

1 **Transpiration, photosynthetic responses, tissue water relations and dry mass**  
2 **partitioning in *Callistemon* plants during drought conditions**

3

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12 **Running title:** Adaptation of *Callistemon* to drought

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24 **ABSTRACT**

25 *Callistemon* is an Australian species used as ornamental plant in Mediterranean regions.  
26 The objective of this research was to analyse the ability of *Callistemon* to overcome  
27 water deficit in terms of adjusting its physiology and morphology. Potted *Callistemon*  
28 *laevis* Anon plants were grown in controlled environment and subjected to drought  
29 stress by reducing irrigation water by 40% compared to the control (irrigated to  
30 container capacity). The drought stress produced the smallest plants throughout the  
31 experiment. After three months of drought, the leaf area, number of leaves and root  
32 volume decreased, while root/shoot ratio and root density increased. The higher root  
33 hydraulic resistance in stressed plants caused decreases in leaf and stem water potentials  
34 resulting in lower stomatal conductance and indicating that water flow through the roots  
35 is a factor that strongly influences shoot water relations. The water stress affected  
36 transpiration (63% reduction compared with the control). The consistent decrease in  $g_s$   
37 suggested an adaptative efficient stomatal control of transpiration by this species,  
38 resulting in a higher intrinsic water use efficiency ( $P_n/g_s$ ) in drought conditions,  
39 increasing as the experimental time progressed. This was accompanied by an  
40 improvement in water use efficiency of production to maintain the leaf water status. In  
41 addition, water stress induced an active osmotic adjustment and led to decreases in leaf  
42 tissue elasticity in order to maintain turgor. Therefore, the water deficit produced  
43 changes in plant water relations, gas exchange and growth in an adaptation process  
44 which could promote the faster establishment of this species in gardens or landscaping  
45 projects in Mediterranean conditions.

46 **Key words:** Water stress; Potted *Callistemon citrinus*; Gas exchange; Pressure-  
47 volume curves

48

49      **Abbreviations:** ET, evapotranspiration;  $F_v/F_m$ , efficiency photosystem II;  $g_s$ ,  
50 stomatal conductance; L, root length;  $P_n$ , net photosynthesis;  $RWC_{tlp}$ , relative water  
51 content at turgor loss point; T, transpiration;  $\epsilon$ , bulk modulus of elasticity;  $\Psi_l$ , leaf water  
52 potential;  $\Psi_{os}$ , leaf osmotic potential at full turgor;  $\Psi_s$ , stem water potential;  $\Psi_{tlp}$ , leaf  
53 water potential at turgor loss point;  $1/Lp$ , root hydraulic resistance.  
54

55 **1. Introduction**

56

57 *Callistemon* belongs to the Myrtaceae family and is the most important Australian  
58 ornamental species, which shown interesting characteristics (rapid growth, abundant  
59 flowering with unusual shapes and brilliant colours and great variety of forms and  
60 volumes) (Mitchem, 1993; Lao and Jiménez, 2002). Most *Callistemon* species have  
61 adapted and been used in Mediterranean conditions, where they show some degree of  
62 tolerance to environmental stresses such as drought (Lippi et al., 2005). They are also  
63 known for their high salt tolerance (Lippi et al., 2003). It is for these reasons that  
64 *Callistemon* has enjoyed considerable success as a flowering shrub for use in gardens  
65 and urban landscaping in the Mediterranean area. However, the prolonged water stress  
66 resulting from low rainfall and high temperatures in summer in this area may alter the  
67 plant's physiological and morphological behaviour, especially in imported species. Such  
68 changes may involve complex functional and structural adaptations to increase the  
69 drought tolerance of the plant: These include plant growth regulation, osmotic  
70 adjustment, decreased stomatal conductance, and changes in the elastic properties  
71 (Zollinger et al., 2006), all of which may improve the plant water status and the  
72 resistance of plants to water stress by limiting water loss in the face of high evaporative  
73 demand (Sánchez-Blanco et al., 2004). However, drought-stress can also decrease  
74 photosynthetic rates and shoot and leaf growth or delay and reduce flower numbers, size  
75 and/or quality (Cameron et al., 1999) which would affect the plant's visual appeal, a  
76 particularly important factor in ornamental plants destined for use in gardens and  
77 landscaping.

78 In these conditions, irrigation management is an important factor and there is  
79 considerable pressure on the ornamental plant industry to produce crops more

80 efficiently, reducing the quantity of water regimes (Sweatt and Davies, 1984) without  
81 losing ornamental characteristics (Cameron et al., 2006). In this sense, monitoring  
82 nursery moisture regimes and understanding morphological and physiological shoot and  
83 root responses of seedlings to water management are critical for optimising the  
84 production of high-quality seedlings (Franco et al., 2006). However, little is known  
85 about these responses in “foreign” Mediterranean ornamental shrubs such as  
86 *Callistemon*.

87         The objective of this study was to evaluate the physiological and whole plant  
88 response of *Callistemon laevis* in control and drought plants under controlled conditions  
89 to contrasting irrigation treatments. The results are evaluated in terms of biomass  
90 partitioning, water use efficiency and water relations to understand the adaptative role  
91 of this species to drought stress.

92

## 93 **2. Materials and methods**

94

### 95 *2.1. Plant material and experimental conditions*

96

97         Six month old rooted cuttings of Australian *Callistemon laevis* Anon  
98 (*Callistemon citrinus splendens* Stapf) were transplanted into 14 cm × 12 cm pots (1.2  
99 L) filled with a mixture of coconut fibre, black peat and perlite (2:1:1) and amended  
100 with osmocote plus (2 g L<sup>-1</sup> substrate) (14:13:13 N,P,K + microelements). The  
101 experiment was carried out in a growth chamber. The environmental conditions of the  
102 chamber for plant growth were selected to simulate natural changes in temperature and  
103 photosynthetic photon flux density. Both parameters gradually increased from 6:00 h to  
104 13:00 h, reaching values of 28 °C and 350 μmol m<sup>-2</sup> s<sup>-1</sup> and then progressively decreased

105 until 20 °C and darkness (22:00 h). The relative humidity ranged between 40 and 60%.  
106 Although the radiation levels present in the growth chamber were lower than those  
107 experienced by the studied plants in the field, we assumed that the specific  
108 photosynthetic active radiation levels used were of secondary importance compared to  
109 the contrast of irrigation treatments. Thus, the results of light response curves to  
110 quantify the degree of limitation in the photosynthesis response of this species showed  
111 high levels of photosynthesis for the light intensity used in this assay, close to half of  
112 the maximum photosynthesis at saturating light.

113

## 114 2.2. *Treatments*

115

116 The plants were watered daily to container capacity during the two weeks prior to  
117 starting the treatments.

118 Plants were grouped into four repetitions (n=4) of eight plants per treatment (64  
119 plants in total, 32 per treatment) and were submitted to two irrigation treatments:  
120 container capacity (control) and drought treatment.

121 To determine the substrate maximum water holding capacity, three samples were  
122 uniformly mixed and packed to a bulk density of 0.165 g cm<sup>-3</sup>. The pots' surfaces were  
123 covered with aluminium foil to prevent water evaporation and the lower parts were  
124 submerged, to half the pot's height, in a water bath and then were left to equilibrate  
125 overnight. The next day, the pots were removed and left to freely drain until drainage  
126 became negligible. Afterward, the fresh weight was recorded and then the substrate was  
127 introduced inside an oven at 105 °C until constant weight. Later, the difference between  
128 the fresh weight and oven-dry weight was measured and consequently a volumetric  
129 water content of 54% was calculated and considered as the substrate's field capacity.

130 In the control treatment, substrate moisture was maintained above and close to  
131 container capacity by daily irrigation. Thus, no significant drainage was obtained in all  
132 days of the experiment. Drought treatment was maintained close to 50% to container  
133 capacity, also by daily irrigation. During the experimental period, drought plants  
134 received around 40% of the amount of water compared with the control treatment. The  
135 water added to each pot during the experimental period was 12.71 L and 5.03 L for  
136 control and drought plants, respectively. The electrical conductivity of water applied  
137 was 0.5 dS m<sup>-1</sup>.

138

### 139 *2.3. Biomass accumulation and electrolyte leakage*

140

141 At the end of both irrigation treatments, five plants per treatment were harvested. The  
142 substrate was gently washed from roots, and the plants were divided into shoots (stems  
143 and leaves) and roots. Leaf numbers and leaf areas were determined using a leaf area  
144 meter (Delta-T Devices Ltd., Cambridge, UK), in the same five plants per treatment.  
145 These were oven dried at 70 °C until they reached a constant mass to measure the  
146 respective dry weights. The roots were cleaned by low pressure water applied through a  
147 flat nozzle. The cleaned root systems were then placed in a metacrylate tray coupled to a  
148 double scanner connected to a computer with a Root System Analyser (Winrhizo LA  
149 1600 Regent Inc., USA). The root systems were put in an oven to dry immediately after  
150 the root length measurements. Root density was determined by dividing the dry weight  
151 by root volume.

152 Plant height was measured weekly during the experimental period and the relative  
153 growth rate was calculated as the rate of increase of height per unit of initial plant  
154 height.

155 The rates of passive ion leakage from stress-sensitive plant tissue can be used as a  
156 measure of alterations of membrane permeability. In our case, ion leakage was  
157 estimated at the end of the experiment, according to the method described by Lafuente  
158 et al., (1991). Thirty leaf discs, each 2 mm in diameter, from each plant, with eight  
159 replicates per treatment, were pooled and incubated in 10 mL 0.3 M mannitol in a 50  
160 mL centrifuge tube. The tubes were shaken at 120 cycles  $\text{min}^{-1}$  and the conductivity of  
161 the solution was measured after 24 h using a Crison Model 524 digital conductivity  
162 meter (Crison Instruments S.A., Barcelona, Spain). Tubes containing the solution were  
163 weighed and heated to boiling for 10 min. After cooling to room temperature, while still  
164 shaking, deionised water was added to restore their initial weight and the total  
165 conductivity was measured after an additional 0.5 h of shaking. Ion leakage rates were  
166 expressed as a percentage of the total conductivity.

167

#### 168 *2.4. Transpiration, stomatal conductance and photosynthesis responses*

169

170 Transpiration (T) and evapotranspiration (ET) were measured gravimetrically  
171 throughout the experimental period, being determined from the difference in weights  
172 (weight after irrigation and weight before irrigating again) using a balance (capacity 5.2  
173 kg and accuracy of 0.01 g, Sartorius, model 5201). Transpiration was measured in three  
174 plants per treatment, in which the surface substrate was covered to avoid loss through  
175 evaporation. Three pots of the same treatment were placed on a balance with a MITRA  
176 programmer that recorded the weight every half an hour. Transpiration and  
177 evapotranspiration were similar due to evaporation from the soil was very low. The pots  
178 had small surface substrate in relation with total leaf area and consequently soil  
179 evaporation was lower than 2% ET.



180 Water use efficiency of production (WUE) was calculated at the end of the  
181 experiment by dividing the increment in the aerial dry weight by the water used (g aerial  
182 dry weight per liter water).

183 Stomatal conductance ( $g_s$ ) and the net photosynthetic rate ( $P_n$ ) were determined in  
184 five plants during the hours of maximum illumination using a gas exchange system (LI-  
185 6400, LI-COR Inc., Lincoln, NE, USA).

186 The values of chlorophyll fluorescence on the adaxial leaf surface were taken after  
187 exposing the leaves to dark for 20 min (Camejo et al., 2005). The values of  $F_v/F_{vm}$  were  
188 read directly in the fluorometer (OS-30 OptiScience Inc., Tyngsboro, MA, USA).

189

## 190 2.5. *Plant water relations*

191

192 During the experiment, leaf water potential ( $\Psi_l$ ) and stem water potential ( $\Psi_s$ ) were  
193 measured in five plants per treatment.  $\Psi_l$  was measured in mature leaves, which were  
194 exposed to direct light for at least 1 h before measurement.  $\Psi_l$  was estimated according  
195 to the method described by Scholander et al., (1965), using a pressure chamber (Soil  
196 Moisture Equipment Co, Santa Barbara, CA, USA), for which leaves were placed in the  
197 chamber within 20 s of collection and pressurised at a rate of 0.02 MPa s<sup>-1</sup> (Turner,  
198 1988).

199  $\Psi_s$  was measured on non-transpiring leaves that had been bagged with both a plastic  
200 sheet and aluminium foil for at least 1 h before measurement in order to prevent leaf  
201 transpiration, in this way leaf water potential equalled stem water potential (Begg and  
202 Turner, 1970). Measurements were made in five plants per treatment.

203 Estimates of the bulk modulus of elasticity ( $\epsilon$ ), leaf osmotic potential at full turgor  
204 ( $\Psi_{os}$ ), leaf water potential at turgor loss point ( $\Psi_{tlp}$ ) and relative water content at turgor

205 loss point ( $RWC_{t\text{lp}}$ ) were obtained at the end of the different irrigation treatments in  
206 three leaves per plant and five plants per treatment, via pressure-volume analysis of  
207 leaves, as outlined by Wilson et al., (1979). The bulk modulus of elasticity ( $\epsilon$ ) at 100%  
208 relative water content was calculated using the formula:

$$209 \quad \epsilon = (RWC_{t\text{lp}} \times \Psi_{os}) / (100 - RWC_{t\text{lp}})$$

210 where  $\epsilon$  is expressed in MPa,  $\Psi_{os}$  is the osmotic potential at full turgor (MPa) and  
211  $RWC_{t\text{lp}}$  is the relative water content at turgor loss point.

212 Leaves were excised in the dark, placed in plastic bags and allowed to reach full  
213 turgor by dipping the petioles in distilled water overnight. Pressure-volume curves were  
214 obtained from periodic measurements of leaf weight and balance pressure as leaves  
215 dried on the bench at constant temperature of 20 °C. The leaf drying period for each  
216 curve was about 3-5 h.

217 Hydraulic resistance ( $1/Lp$ ) was determined at the end of the experimental period in  
218 five plants per treatment as the inverse of the root hydraulic conductivity ( $Lp$ ), measured  
219 according to Ramos and Kaufmann (1979). Plants were de-topped and the substrate was  
220 carefully washed from the roots, which were submerged in a container of water and  
221 placed in the pressure chamber with the cut stump exposed. The air pressure in the  
222 chamber was increased at an approx. rate of 0.4 MPa  $\text{min}^{-1}$ , up to a final pressure of 0.8  
223 MPa. A small piece of plastic tubing was fitted to the stump and the exudate was  
224 collected every 5 min and its volume measured. After the exudation measurements, the  
225 root systems were placed in an oven at 80 °C until they reached a constant dry weight.  
226 Root hydraulic conductivity was calculated using the formula:

$$227 \quad Lp = J / (P \times W)$$

228 where  $L_p$  is expressed in  $\text{mg g}^{-1} \text{s}^{-1} \text{MPa}^{-1}$ ,  $P$  is the applied hydrostatic pressure  
229 (MPa),  $W$  is the dry weight of the root system (in g), and  $J$  is the water flow rate through  
230 the entire root system (in  $\text{mg s}^{-1}$ ).

231

## 232 2.6. Statistical analyses

233

234 The data were analysed by one-way ANOVA using Statgraphics Plus for Windows  
235 5.1 Software (Manugistics Ltd., Rockville, MD, USA). Ratio and percentage data were  
236 subjected to an arcsine square-root transformation before statistical analysis to ensure  
237 homogeneity of variance Treatment means were separated with Duncan's Multiple  
238 Range Test ( $P \leq 0.05$ ).

239

## 240 3. Results

241

### 242 3.1. Biomass accumulation and electrolyte leakage

243

244 Water deficit was seen to have significantly altered *Callistemon* plant growth by the  
245 end of the experiment; although the changes differed depending on the plant organ  
246 studied (Fig. 1). The greatest accumulation of dry matter in relation to total plant dry  
247 matter was seen in the leaves of the control plants and in the roots of stressed plants.  
248 The total dry matter of drought-treated plants was 47% of the control values (Table 1);  
249 both total leaf area and the number of leaves decreased to 41 and 50%, respectively,  
250 compared with control plants. However, the root/shoot ratio increased in the plants  
251 grown under drought conditions. Water deficit had a significant effect on root  
252 morphology (Table 2). Total root length decreased with water stress (27%), a reduction

253 observed in all sizes of root. In relation to the root distribution, water deficit increased  
254 the percentage of fine roots and decreased those with a diameter higher than 0.5 mm. In  
255 general, stressed plants showed a reduced root volume, although root dry weight was  
256 not modified, with the result that root density increased.

257 Plant height was significantly inhibited from the beginning of the deficit treatment  
258 (Fig. 2A), which produced the smallest plants throughout the experiment. At the end of  
259 the experiment the reductions were around 30% compared with the control. A similar  
260 pattern in the relative growth rate for both treatments was observed (Fig. 2B) and two  
261 growth periods being evident during the experiment (8 and 14 weeks), although the  
262 stressed plants showed a certain delay compared with the control.

263 Membrane damage, assessed by ion leakage of the control and drought-exposed  
264 plants was significantly higher in the latter (Table 1).

265

### 266 *3.2. Transpiration, stomatal conductance and photosynthesis responses*

267

268 The evolution of transpiration (T) along the study period is showed in Fig. 3. In the  
269 control, daily T values fluctuated throughout the experiment between 43 mL d<sup>-1</sup> and 150  
270 mL d<sup>-1</sup> (Fig. 3A). Daily T values increase coinciding with the two growth periods  
271 (Figure 2A). As regards cumulative data, the transpiration at the beginning of the  
272 experiment (1-7 weeks) was close to 60% that the level reached throughout the rest of  
273 the experimental period. In contrast, in the drought treatment, the daily T level was  
274 more maintained in the experiment reaching mean values of around 47 ml/d (about 40%  
275 of the control). The behaviour of the transpiration rate on a representative day of the  
276 period can be seen in Fig. 3B. T was higher during the morning and decreased during  
277 the afternoon. The highest value was reached between 13 and 17 h especially in control

278 plants (7.2 mL per 30 min), coinciding with the highest temperature (28 °C) after which,  
279 transpiration decreased. In drought plants, the transpiration curve was more stable  
280 throughout the day, independently of temperature changes. Daily accumulated T  
281 showed a maximum of 150 mL pot<sup>-1</sup> and 47 mL pot<sup>-1</sup> in the control and drought  
282 treatments, respectively.

283 Water stress affected stomatal functionality (Fig. 4): stomatal conductance ( $g_s$ ) and  
284 the net photosynthetic rate ( $P_n$ ) decreased in drought-exposed plants in relation to the  
285 control treatment, although intrinsic water use efficiency was different in each  
286 treatment. The values of  $P_n/g_s$  in the water deficit plants were higher than those of the  
287 control plants throughout the experimental period. As the experimental time progressed  
288 greater intrinsic water use efficiency (higher  $P_n/g_s$ ) was observed in the stressed plants.  
289 Also, exposing plants to water stress increased water use efficiency of production  
290 (WUE), expressed as dry weight per unit of water consumption (3.61 g L<sup>-1</sup> and 4.68 g L<sup>-1</sup>  
291 for control and stressed plants, respectively). The  $F_v/F_m$  values were not affected by  
292 the drought treatment (Table 1).

293

### 294 3.3. Plant water relations

295

296 Leaf water potential ( $\Psi_l$ ), and stem water potential ( $\Psi_s$ ) values were recorded during  
297 the hours of maximum illumination (Fig. 5A, B). In the control treatment  $\Psi_l$  and  $\Psi_s$   
298 values were always higher than in water deficit treatment (Fig. 5A). For each treatment,  
299  $\Psi_s$  showed less negative values than those found for  $\Psi_l$ . The differences between  $\Psi_s$  and  
300  $\Psi_l$  measured simultaneously of the same plant were higher for the control treatment  
301 (Fig. 5A, B). The water deficit applied produced increases in root hydraulic resistance,

302 with values of 1.02 and 3.20 g MPa s mg<sup>-1</sup> recorded for the control and drought  
303 treatments, respectively (Table 3).

304 The parameters derived from the pressure-volume curve for control and stressed  
305 plants are shown in Table 3. Both leaf osmotic potential values at full turgor ( $\Psi_{os}$ ) and  
306 leaf water potential at turgor loss point ( $\Psi_{tlp}$ ) decreased markedly two fold in the water  
307 deficit plants. The difference between the  $\Psi_{os}$  values obtained for the control and deficit  
308 irrigated plants were taken as an estimate of the osmotic adjustment (1.58 MPa for  
309 water stress). The point of zero turgor occurred at much lower water potential values (-  
310 4.09 MPa). In contract, the bulk modulus of elasticity ( $\epsilon$ ) increased (75%) in the water  
311 deficit treatment.

312

#### 313 **4. Discussion**

314

315 Leaf growth is often more reduced than root growth as a result of water stress (Hsiao  
316 and Xu, 2000; Franco et al., 2006), indicating that shoots and roots respond differently  
317 to drought (Bacelar et al., 2007; Álvarez et al., 2009). This was confirmed in our  
318 conditions because the application of a water deficit to the plant substrate led a decrease  
319 in aerial dry matter accumulation, leaf area and height while the contrary effect on root  
320 mass was seen, provoking a redistribution of dry matter in favour of the roots at the  
321 expense of shoots (higher root/shoot ratio) (Montero et al., 2001; Sánchez-Blanco et al.,  
322 2009). These changes, which have been described in other ornamental species (Shao et  
323 al., 2008; Mugnai et al., 2005) can be considered as a morphological adaptation of the  
324 plant to water stress to reduce the evaporative surface area (de Herralde et al., 1998) and  
325 to induce a lower consumption of water (Bañón et al., 2004). On the other hand, the  
326 plants growth in pots under water deficit had appreciable and rapid response in the

327 relative growth rate, with plant height decreasing even under climatic conditions of  
328 moderate evaporative demand. Some authors have suggested that to detect water stress  
329 in plants can be different when the plants grown in pots, with restricted root volume  
330 than in soil-cultivated plants (Gallardo et al., 2006; Miralles et al., 2009). These results  
331 are useful because the application of drought treatment during nursery production can  
332 be used as a technique to reduce the excessive growth in ornamental plants without  
333 applying plant growth retardants (Morvant et al., 1998).

334 Transpiration and, consequently water consumption in the water stressed plants  
335 decreasing substantially. In plants subjected to no water restriction, transpiration was  
336 greater at the time of higher water demand (higher temperature and illumination  
337 conditions), which is agrees with the observation of numerous authors (Alarcón et al.,  
338 2000; Montero et al., 2001; Nicolás et al., 2005).

339 Water stress appeared to affect transpiration (reduction of 63% of the control) as seen  
340 from the substantial decrease in stomatal conductance in these plants during the  
341 experimental period (values below  $60 \text{ mmol m}^{-2} \text{ s}^{-1}$  vs 130 in control). This response  
342 could affect to net  $\text{CO}_2$  assimilation rate, leading to lower plant biomass production  
343 (Brugnoli and Bjorkman, 1992; Mugnai et al., 2009). Thus, the fact that  $F_v/F_m$  values  
344 were maintained at 0.80 in both treatments throughout the experimental period  
345 demonstrates the lack of drought-induced damage to PSII photochemistry, suggesting  
346 that *Callistemon laevis* is a drought-tolerant species (Genty et al., 1987; Mugnai et al.,  
347 2009). The intrinsic water use efficiency ( $P_n/g_s$ ) progressively increased in the water  
348 stressed plants throughout the period, indicating a predominant stomatal control over  
349 photosynthesis (Gulías et al., 2009). The consistent decrease in  $g_s$  suggested an  
350 adaptative efficient stomatal control of transpiration by this species (Hessini et al.,  
351 2008). In this sense, most woody species increase their intrinsic water use efficiency,

352 CO<sub>2</sub> assimilation remaining proportionally higher than water vapor loss from the  
353 stomata as an additional drought acclimatation. The advantage in the case of these  
354 plants is that controlled drought may lead to an accumulation of carbohydrate reserves  
355 in the plants and together with the increased root: shoot ratio and root density could  
356 promote a more rapid establishment of ornamental plants in the garden or landscape  
357 (Cameron et al., 2006; Franco et al., 2006). Also, water use efficiency of production  
358 (WUE) measured as dry weight per unit of water used, improved in the stressed plants,  
359 an observation that has been associated with the application of deficit irrigation regimes  
360 (Cameron et al., 2006; Álvarez et al., 2009) to maintain leaf water status of these  
361 species (Hessini et al., 2008).

362 In this study, the reduction in plant growth and  $g_s$  caused by the drought could be  
363 related to changes in the plant water status. The higher root hydraulic resistance in  
364 stressed plants may have caused the leaf and stem water potentials decrease, which  
365 caused a substantial fall in stomatal conductance (Pereira and Chaves, 1993; Munné-  
366 Bosch et al., 1999). It has been reported that the threshold level for the decline of water  
367 potential to cause a decrease in stomatal opening ranges from -0.7 to -1.2 MPa for  
368 different ornamental species (Ackerson, 1985; Sánchez-Blanco et al., 2009). The fact  
369 that the  $\Psi_l$  values were always lower than  $\Psi_s$  is because  $\Psi_l$  reflected a combination of  
370 many factors such as environmental conditions, soil water availability, hydraulic  
371 conductivity and stomatal regulation, while  $\Psi_s$  is more directly related to whole plant  
372 transpiration and root hydraulic conductivity (Choné et al., 2001). The stem water  
373 potential has been successfully used as a water deficit indicator in fruit crops (Garnier  
374 and Berger, 1985; McCutchan and Shackel, 1992). In our conditions, the difference  
375 between  $\Psi_s$  and  $\Psi_l$  could be a good indicator of shoot transpiration in these plants, since



376 the pattern of these differences throughout the experimental was similar to those  
377 observed for transpiration.

378 The significantly lower values of  $\Psi_{os}$  in stressed callistemon plants suggest an active  
379 osmotic adjustment in leaves and, besides, water stress also induced a decrease in leaf  
380 tissue elasticity. Many species show these responses as tolerance mechanisms to  
381 drought in order to maintain turgor (Meinzer et al., 1990; Hessini et al., 2008).

382 In conclusion, the tolerance of *C. laevis* to the drought was related to morphological  
383 and physiological adaptations: That is the ability to adjust osmotic potential to enhance  
384 rigidity and to modify leaf gas exchange, is accompanied by a capacity for  
385 photosynthesis and to reduce water losses through transpiration. The reductions in aerial  
386 dry weight (leaf area, leaf number and height), together with increases in the root: shoot  
387 ratio and root density, could promote the more rapid establishment of these species in  
388 Mediterranean conditions.

389

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391

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394

### 395 **References**

396

397 Alarcón, J.J., Domingo, R., Green, S., Sánchez-Blanco, M.J., Rodríguez, P., Torrecillas,  
398 A., 2000. Sap flow as an indicator of transpiration and the water status of young  
399 apricot trees. *Plant Soil* 227, 77-85.

400 Álvarez, S., Navarro, A., Bañón, S., Sánchez-Blanco, M.J., 2009. Regulated deficit  
401 irrigation in potted *Dianthus* plants: effects of severe and moderate water stress on  
402 growth and physiological responses. *Sci. Hort.* 122, 579-585.

403 Ackerson, R.C., 1985. Osmoregulation in cotton in response to water stress. *Plant*  
404 *Physiol.* 77, 309-312.

405 Bacelar, E.A., Santos, D.L., Moutinho-Pereira, J.M., Lopes, J.L., Gonçalves, B.C.,  
406 Ferreira, T.C., Correira, C.M., 2007. Physiological behaviour, oxidative damage and  
407 antioxidative protection of olive trees grown under different irrigation regimes. *Plant*  
408 *Soil* 292, 1-12.

409 Bañón, S., Fernández, J.A., Franco, J.A., Torrecillas, A., Alarcón, J.J., Sánchez-Blanco,  
410 M.J., 2004. Effects of water stress and night temperature pre-conditioning on water  
411 relations and morphological and anatomical changes of *Lotus creticus* plants. *Sci.*  
412 *Hort.* 101, 333-342.

413 Begg, J.E., Turner, N.C., 1970. Water potential gradients in field tobacco. *Plant Physiol.*  
414 46, 343-346.

415 Brugnoli, E., Bjorkman, O., 1992. Growth of cotton under continuous salinity stress:  
416 influence on allocation pattern, stomatal and not-stomatal components of  
417 photosynthesis and dissipation of excess light energy. *Planta* 187, 335-347.

418 Camejo, D., Rodríguez, P., Morales, M.A., Dell'Amico, J., Torrecillas, A., Alarcón, J.J.,  
419 2005. High temperature effects on photosynthetic activity of two tomato cultivars  
420 with different heat susceptibility. *J. Plant Physiol.* 162, 281-289.

421 Cameron, R.W.F., Harrison-Murray, R.S., Scott, M.A., 1999. The use of controlled  
422 water stress to manipulate growth of container-grown *Rhododendron* cv. Hoppy. *J.*  
423 *Hort. Sci. Biotechnol.* 74, 161-169.

424 Cameron, R.W.F., Harrison-Murray, R.S., Atkinson, C.J., Judd, H.L., 2006. Regulated  
425 deficit irrigation: a means to control growth in woody ornamentals. *J. Hort. Sci.*  
426 *Biotechnol.* 81, 435-443.

427 Choné, X., Van Leeuwen, C., Dubourdieu, D., Gaudillère, J.P., 2001. Stem water  
428 potential is a sensitive indicator of grapevine water status. *Ann. Bot.* 87, 477-483.

429 de Herralde, F., Biel, C., Savé, R., Morales, M.A., Torrecillas, A., Alarcón, J.J.,  
430 Sánchez Blanco, M.J., 1998. Effect of water and salt stresses on the growth, gas  
431 exchange and water relations in *Argyranthemum coronopifolium* plants. *Plant Sci.*  
432 139, 9-17.

433 Franco, J.A., Martínez-Sánchez, J.J., Fernández, J.A., Bañón, S., 2006. Selection and  
434 nursery production of ornamental plants for landscaping and xerogardening in semi-  
435 arid environments. *J. Hort. Sci. Biotechnol.* 81, 3-17.

436 Gallardo, M., Thompson, R. B., Valdez, L. C., Fernández, M. D., 2006. Response of  
437 stem diameter variations to water stress in greenhouse-grown vegetables crops. *J.*  
438 *Hort. Sci. Biotechnol.* 81, 483-495.

439 Garnier, E., Berger, A., 1985. Testing water potential in peach trees as an indicator of  
440 water stress. *J. Hort. Sci. Biotechnol.* 60, 47-56.

441 Genty, B., Briantais, J.M., Viera da Silva, J.B., 1987. Effects of drought on primary  
442 photosynthetic processes of cotton leaves. *Plant Physiol.* 83, 360-374.

443 Gulías, J., Cifre, J. Jonasson, S., Medrano, H., Flexas, J., 2009. Seasonal and inter-  
444 annual variations of gas exchange in thirteen woody species along a climatic  
445 gradient in the Mediterranean island of Mallorca. *Flora* 204, 169-181.

446 Hessini, K., Ghandour, M., Albouchi, A., Soltani, A., Werner, K.H., Abdelly, C., 2008.  
447 Biomass production, photosynthesis, and leaf water relations of *Spartina alterniflora*  
448 under moderate water stress. *J. Plant Res.* 121, 311-318.

449 Hsiao, T.C., Xu, L.K., 2000. Predicting water use efficiency in crops. *Acta Hort.* 537,  
450 199-206.

451 Lafuente, M.T., Belver, A., Guye, M.G., Saltveit Jr, M.E., 1991. Effect of the  
452 temperature conditioning on chilling injury of cucumber cotyledons. *Plant Physiol.*  
453 95, 443-449.

454 Lao, M.T., Jiménez, S., 2002. Estudio comparativo del comportamiento productivo de  
455 *Callistemon citrinus*. In: I Jornadas Ibéricas de plantas ornamentales. Servicio de  
456 Publicaciones y Divulgación. Consejería de Agricultura y Pesca. Junta de  
457 Andalucía. pp 113-120.

458 Lippi, G., Serra, G., Vernieri, P., Tognoni, F., 2003. Response of potted *Callistemon*  
459 species to high salinity. *Acta Hort.* 609, 247-250.

460 Lippi, G., Vernieri, P., Serra, G., Carrai, C., 2005. *Callistemon* spp-Resistenza agli  
461 stress ambientali. *Clamer Informa* 11, 7-12.

462 McCutchan, H., Shackel, K.A., 1992. Stem water potential as a sensitive indicator of  
463 water stress in prune trees. *J. Am. Soc. Hortic. Sci.* 117, 607-611.

464 Meinzer, F.C., Grantz, D.A., Goldstein, G., Saliendra, N.Z., 1990. Leaf water relations  
465 and maintenance of gas exchange in coffee cultivars grown in drying soil. *Plant*  
466 *Physiol.* 94, 1781-1787.

467 Miralles, J., Nortes, P., Sánchez-Blanco, M.J., Martínez-Sánchez, J.J., Bañón, S., 2009.  
468 Above ground and pot-in-pot production systems in native Myrtle. *Transactions of*  
469 *the ASABE* 52, 93-101.

470 Mitchem, C.M., 1993. *Callistemon*: the beautiful bottlebrushes. *Plantsman*, 15, 29-41.

471 Montero, J.I., Antón, A., Muñoz, P., Lorenzo, P., 2001. Transpiration from geranium  
472 grown under high temperatures and low humidities in greenhouses. *Agric. For.*  
473 *Meteorol.* 107, 323-332.

474 Morvant, J.K., Dole, J.M., Cole, J.C., 1998. Irrigation frequency and system affect  
475 Poinsettia growth, water use and runoff. Hort Sci. 33, 42-46.

476 Mugnai, S., Vernieri, P., Malorgio, F., Serra, G., 2005. Response of some ornamental  
477 shrubs to different soil water conditions. Adv. Hort. Sci. 19, 94-100.

478 Mugnai, S., Ferrante, A., Petrognani, L., Serra, G., Vernieri, P., 2009. Stress-induced  
479 variation in leaf gas exchange and chlorophyll a fluorescence in *Callistemon* plants.  
480 Res. J. Biol. Sci. 4, 913-921.

481 Munné-Bosch, S., Nogués, S., Alegre, L., 1999. Diurnal variations of photosynthesis  
482 and dew absorption by leaves in two evergreen shrubs growing in Mediterranean  
483 field conditions. New Phytol. 144, 109-119.

484 Nicolás, E., Torrecillas, A., Ortuño, M.F., Domingo, R., Alarcón, J.J., 2005. Evaluation  
485 of transpiration in adult apricot trees from sap flow measurements. Agric. Water  
486 Manage. 72, 131-145.

487 Pereira, J.S., Chaves, M.M., 1993. Plant water deficits in Mediterranean ecosystems, in:  
488 Smith, J.A.C., Griffiths, H. (Eds.), Water deficits. Plant responses from cell to  
489 community. Bios Scientific Publishers, Oxford, UK, pp. 237-252.

490 Ramos, C., Kaufman, M.R., 1979. Hydraulic resistance of rouge lemon roots. Physiol.  
491 Plant. 45, 311-314.

492 Sánchez-Blanco, M. J., Ferrández, T., Navarro, A., Bañón, S., Alarcón, J.J., 2004.  
493 Effects of irrigation and air humidity preconditioning on water relations, growth and  
494 survival of *Rosmarinus officinalis* plants during and after transplanting. J. Plant  
495 Physiol. 161, 1133–1142.

496 Sánchez-Blanco, M.J., Álvarez, S., Navarro, A., Bañón S., 2009. Changes in leaf water  
497 relations, gas exchange, growth and flowering quality in potted geranium plants  
498 irrigated with different water regimes. J. Plant Physiol. 166, 467-476.

499 Scholander, P.F., Hammel, H.T., Bradstreet, E.D., Hemingsen, E.A., 1965. Sap pressure  
500 in vascular plants. *Science* 148, 339-346.

501 Shao, H.B., Chu, L.Y., Jaleel, C.A., Zhao, C.X., 2008. Water-deficit stress-induced  
502 anatomical changes in higher plants. *Compt. Rend. Biol.* 331, 215-225.

503 Sweatt, M.R., Davies, Jr. F.T., 1984. Mycorrhizae, water relations, growth, and nutrient  
504 uptake of geranium grown under moderately high phosphorus regimes. *J. Am. Soc.*  
505 *Hort. Sci.* 109, 210-213.

506 Turner, N.C., 1988. Measurement of plant water status by the pressure chamber  
507 technique. *Irrig. Sci.* 9, 289-308.

508 Wilson, J.R., Fisher, M.J., Schulze, E.D., Dolby, G.R., Ludlow, M.M., 1979.  
509 Comparison between pressure-volume and dewpoint-hygrometry techniques for  
510 determining the water relations characteristics of grass and legume leaves.  
511 *Oecologia* 41, 77-88.

512 Zollinger, N., Kjelgren, R., Cerny-Koenig, T., Kopp, K., Koenig, R., 2006. Drought  
513 responses of six ornamental herbaceous perennials. *Sci. Hort.* 109, 267-274.

514

515 **FIGURE CAPTIONS**

516 **Fig. 1.** Partitioning mass in *Callistemon* plants subjected to control and water stress at  
517 the end of the experiment. Each histogram represents the mean of five values and the  
518 vertical bars indicate standard errors.

519 **Fig. 2.** Plant height (A), and relative growth rate (B) in *Callistemon* plants subjected to  
520 control and water stress during the experimental period. Values are means (n = 32) and  
521 the vertical bars indicate standard errors.

522 **Fig. 3.** Transpiration during the experimental period (T, A) and daily transpiration  
523 during a representative day of the period (B) in *Callistemon* plants subjected to control  
524 and water stress.

525 **Fig. 4.** Evolution of the intrinsic water use efficiency ( $P_n/g_s$ ) in *Callistemon* plants  
526 subjected to control and water stress during the experimental period.

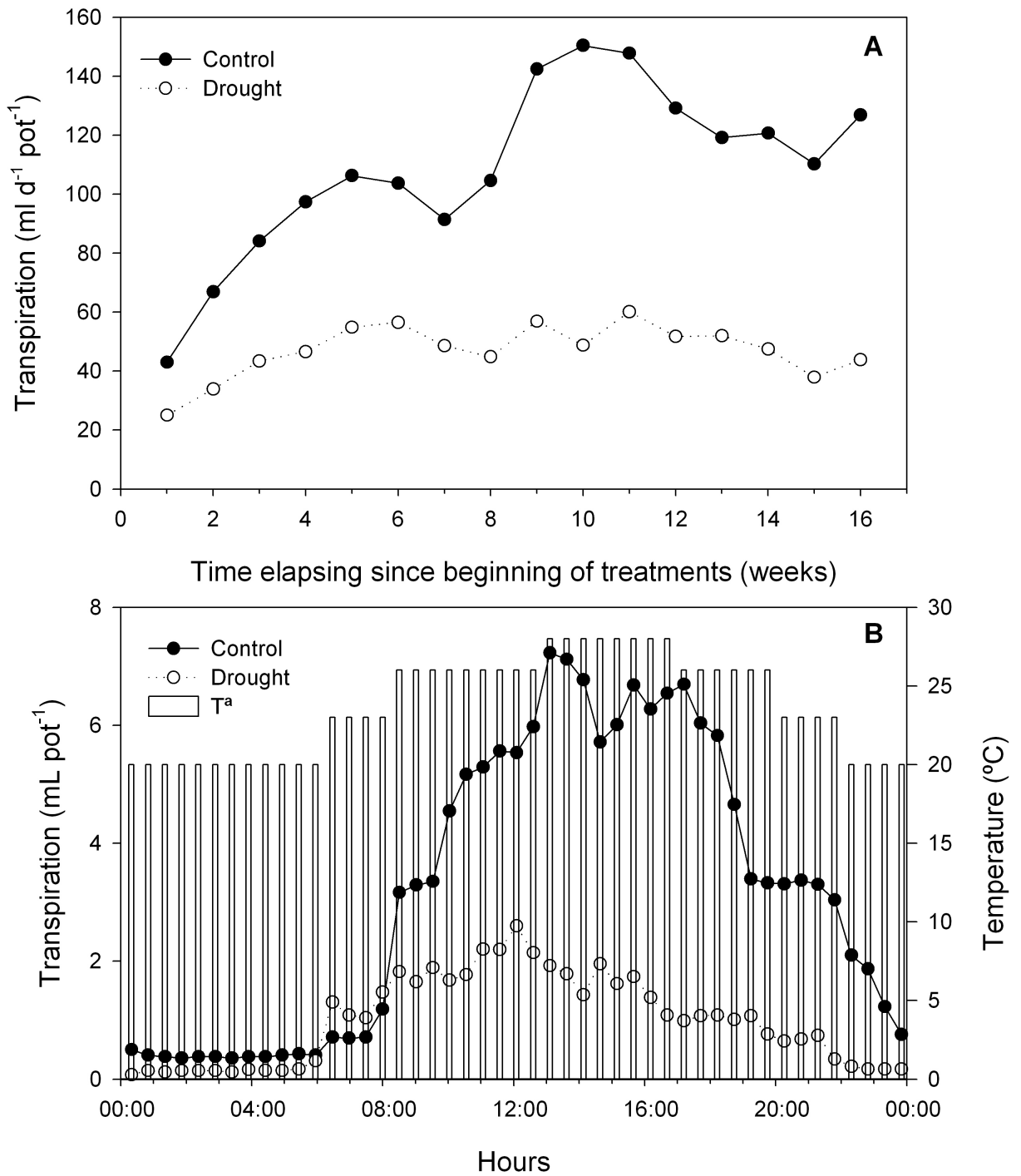
527 **Fig. 5.** Evolution of the leaf water potential ( $\Psi_l$ , A) and stem water potential ( $\Psi_s$ , B) in  
528 *Callistemon* plants subjected to control and water stress during the experimental period.  
529 Each histogram represents the mean of five values and the vertical bars indicate  
530 standard errors.

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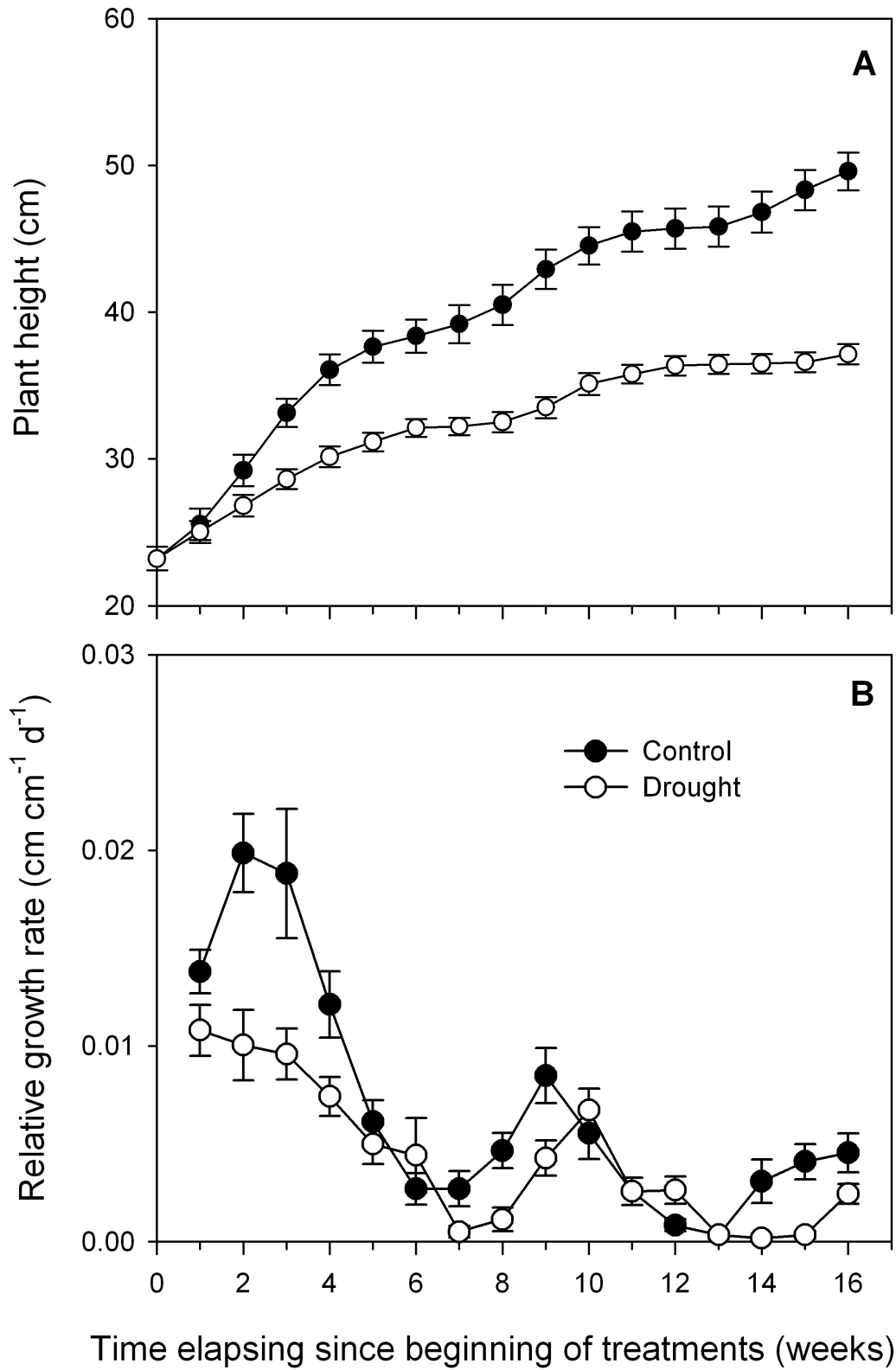
534 **Fig. 1**



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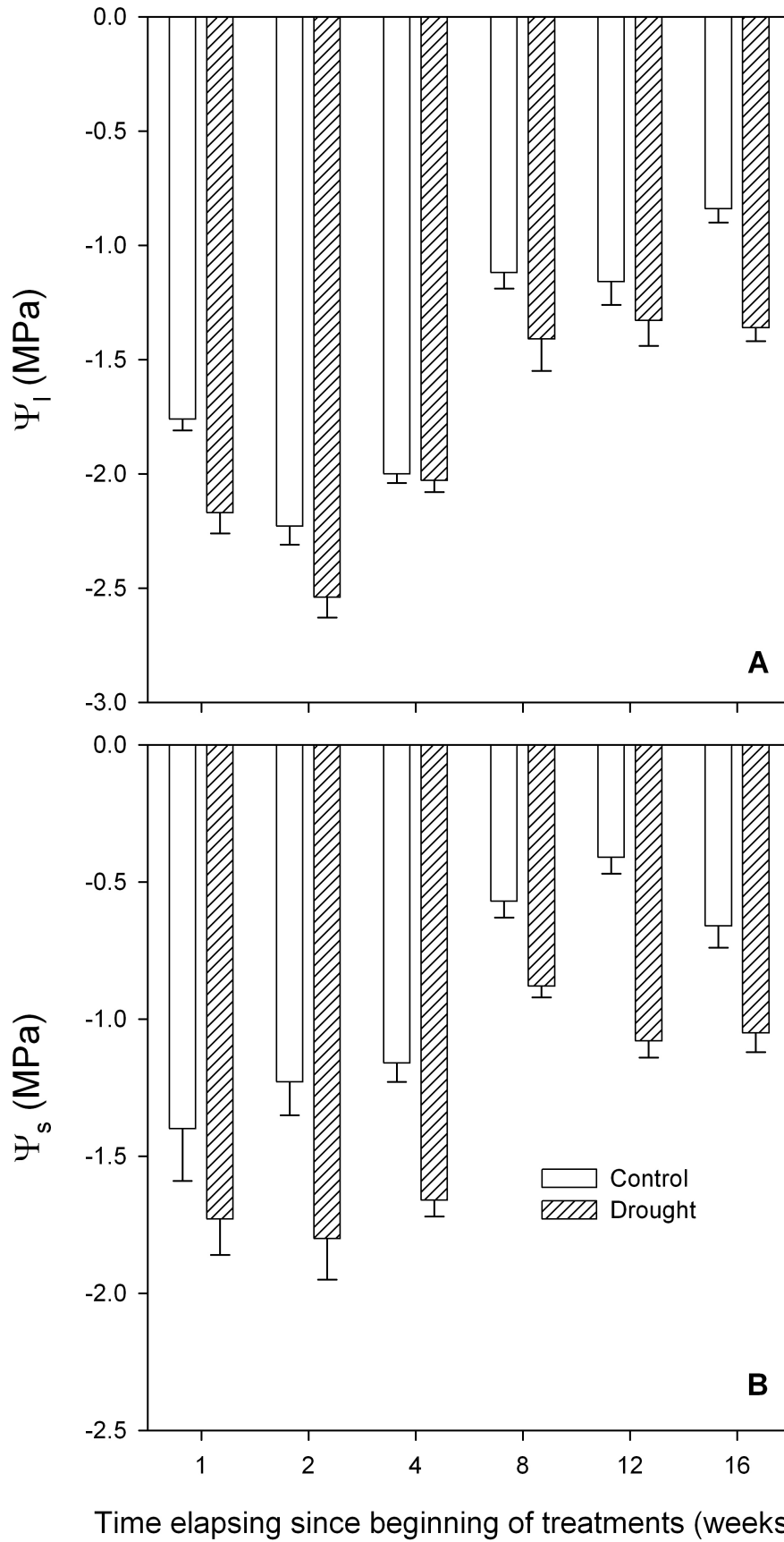
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540 **Fig. 3**

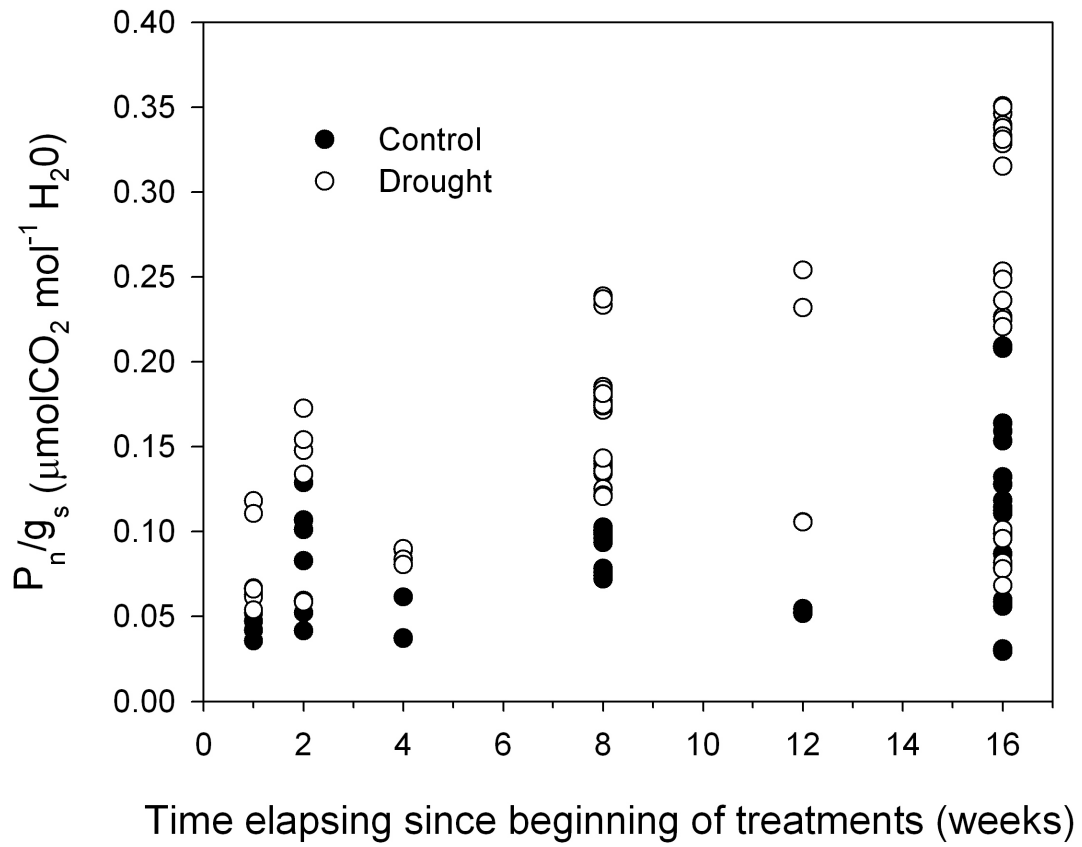


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542



545 **Fig. 5**



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547

548 **Table 1**

549 Growth parameters, ion leakage and chlorophyll fluorescence ( $F_v/F_m$ ) in potted

550 *Callistemon* plants subjected to control and water stress at the end of the experiment.

551 Each value is the mean of five plants per treatment.

552

Parameters	Treatments		Significance
	Control	Drought	
Total dry weight (g plant <sup>-1</sup> )	51.01±2.03	24.36±0.30	***
Root/shoot ratio	0.20±0.01	0.42±0.02	***
Number of leaves	444±51.7	224±15.5	**
Total leaf area (cm <sup>2</sup> )	2913.7±125.0	1183.0±55.9	***
Total ion leakage (%)	23.96±0.04	34.19±0.07	*
$F_v/F_m$	0.79±0.06	0.81±0.03	ns

553 \*P <0.05, \*\*P <0.01 and \*\*\*P <0.001.

554 **Table 2**

555 Root morphology in potted *Callistemon* plants subjected to control and water stress at  
 556 the end of the experiment. Each value is the mean of five plants per treatment.

557

Parameters	Treatments		Significance
	Control	Drought	
Total root length (cm)	3556±151	2595±154	**
L $\phi$ <0.5 mm (%)	49.28±0.02	55.33±0.01	*
L $0.5<\phi<2.0$ mm (%)	38.09±0.01	33.86±0.01	*
L $\phi>2.0$ mm (%)	12.15±0.01	10.24±0.01	*
Root volume (cm <sup>3</sup> )	4.74±0.46	2.96±0.13	**
Root dry weight (g)	8.59±0.50	7.42±0.37	ns

558 \*P <0.05, \*\*P <0.01 and \*\*\*P <0.001.

559

560 **Table 3**

561 Leaf water relations parameters derived from pressure-volume curves and root hydraulic  
 562 resistance ( $1/L_p$ ) in *Callistemon* plants subjected to control and water stress at the end of  
 563 the experiment. Each value is the mean of five plants per treatment.  
 564

Parameters	Treatments		Significance
	Control	Drought	
$\Psi_{os}$ (MPa)	-1.77±0.07	-3.35±0.07	***
$\Psi_{tlp}$ (Mpa)	-2.27±0.11	-4.09±0.10	***
$\varepsilon$ (Mpa)	8.27±0.87	14.40±1.57	*
$1/L_p$ (g s MPa mg <sup>-1</sup> )	1.02±0.10	3.20±0.44	**

565 \*P <0.05, \*\*P <0.01 and \*\*\*P <0.001.

566