fresh leaves per measured plant was selected and taken in plastic bags to calculate
171 leaf mass per area (LMA), the relative water content (RWC) and leaf dry matter content (LDMC). In the
172 laboratory, fresh leaves were first weighed, and then their leaf surface scanned, and the leaf area calculated
173 using Midebmp (R. Ordiales, CSIC, Spain, 2000). Then, the leaves were taken to full water saturation and
174 weighed again after 24 hours. Finally, the leaves were dried for 24 h at 80 °C and weighed again. LMA was
175 measured as the ratio of leaf dry mass to leaf area (gm^{-2}). Following several authors this trait is a good index
176 of the plant strategies to face the local environmental conditions (Westoby et al., 2002).

177 Relative Water Content (RWC) was calculated using the following equation (Saura-Mas and Lloret,
178 2007) and it represents a good index of leaf water status: $\text{RWC} = (\text{Fresh leaf mass} - \text{Dry leaf mass}) \times 100$
179 $/(\text{Fully water-saturated leaf mass} - \text{Dry leaf mass})$.

180 Leaf Dry Matter Content (LDMC) is related to tissues density and plant productivity (Garnier et al.,
181 2001), it was calculated using the following equation: $\text{LDMC} = (\text{Dry leaf mass} / \text{Fully water-saturated leaf}$
182 $\text{mass}) (\text{mg/g})$.

183

184 **2.5. Physiological Traits**

185 Chlorophyll fluorescence kinetics of leaves was assessed *in situ* by means of the pulse-amplitude
186 modulation technique using a portable fluorometer (mini-PAM, Walz, Effeltrich, Germany). Fluorescence
187 was excited by a pulse of modulated red light from a light-emitting diode (LED, type H-3000 Stanley)
188 connected to a fibreoptic.

189 Maximum quantum yield of PSII was determined from the ratio of variable to maximal fluorescence,
190 $F_v/F_m = (F_m - F_o)/F_m$, where F_o and F_m denote basal and maximal fluorescence of dark-adapted leaves over
191 20 min, a period found to be sufficient to allow complete reoxidation of the PSII reaction centers. F_o was

192 determined with a modulated measuring light from an LED at a 600 Hz frequency. Maximal fluorescence
193 (F_m) was obtained with a brief saturating light pulse. Effective quantum yield (Φ_{PSII}), or photochemical
194 efficiency of PSII in a light-adapted state, was estimated on environment conditions light-adapted leaves
195 with leaf clip-holder as $\Phi_{PSII} = (F'_m - F)/F'_m$ where F'_m = maximal and F = steady-state fluorescence under
196 actinic irradiance (Genty et al., 1989). Non photochemical quenching (NPQ) was calculated as: $NPQ = (F_m -$
197 $F'_m)/F'_m$ (Maxwell and Johnson, 2000). NPQ is linearly related to heat dissipation and lies on a scale 0–
198 infinity. In a typical plant, values are usually in the range 0.5–3.5 at saturating light intensities; however,
199 this varies markedly between species and on the previous history of the plant (Maxwell and Johnson, 2000).

200 Leaf gas exchange measurements were performed the same days as fluorescence measurements, by
201 means of a gas exchange CO_2/H_2O analyzer LI-6400 (LI-COR Inc., Neb., USA). Measurements conditions
202 inside the leaf-chamber were $1400 \mu mol m^{-2} s^{-1}$ PPFD, 1.0-1.5 kPa VPD and $400 \mu mol mol^{-1} CO_2$. The
203 system provides the values of net photosynthetic rate (A), transpiration rate (E), stomatal conductance (g_s)
204 and internal CO_2 concentration (C_i). Water use efficiency (WUE) was calculated as the ratio of CO_2 uptake
205 per H_2O transpired or A/E , intrinsic WUE as A/g_s and carboxylation efficiency as A/C_i . Measurements were
206 conducted on leaves from terminal shoots and all results were expressed on an area basis, calculated using
207 Midebmp (R. Ordiales, CSIC, Spain, 2000), from scanned images of the leaves from each measured shoot.

208 Fluorescence and leaf gas exchange measurements were carried out in two leaves per plant, between
209 11h am and 13h pm.

210 In total 2 leaves x 5 plants x 2 treatments x 4 populations were measured in each sampling day.

211

212 **2.6. Statistical Analysis**

213 After testing for normality of the data using a Kolmogorov–Smirnov test, one-way ANOVA was
214 used to compare differences of each trait among populations, before the water stress experiment. In the
215 drought stress experiment, the temporal differences of each trait in each population and treatment were
216 analysed by repeated measures ANOVA (Potvin et al., 1990), in which the within-subject factor was time
217 (with four categories) and the between-subject factor were population and treatment. In these analyses, the
218 replicates were the individuals, so that there were five replicates per population and treatment. In the cases
219 where Mauchly's sphericity was not assumed, we applied Huynh–Feldt's correction. The data from the fifth
220 sampling day, in which plants had been re-watered, was excluded from the analyses. In order to compare
221 individual differences between control and stressed plants, for each variable in each sample day and

222 temporal differences in each population (independent of the treatment), we have also used one-way
 223 ANOVA. Tukey's Post-hoc analyses were performed to test for within-group differences for each variable.
 224 Pearson's correlation was calculated for pair-comparisons between the different variables. A multivariate
 225 principal component analysis (PCA) was performed with the individual physiological and morphological
 226 trait measurements at the end of the drought period, in order to integrate the complete set of traits measured
 227 in the plants during the last week of drought-treatment. In relation to the relative position of individuals in
 228 the space defined by axis I and II of the analysis, we aim to define the patterns of control and stressed plants
 229 from the different populations. All the analyses were performed with IBM-Statistics 22.

230

231 **3. Results**

232

233 **3.1. Comparison among populations before the water stress**

234 Although the plants were grown under the same greenhouse-conditions, they exhibited differences
 235 in several studied variables according to their population's origin.

236 Stomatal density presented significant differences among populations (Table 2). Native and West-
 237 Med populations exhibited the highest stomatal density (approximately 200 stomatas mm⁻²), while in East-
 238 Med and Atlantic populations stomatal density ranged between 160 and 170 stomatas mm⁻², differences
 239 among populations were significant at $p < 0.05$ (Table 2).

240 In relation to leaf morphological traits, there were significant differences in LMA ($p < 0.01$), with
 241 plants from West-Med population exhibiting the highest values, while LDMC was higher in Native than in
 242 the other populations, but differences were only significant between Native and Atlantic populations ($p <$
 243 0.05). On the other hand, RWC was similar for all populations (Table 2).

244

245 Table 2. Morphological leaf traits of the different populations (average \pm sd) and the significance for one-
 246 way ANOVA. Stomatal density (stomatas.mm⁻²), LMA (leaf mass area, gm⁻²), LDMC (leaf dry matter
 247 content mg g⁻¹), RWC (%). Capital letters indicate Tukey post-hoc differences ($p < 0.05$) between
 248 populations for each variable.

249

	P value	Native	Atlantic	West-Med	East-Med
Stomatal-density	< 0.05	199.9 \pm 48.2 A	170.4 \pm 34.0 AB	202.6 \pm 29.6 A	160.9 \pm 23.4 B

LMA	< 0.01	42.9±5.8 A	42.7±6.9 A	50.1±7.7 B	42.6±7.3 A
LDMC	< 0.05	124.9±23.2 B	109.0±18.3 A	115.1±9.9 AB	120.5±18.6 AB
RWC	0.11	85±6	84±4	88±4	85±3

250

251 *3.2. Water Stress treatment*

252 Some of the measured traits presented significant differences for population and treatment along the 36
 253 days of water deficit. The following traits, F_v/F_m , NPQ, CO_2 assimilation (A), leaf conductance (g_s),
 254 intrinsic WUE (WUE_i) and RWC presented significant temporal effects during the water stress experiment,
 255 which is shown by the within-subject repeated-measures ANOVA (Table 3a). The interaction Time x
 256 Population was significant for F_v/F_m , A, g_s , WUE_i , LMA and LDMC, which evidences as some of the
 257 populations presented temporal differences for these traits. The interaction Time x Treatment was
 258 significant for F_v/F_m , NPQ, A, g_s , WUE_i and RWC which shows as the treatment exerted significant
 259 temporal differences for these variables. Finally, there were significant differences in the third order
 260 interaction Time x Population x Treatment for four of the traits, F_v/F_m , g_s , WUE_i and LDMC, revealing as
 261 the studied populations presented different temporal responses to the water stress treatment for these traits
 262 (Table 3a).

263 For all study traits there were significant differences among populations in the analysis of between-subjects'
 264 effects of the repeated-measures ANOVA (Table 3b), which shows as populations exhibited significant
 265 differences of these traits. The effect of treatment was significant for F_v/F_m , NPQ, A, g_s , WUE_i , LMA and
 266 RWC, as these traits were significantly affected by the water stress. However, populations only exhibited
 267 specific differences in response to treatment for WUE_i , LMA and LDMC as it is shown in the significant
 268 interaction Population x Treatment (Table 3b).

269

270 Table 3. Repeated-measures ANOVA on the effects of time, population and treatment along the 4 weeks
 271 of water withholding. Significant differences, below the $P < 0.05$ threshold, are marked in bold in the table.
 272 The acronyms used in the table indicate the following variables: F_v/F_m : maximum quantum yield; Φ_{PSII}
 273 effective quantum yield; NPQ non photochemical quenching; A net photosynthetic rate; g_s stomatal
 274 conductance; WUE_i intrinsic water use efficiency A/g_s ; LMA leaf mass area; LDMC leaf dry matter
 275 content; RWC relative water content.

276 a) Within-subjects' effects of Time and interaction Time x Population, Time x Treatment and Time x
 277 Population x Treatment.

	Time		Time x Population		Time x Treatment		Time x Population x Treatment	
	F	P	F	P	F	P	F	P
F _v /F _m	5.258	0.002	3.121	0.002	3.452	0.020	2.340	0.019
Φ _{PSII}	2.175	0.096	1.309	0.243	0.535	0.660	0.640	0.760
NPQ	4.329	0.007	1.325	0.234	5.119	0.003	1.721	0.095
A	49.012	<0.001	4.367	0.001	48.939	<0.001	1.000	0.443
g _s	19.392	<0.001	4.144	0.001	17.500	<0.001	3.272	0.006
WUE _i	97.161	<0.001	13.464	<0.001	79.986	<0.001	7.096	<0.001
LMA	0.660	0.579	2.559	0.011	0.285	0.836	0.735	0.091
LDMC	0.898	0.445	2.944	0.004	0.364	0.779	2.759	0.007
RWC	3.337	0.023	1.386	0.205	7.927	<0.001	1.236	0.282

278

279 b) Between-subjects' effects of population, treatment and the interaction population x treatment.

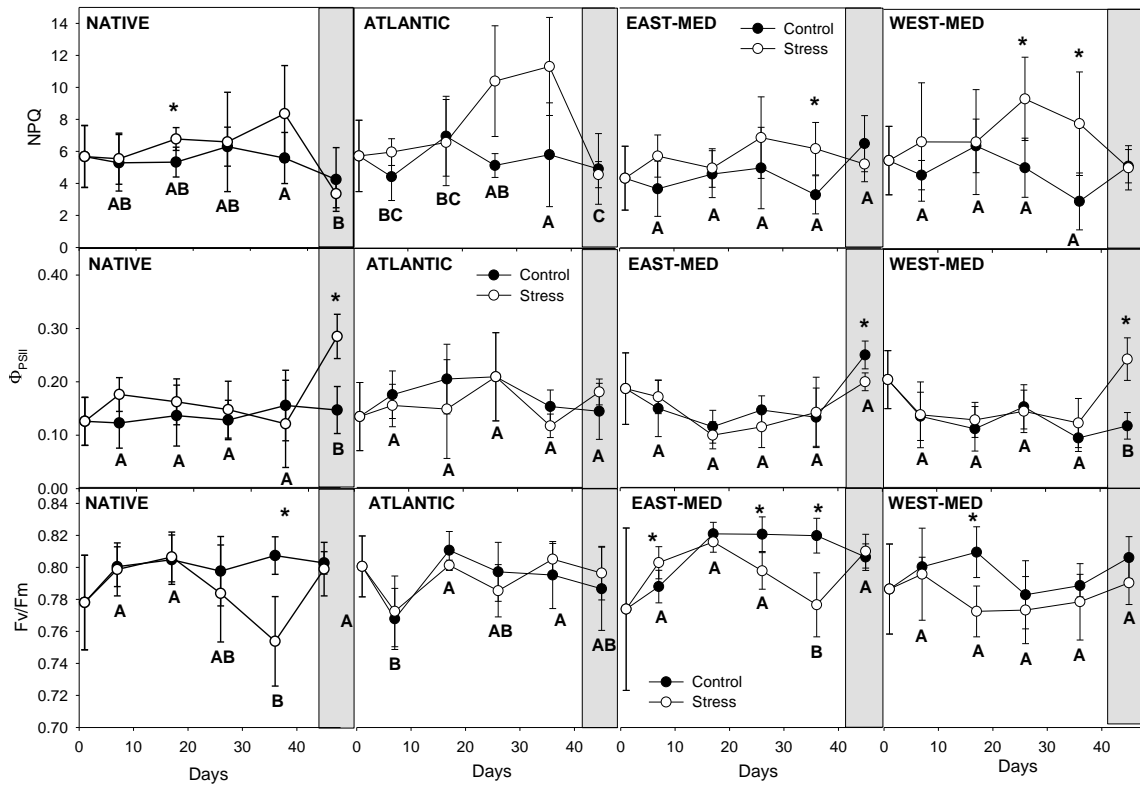
	Population		Treatment		Population x Treatment	
	F	P	F	P	F	P
F _v /F _m	5.822	0.003	14.683	0.001	1.247	0.309
Φ _{PSII}	5.102	0.005	0.124	0.727	1.852	0.158
NPQ	4.735	0.008	27.955	<0.001	2.074	0.123
A	11.139	<0.001	126.298	<0.001	1.970	0.138
g _s	12.229	<0.001	224.086	<0.001	2.308	0.095
WUE _i	21.794	<0.001	26.652	<0.001	5.193	0.005
LMA	13.023	<0.001	5.138	0.030	7.610	0.001
LDMC	12.574	<0.001	0.090	0.766	9.612	<0.001
RWC	4.721	0.008	9.225	0.005	0.714	0.551

280

281 3.2.1. Chlorophyll fluorescence: F_v/F_m, Φ_{PSII}, NPQ

282 During the 36 days of the drought treatment, maximum quantum yield (F_v/F_m) dropped in Native
 283 and East-Med populations, revealing significant differences between control and stressed plants at the end
 284 of the experiment. The significant interaction Time x Population x Treatment showed that populations
 285 presented different temporal responses to the treatment (Table 3a). After re-watering all the plants
 286 recovered well (Fig 3). Effective quantum yield (Φ_{PSII}) only presented differences among populations; but
 287 did not show differences in response to the treatment, although there were significant differences between
 288 stressed and control plants after the recover treatment in Native population (Table 3, Fig 3). NPQ appears
 289 to be significantly affected by Treatment x Time, regardless of population (Table 3). During the experiment,

290 it seems that NPQ increased with time in stressed plants, and this trend appears similar in all populations,
 291 as seen in Figure 3.



292

293

294 **Fig. 3.** Changes in fluorescence variables in control and stressed plants from the four populations of *O.*
 295 *drummondii* during the water deficit experiment. Shadow represents plant response after re-watering. For
 296 each variable, significant differences between control and stressed plants for a single day are shown with *
 297 ($p < 0.05$) and capital letters evidence temporal differences for each population (control + stressed plants)
 298 along the experiment ($p < 0.05$).

299

300 3.2.2. Gas exchange variables: *A*, *gs*, *WUEi*

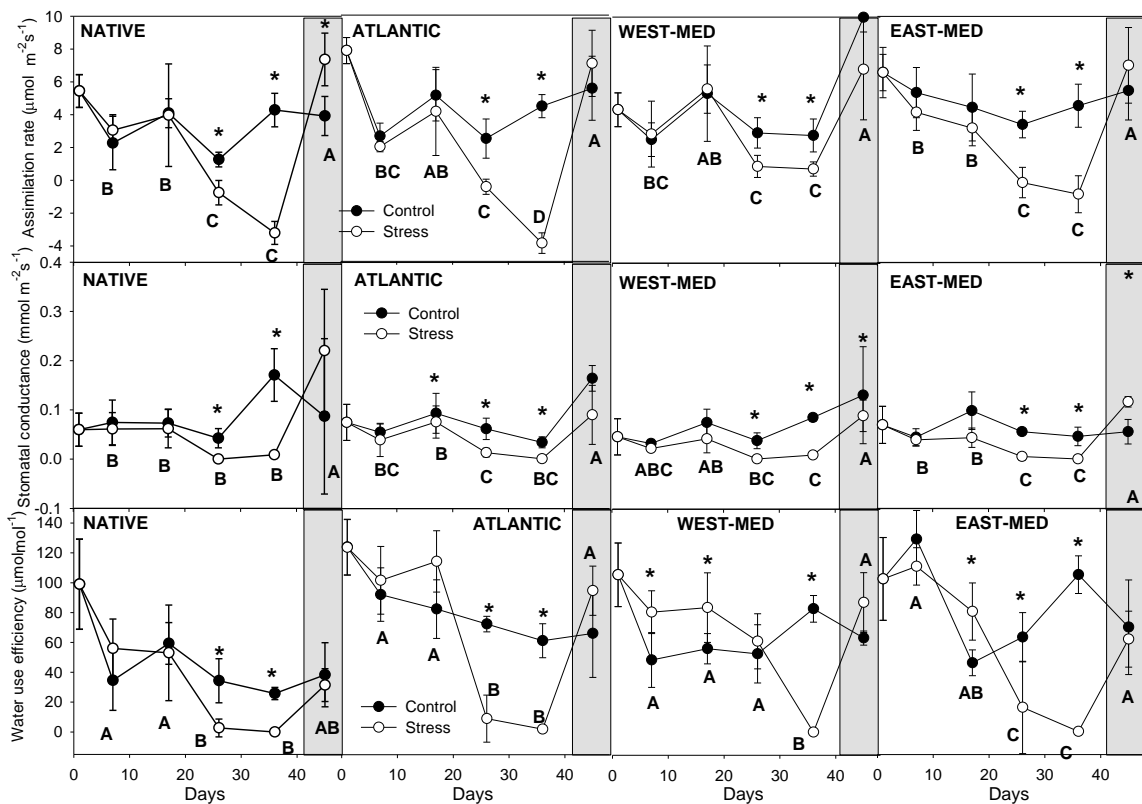
301 The effect of the drought treatment was more evident regarding gas exchange variables.
 302 Photosynthetic CO_2 assimilation (*A*) decreased from day 17 of water deficit and the differences between
 303 control and stressed plants were significant for all populations (Figure 4). The significant interaction Time
 304 x Treatment (Table 3a) evidences as stressed plants reduced *A* with time and this happened for all
 305 populations. However, there is also a significant interaction Time x Population (Table 3a), which suggests
 306 as the temporal response to the treatment is not the same in the 4 populations, with a lower decrease in

307 West-Med populations, as it is well manifested in Figure 4. With the exception of West-Med, carbon
 308 balance was negative for all populations at the last day of water deficit.

309 There were also significant differences in stomatal conductance (g_s) between control and stressed
 310 plants during the treatment and different responses among populations (See Table 3 a and b; Figure 4). It
 311 seems that the different populations exhibited different temporal responses to the water stress treatment, as
 312 it is shown by the significant third order interaction Time x Population x Treatment

313 Although A and g_s decreased in response to water withholding, the effect was stronger in WUEi.
 314 This trait presented significant differences in all the effects of repeated-measures ANOVA (Table 3 a and
 315 b) and significant levels between control and stressed plants at the end of the treatment (Figure 4). The
 316 significant third order interaction is showing as populations exhibited different temporal responses for the
 317 treatment (Table 3a). After re-watering the stressed plants recovered to similar values than control plants.

318 There was a negative significant correlation between NPQ and photosynthetic assimilation ($r=-$
 319 0.436, $p < 0.01$), which suggested that A decreases in response to the increase of NPQ during the water
 320 stress period.



321

322

323 **Fig. 4.** Changes in gas exchange variables in control and stressed plants from the four populations of *O.*
324 *drummondii* during the water deficit experiment. Shadow represents plant response after re-watering. For
325 each variable, significant differences between control and stressed plants for a single day are shown with *
326 ($p < 0.05$) and capital letters evidence temporal differences for each population (control + stressed plants)
327 along the experiment ($p < 0.05$).

328

329 3.2.3. Relative water content

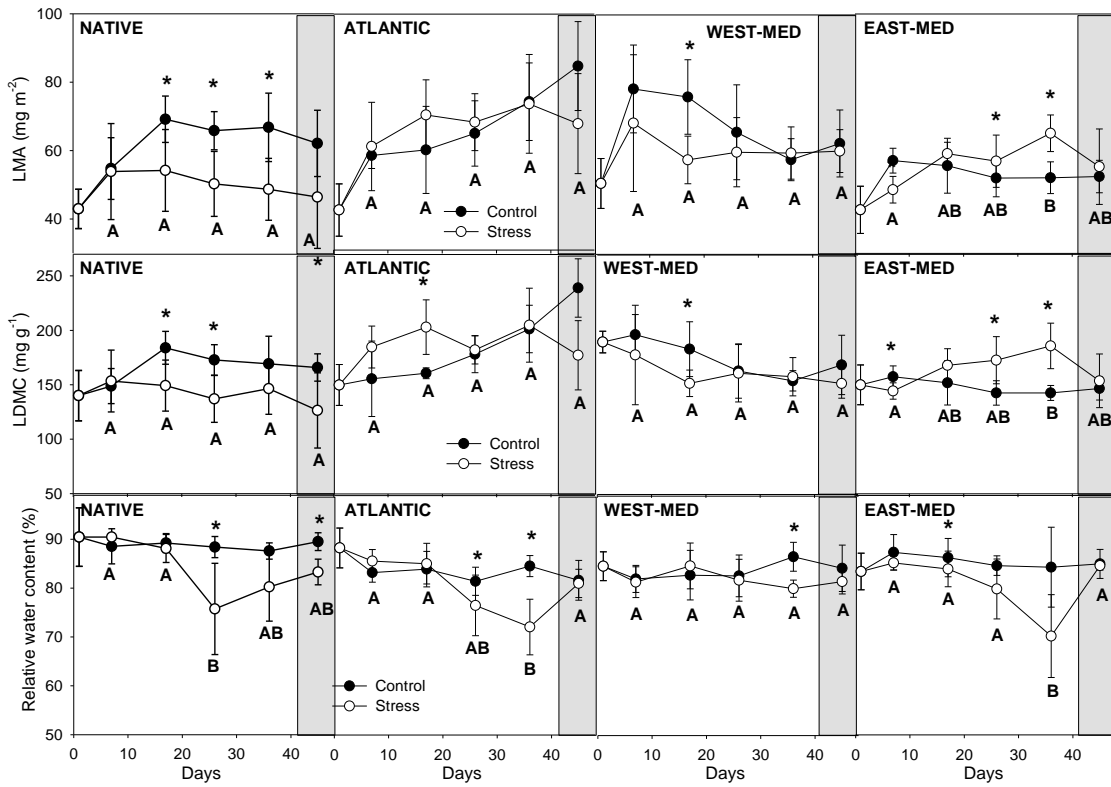
330 RWC descended along the water withholding period in all populations, with significant differences
331 in Time and Time x Treatment (Table 3a) and in Populations and Treatment (Table 3b), which revealed as
332 the for studied populations responded in similar way to the water stress. In spite of this, plants from West-
333 Med population maintained the highest values of RWC along the experiment and which never dropped
334 below 80% along the 36 days of water withholding. In the other populations RWC descended to 70% in the
335 last day of the stress treatment, with significant differences with the control plants (Fig 5). After re-watering,
336 all plants recovered well, except for Native populations in which RWC of stressed plants still maintained
337 significant lower values than control plants. (Fig 5).

338

339 3.2.4. LDMC and LMA

340 Temporal LDMC responses to the water stress were different among populations, as it is shown by
341 the significant interaction Time x Population (Table 3a). It appears that differences in LDMC between
342 control and stressed plants appeared at different times for each population, as it is shown by the significant
343 third order interaction Time x Treatment x Population (Table 3a) and the temporal results of stressed and
344 control plants (Figure 5). It seems that at the end of the drought period differences between control and
345 stressed plants only occurred in East- Med population. After re-watering all stressed plants recovered the
346 values of the control plants (Figure 5).

347 LMA responses to the water stress were different among populations as it is evidenced in Figure 5,
348 only showing significant differences in the interaction Time x Population (Table 3a); it seems that in most
349 populations the overall LMA remained fairly constant through time except for Atlantic populations that
350 seemed to increase. After re-watering all stressed plants recovered the values of the control plants (Figure
351 5).



352

353

354 **Fig. 5.** Changes in LMA, LDMC and RWC in control and stressed plants from the four populations of *O.*
 355 *drummondii* during the water deficit experiment. Shadow represents plant response after re-watering. For
 356 each variable, significant differences between control and stressed plants for a single day are shown with *
 357 ($p < 0.05$) and capital letters evidence temporal differences for each population (control + stressed plants)
 358 along the experiment ($p < 0.05$)

359

360

361

362

363

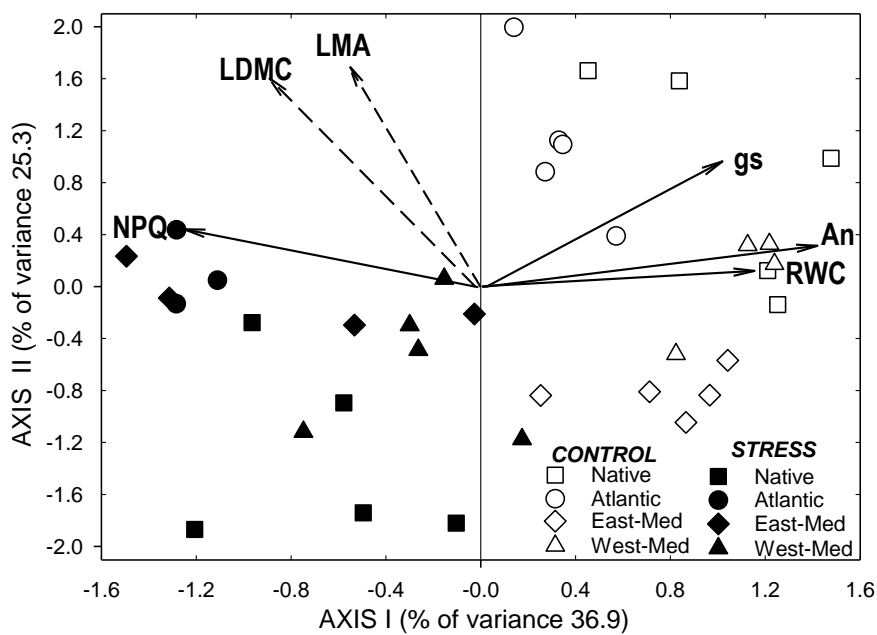
364

365

366

367

368



369 **Fig. 6.** Results of the PCA of the individual physiological and morphological traits at the end of the drought
370 experiment for stressed and control plants. Solid arrows indicate variables correlated with axis 1 and dashed
371 arrows with axis 2 of the analysis.

372

373 3.2.5. Principal component analysis

374 In the axis 1 of PCA, control plants are separated from those subjected to the water deficit treatment,
375 independent of the population origin (36.9% of the total variance). Control plants (located in the positive
376 end of axis 1) are characterized for having higher photosynthetic rates, leaf conductance and RWC, while
377 stressed plants exhibited higher NPQ. However, axis 2 (25.3% total variance) separate the different
378 populations of control plants in relation to leaf traits, especially LMA and LDMC in the positive end of
379 axis 2 (Figure 6). Plants from Native and Atlantic populations are located in the positive end of axis 2,
380 while East-Mediterranean plants are located in the negative end and West-Mediterranean plants are placed
381 in an intermediate position (Figure 6), this result is also supported by the between-subject repeated measures
382 ANOVA, in which there were significant differences among Populations and the interaction Population x
383 Treatment (Table 3b).

384

385 4. Discussion

386 Our results evidence that *O. drummondii* plants maintain singular morphological and functional
387 differences in relation to their original population, despite having germinated and grown under the same
388 environmental conditions of the greenhouse, showing significant differences in stomatal density, LMA and
389 LDMC. Moreover, the four study populations exhibited different responses to the water stress as we have
390 evidenced with the repeated-measures ANOVA, presenting significant differences in different levels of this
391 analysis and significant third order interaction in F_v/F_m , g_s , WUE_i and LDMC; but, all populations showed
392 a high capacity of recover after the period of water withholding.

393 In stressed plants, F_v/F_m decreased during the drought treatment but, the values never dropped below
394 0.750 (observed in Native population in the week 4), suggesting no clear signs of photoinhibition; which
395 are usually considered when they dropped below 0.7 (Maxwell and Johnson, 2000). The significant third-
396 order interaction for this trait revealed as the temporal responses for the water stress were different among
397 the populations. After re-watering, all plants recovered to achieve similar values of the control plants in a
398 ten-day period. Effective quantum yield only presented differences among populations, but did not show

399 significant differences between stressed and control plants along the experiment, which evidenced the
400 absence of photoinhibition. In many studies, low water availability may predispose leaves to
401 photoinhibition, probably through stomatal restriction of CO₂ supply to photosynthetic reaction centers
402 (Álvarez-Cansino et al., 2010; Werner et al., 2002). However, our results suggest the absence of permanent
403 photochemical damage in any population, probably because our plants did not experience an excess of
404 radiation as they were growing under greenhouse conditions. Under field conditions, West-Med populations
405 of *O. drummondii* experienced a significant decrease of photochemical efficiency in winter, probably
406 because low temperatures might cause photoinhibition through an inhibition of electron transport rate
407 (Zunzunegui et al., 2020). These authors concluded that cold photoinhibition might be due to the tropical
408 origin of this species.

409 In terrestrial plants, stomata are key determinants of the trade-off between photosynthetic carbon
410 fixation and water transpired, therefore stomatal control is an efficient mechanism to avoid water loss in
411 many species of arid and semiarid regions (Correia and Ascensão, 2017; Correia and Diaz Barradas, 2000;
412 Díaz-Barradas et al., 2010). In our study, the decrease in stomatal conductance occurred significantly in all
413 populations after 17 days of the beginning of the water deprivation treatment, as a consequence there was
414 a parallel decrease in photosynthetic CO₂ assimilation in all populations, which indicates that
415 photosynthesis is mostly dependent of leaf conductance. Only the population of West-Med maintained a
416 positive carbon balance at the end of the drought period, these results coincide with field studies of *O.*
417 *drummondii* in the same area (Zunzunegui et al., 2020), which emphasizes the capacity of this population
418 to tolerate a prolonged water stress period and may contribute to their success invading Mediterranean
419 dunes (Gallego-Fernández et al., 2019). Although they share the same climate, this tolerance to the
420 reduction in water availability was not found in the East-Med population, probably new field studies would
421 help to solve this question. In our study, due to the more pronounced reduction in CO₂ assimilation than in
422 leaf conductance, WUE_i decrease significantly at the end of drought stress period in the four populations,
423 presenting significant differences at all the levels of the repeated-measures ANOVA.

424 The negative correlation between NPQ and A suggests that *O. drummondii* possess an efficient
425 system of thermal dissipation of the energy, when this energy cannot be assimilated through the electron
426 transport chain. NPQ is a regulatory mechanism of photosynthetic organisms (Demmig-Adams et al., 1996;
427 Sello et al., 2019), but its relative weight is variable and depends on the specific species and environmental
428 niche. The results of this study suggest that, the significant increase of NPQ might contribute for the

429 photochemical regulation of water stressed plants (especially in the case of Atlantic and West-Med
430 populations); this mechanism is achieved by means of thermal dissipation of the excess of energy through
431 xanthophyll cycle (Demmig-Adams et al., 1996; Demmig-Adams and Adams III, 2002; Peñuelas et al.,
432 2004). Further studies would be necessary to find out the role of this cycle in the light responses of *O.*
433 *drummondii*.

434 As it was expected, RWC decreased after one month of water stress treatment and recover well after
435 re-watering. The decrease was similar in Native, Atlantic and East-Med populations reaching values close
436 to 70%, but in the West-Med population RWC only decreased to 80% (significant differences in the
437 interaction Time x Treatment). These results coincide with those of gas exchange, and evidence that West-
438 Med populations presented a higher tolerance to water stress than the other studied populations. Structural
439 traits as LMA and LDMC exhibited fluctuations along the water stress treatment (evidenced by the
440 significant interaction Time x Population and Population x Treatment in the repeated-measures ANOVA),
441 but there was no clear pattern in response to either the water stress or the population origin. We could expect
442 an increase in these traits facing water stress, as occurs in Mediterranean *Cistus* which exhibit significant
443 differences between summer and winter leaves (Correia and Ascensão, 2017), but we couldn't find any
444 clear pattern, probably because we would need more time to find structural changes in leaves.

445 The finding of functional traits that facilitate invasibility is an important topic in ecology. In the case
446 of coastal dunes in Italy, large leaf area, reproductive period in late summer, a biennial/annual life cycle
447 and thick, long roots were useful traits for the successful colonisation of the most invasive species along
448 the mobile coastal dunes (Stanisci et al., 2010). In a common garden experiment, using 15 different
449 genotypes of *Phalaris arundinacea* from both its native (European) and invasive (North American) range,
450 the authors have not found clear physiological differences between native and invasive genotypes. Instead,
451 their results support that morphological traits and defensive secondary compound metabolism may play a
452 more important role in the invasion success of *P. arundinacea* (Brodersen et al., 2008). In our study, the
453 results of the PCA analysis evidenced that under water stress, all the plants experiment an increase in NPQ
454 and a reduction of RWC, A and gs (negative versus positive axis of the PCA), although plants from the 4
455 populations exhibited functional trait variability (with and without drought stress). Regarding control
456 plants, there are some morphological differences (in LMA and LDMC) between Native and Atlantic from
457 East Mediterranean plants.

458 This study also highlights some significant differences between West-Med population and the
459 others, but there are not clear differences between Mediterranean populations and the others. Before water
460 stress treatment, LMA was significantly higher in West-Mediterranean plants, which might be a
461 morphological trait in response to water stress (Correia and Ascensão, 2017). Field studies have revealed
462 that West-Med populations of *O. drummondii* are well acclimated to Mediterranean conditions exhibiting
463 lower LDMC, higher assimilation rates and water performance than some native species (Zunzunegui et
464 al., 2020). This site corresponds to a recent deposition dune area in which *O. drummondii* has spread over
465 all the gradient from the beach to inner dunes, reaching the highest density of 4.5 ± 1.9 individuals. m⁻² in
466 the inner dunes (Gallego-Fernández et al., 2019). It is estimated that the total number of individuals, in this
467 area, ranges between 3-4 million plants extended over 100 hectares, but unfortunately, we don't have
468 demographic data from the East-Med population, although the population densities are much lower than in
469 SW Spain (Bar Kutiel, personal communication).

470 In spite of having a tropical or sub-tropical origin, the results of this study suggest that *O.*
471 *drummondii* is a drought-tolerant species, with high recovery capacity and exhibiting different functional
472 trait variability in relation to population origin.

473

474 **5. Conclusion**

475 Our results largely confirm the ability of *O. drummondii* to modify its physiological traits to local
476 environmental characteristics, and these modified traits remain when we grow plants from different
477 biogeographical origins under the same greenhouse conditions. Our initial hypothesis that plants growing
478 in Mediterranean climate regions can survive water deficits more effectively than those from wet climates
479 is not totally supported by our results. In spite of West-Med plants presenting better physiological status
480 under water stress, East-Med population did not turn out to be better adapted to water deficit than the native
481 population. *O. drummondii* ability to thrive in a wide range of habitats modifying functional traits might be
482 the clue for its invasive capacity, becoming an aggressive invasive species of coastal dunes in a wide climate
483 range.

484

485

486

487

488 **Author contribution statement**

489 All the authors have contributed in a similar way to the development of the experimental work and the
490 manuscript.

491

492 **Acknowledgments**

493 We thank Pua Bar Kutiel (Israel) and Marta Torca (Zarautz) for seed collection. We thank the Green House
494 Service of the Seville University for their support and two students Angela Fraidias and Ursula García
495 Conde for their collaboration in the experimental work. We thank an anonymous referee for his/her valuable
496 advices which have contributed for the improving of the manuscript. This study was funded by the
497 Ministerio de Economía y Competitividad (MINECO Project CGL2015-65058-R co-funded by FEDER).

498

499 **References**

500 Álvarez-Cansino, L., Zunzunegui, M., Díaz Barradas, M.C., Esquivias, M.P., 2010. Physiological
501 performance and xylem water isotopic composition underlie gender-specific responses in the
502 dioecious shrub *Corema album*. *Physiol. Plant.* 140, 32–45. [https://doi.org/10.1111/j.1399-](https://doi.org/10.1111/j.1399-3054.2010.01382.x)
503 [3054.2010.01382.x](https://doi.org/10.1111/j.1399-3054.2010.01382.x)

504 Antunes, C., Pereira, A.J., Fernandes, P., Ramos, M., Ascensão, L., Correia, O., Máguas, C., 2018.
505 Understanding plant drought resistance in a Mediterranean coastal sand dune ecosystem: Differences
506 between native and exotic invasive species. *J. Plant Ecol.* 11, 26–38.
507 <https://doi.org/10.1093/jpe/rtx014>

508 Brodersen, C., Lavergne, S., Molofsky, J., 2008. Genetic variation in photosynthetic characteristics among
509 invasive and native populations of reed canarygrass (*Phalaris arundinacea*). *Biol. Invasions* 10,
510 1317–1325. <https://doi.org/10.1007/s10530-007-9206-x>

511 Brook, B.W., Sodhi, N.S., Bradshaw, C.J.A., 2008. Synergies among extinction drivers under global
512 change. *Trends Ecol. Evol.* 23, 453–460. <https://doi.org/10.1016/j.tree.2008.03.011>

513 Castillo, S.A., Moreno-Casasola, P., 1996. Coastal sand dune vegetation: An extreme case of species
514 invasion. *J. Coast. Conserv.* 2, 13–22. <https://doi.org/10.1007/BF02743033>

515 Castro-Díez, P., Godoy, O., Alonso, A., Gallardo, A., Saldaña, A., 2014. What explains variation in the
516 impacts of exotic plant invasions on the nitrogen cycle? A meta-analysis. *Ecol. Lett.* 17, 1–12.
517 <https://doi.org/10.1111/ele.12197>

518 Correia, O., Ascensão, L., 2017. Summer semi-deciduous species of the Mediterranean landscape: a
519 winning strategy of *Cistus* species to face the predicted changes of the Mediterranean climate., in:
520 Plant Biodiversity: Monitoring, Assessment and Conservation. pp. 195–217.
521 <https://doi.org/10.1079/9781780646947.0195>

522 Correia, O., Diaz Barradas, M.C., 2000. Ecophysiological differences between male and female plants of
523 *Pistacia lentiscus* L. *Plant Ecol.* 149. <https://doi.org/10.1023/A:1026588326204>

524 Crous, C.J., Jacobs, S.M., Esler, K.J., 2012. Drought-tolerance of an invasive alien tree, *Acacia mearnsii*
525 and two native competitors in fynbos riparian ecotones. *Biol. Invasions* 14, 619–631.
526 <https://doi.org/10.1007/s10530-011-0103-y>

527 Davidson, A.M., Jennions, M., Nicotra, A.B., 2011. Do invasive species show higher phenotypic plasticity
528 than native species and, if so, is it adaptive? A meta-analysis. *Ecol. Lett.* 14, 419–431.
529 <https://doi.org/10.1111/j.1461-0248.2011.01596.x>

530 Demmig-Adams, B., Adams III, W.W., 2002. Antioxidants in photosynthesis and human nutrition. *Science*
531 298, 2149–53. <https://doi.org/10.1126/science.1078002>

532 Demmig-Adams, B., Gilmore, A.M., Adams, W.W., 1996. In vivo functions of carotenoids in higher plants.
533 *Faseb J.* 10, 403–412. <https://doi.org/10.1586/14760584.2014.862499>

534 Díaz-Barradas, M.C., Zunzunegui, M., Ain-Lhout, F., Jáuregui, J., Boutaleb, S., Álvarez-Cansino, L.,
535 Esquivias, M.P., 2010. Seasonal physiological responses of *Argania spinosa* tree from Mediterranean
536 to semi-arid climate. *Plant Soil* 337, 217–231. <https://doi.org/10.1007/s11104-010-0518-8>

537 Díaz-Barradas, M.C., Zunzunegui, M., Álvarez-Cansino, L., Esquivias, M.P., Collantes, M.B., Cipriotti,
538 P.A., 2015. Species-specific effects of the invasive *Hieracium pilosella* in Magellanic steppe
539 grasslands are driven by nitrogen cycle changes. *Plant Soil* 397. [https://doi.org/10.1007/s11104-015-](https://doi.org/10.1007/s11104-015-2608-0)
540 2608-0

541 Ehrenfeld, J.G., 2010. Ecosystem Consequences of Biological Invasions. *Annu. Rev. Ecol. Evol. Syst.* 41,
542 59–80. <https://doi.org/10.1146/annurev-ecolsys-102209-144650>

543 Funk, J.L., Vitousek, P.M., 2007. Resource-use efficiency and plant invasion in low-resource systems.
544 *Nature* 446, 1079–1081. <https://doi.org/10.1038/nature05719>

545 Gallego-Fernández, J.B., Martínez, M.L., García-Franco, J.G., Zunzunegui, M., 2019. The impact on plant
546 communities of an invasive alien herb, *Oenothera drummondii*, varies along the beach-coastal dune
547 gradient. *Flora* 260, 151466. <https://doi.org/10.1016/j.flora.2019.151466>

548 García-Mora, M.R., Gallego-Fernández, J.B., García-Novo, F., 1999. Plant functional types in coastal
549 foredunes in relation to disturbance. *J. Veg. Sci.* 10, 27-34.

550 Garnier, E., Shipley, B., Roumet, C., Laurent, G., Shipley, T.B., 2001. A Standardized Protocol for the
551 Determination of Specific Leaf Area and Leaf Dry Matter A standardized protocol for the
552 determination of specific leaf area and leaf dry matter content. *Funct. Ecol.* 15, 688–695.
553 <https://doi.org/10.1046/j.0269-8463.2001.00563.x>

554 Genty, B., Briantais, J.M., Baker, N.R., 1989. The relationship between the quantum yield of photosynthetic
555 electron transport and quenching of chlorophyll fluorescence. *Biochim. Biophys. Acta - Gen. Subj.*
556 990, 87–92. [https://doi.org/10.1016/S0304-4165\(89\)80016-9](https://doi.org/10.1016/S0304-4165(89)80016-9)

557 Hesp, P.A., 1991. Ecological processes and plant adaptations on coastal dunes. *J. Arid Environ.* 21, 165–
558 191. [https://doi.org/10.1016/s0140-1963\(18\)30681-5](https://doi.org/10.1016/s0140-1963(18)30681-5)

559 Jørgensen, R.H., Kollmann, J., 2009. Invasion of coastal dunes by the alien shrub *Rosa rugosa* is associated
560 with roads, tracks and houses. *Flora Morphol. Distrib. Funct. Ecol. Plants* 204, 289–297.
561 <https://doi.org/10.1016/j.flora.2008.03.002>

562 Liao, C., Peng, R., Luo, Y., Zhou, X., Wu, X., Fang, C., Chen, J., Li, B., 2008. Altered ecosystem carbon
563 and nitrogen cycles by plant invasion: A meta-analysis. *New Phytol.* 177, 706–714.
564 <https://doi.org/10.1111/j.1469-8137.2007.02290.x>

565 Maxwell, K., Johnson, G.N., 2000. Chlorophyll fluorescence—a practical guide. *J. Exp. Bot.* 51, 659–668.
566 <https://doi.org/10.1093/jxb/51.345.659>

567 Morais, M.C., Freitas, H., 2012. The acclimation potential of *Acacia longifolia* to water stress: Implications
568 for invasiveness. *Plant Sci.* 196, 77–84. <https://doi.org/10.1016/j.plantsci.2012.08.007>

569 Mozdzer, T.J., Zieman, J.C., 2010. Ecophysiological differences between genetic lineages facilitate the
570 invasion of non-native *Phragmites australis* in North American Atlantic coast wetlands. *J. Ecol.* 98,
571 451–458. <https://doi.org/10.1111/j.1365-2745.2009.01625.x>

572 Naidoo, G., Naidoo, K.K., 2018. Drought stress effects on gas exchange and water relations of the invasive
573 weed *Chromolaena odorata*. *Flora Morphol. Distrib. Funct. Ecol. Plants* 248, 1–9.
574 <https://doi.org/10.1016/j.flora.2018.08.008>

575 Novoa, A., González, L., Moravcová, L., Pyšek, P., 2013. Constraints to native plant species establishment
576 in coastal dune communities invaded by *Carpobrotus edulis*: Implications for restoration. *Biol.*
577 *Conserv.* 164, 1–9. <https://doi.org/10.1016/j.biocon.2013.04.008>

578 Paini, D.R., Sheppard, A.W., Cook, D.C., De Barro, P.J., Worner, S.P., Thomas, M.B., 2016. Global threat
579 to agriculture from invasive species. *Proc. Natl. Acad. Sci.* 113, 7575–7579.
580 <https://doi.org/10.1073/pnas.1602205113>

581 Pardini, E.A., Vickstrom, K.E., Knight, T.M., 2015. Early successional microhabitats allow the persistence
582 of endangered plants in coastal sand dunes. *PLoS One* 10, 1–15.
583 <https://doi.org/10.1371/journal.pone.0119567>

584 Peñuelas, J., Munne-Bosch, S., Llusia, J., Filella, I., 2004. Leaf reflectance and pho- and antioxidant
585 protection in field-grown summer-stressed *Phillyrea* of oxidative Optical signals of oxidative stress?
586 *New Phytol.* 162, 115–124. <https://doi.org/10.1111/j.1469-8137.2004.01007.x>

587 Potvin, C., Lechowicz, M.J., Tardif, S., 1990. The Statistical Analysis of Ecophysiological Response
588 Curves Obtained from Experiments Involving Repeated Measures. *Ecology* 71, 1389–1400.
589 <https://doi.org/10.2307/1938898>

590 Pyšek, P., Jarošík, V., Hulme, P.E., Pergl, J., Hejda, M., Schaffner, U., Vilà, M., 2012. A global assessment
591 of invasive plant impacts on resident species, communities and ecosystems: The interaction of impact
592 measures, invading species' traits and environment. *Glob. Chang. Biol.* 18, 1725–1737.
593 <https://doi.org/10.1111/j.1365-2486.2011.02636.x>

594 Pyšek, P., Richardson, D.M., 2010. Invasive Species, Environmental Change and Management, and Health.
595 *Annu. Rev. Environ. Resour.* 35, 25–55. <https://doi.org/10.1146/annurev-environ-033009-095548>

596 Rascher, K.G., Große-Stoltenberg, A., Máguas, C., Werner, C., 2011. Understory Invasion by *Acacia*
597 *longifolia* Alters the Water Balance and Carbon Gain of a Mediterranean Pine Forest. *Ecosystems*
598 14, 904–919. <https://doi.org/10.1007/s10021-011-9453-7>

599 Richardson, D.M., Pyšek, P., 2006. Plant invasions: Merging the concepts of species invasiveness and
600 community invasibility. *Prog. Phys. Geogr.* 30, 409–431.
601 <https://doi.org/10.1191/0309133306pp490pr>

602 Santoro, R., Jucker, T., Carboni, M., Acosta, A.T.R., 2012. Patterns of plant community assembly in
603 invaded and non-invaded communities along a natural environmental gradient. *J. Veg. Sci.* 23, 483–
604 494. <https://doi.org/10.1111/j.1654-1103.2011.01372.x>

605 Sello, S., Meneghesso, A., Alboresi, A., Baldan, B., Morosinotto, T., 2019. Plant biodiversity and regulation
606 of photosynthesis in the natural environment. *Planta* 249, 1217–1228.
607 <https://doi.org/10.1007/s00425-018-03077-z>

608 Stanisci, A., Acosta, A.T.R., Di Iorio, A., Vergalito, M., 2010. Leaf and root trait variability of alien and
609 native species along Adriatic coastal dunes (Italy). *Plant Biosyst.* 144, 47–52.
610 <https://doi.org/10.1080/11263500903454252>

611 Vilagrosa, A., Hernández, E.I., Luis, V.C., Cochard, H., Pausas, J.G., 2013. Physiological differences
612 explain the co-existence of different regeneration strategies in Mediterranean ecosystems. *New*
613 *Phytol.* 201, 1277–1288. <https://doi.org/10.1111/nph.12584>

614 Vitousek, P.M., Mooney, H. a, Lubchenco, J., Melillo, J.M., 1997. Human Domination of Earth' s
615 Ecosystems. *Science* (80-.). 277, 494–499. <https://doi.org/10.1126/science.277.5325.494>

616 Werner, C., Correia, O., Beychlag, W., 2002. Characteristic patterns of chronic and dynamic
617 photoinhibition of different functional groups in a Mediterranean ecosystem. *Funct. Plant Biol.* 29,
618 999–1011.

619 Westoby, M., Falster, D.S., Moles, A.T., Vesk, P.A., Wright, I.J., 2002. Plant Ecological Strategies: Some
620 Leading Dimensions of Variation Between Species. *Annu. Rev. Ecol. Syst.* 33, 125–159.
621 <https://doi.org/10.1146/annurev.ecolsys.33.010802.150452>

622 Zunzunegui, M., Ruiz-Valdepeñas, E., Sert, M.A., Díaz-Barradas, M.C., Gallego-Fernández, J.B., 2020.
623 Field comparison of ecophysiological traits between an invader and a native species in a
624 Mediterranean coastal dune. *Plant Physiol. Biochem.* 146, 278–286.
625 <https://doi.org/10.1016/j.plaphy.2019.11.032>

626

627