

1 **Running title:** Response of callistemon to deficit irrigation

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3 **Changes in growth rate, root morphology and water use efficiency of potted *Callistemon citrinus***
4 **plants in response to different levels of water deficit**

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24 **Abstract**

25 *Callistemon* is widely used as a flowering shrub in gardening and landscaping in the Mediterranean area.

26 However, prolonged or severe water stress may alter its physiological and morphological behaviour.

27 *Callistemon citrinus* plants were grown in nursery conditions and subjected to three irrigation treatments:

28 a control (watered to container capacity) and two water deficit treatments of 50 and 25% of the amount of

29 water supplied in the control treatment (moderate and severe deficit irrigation, respectively). After 53

30 weeks, the moderate deficit irrigation plants showed a lower relative growth rate but increased root/shoot

31 ratio, improved the root system and increased water use efficiency, while flowering and leaf colour were

32 unaffected. However, severe deficit irrigation reduced flowering and affected leaf colour. Both deficit

33 irrigation treatments reduced stomatal conductance, suggesting an efficient and adaptive stomatal control

34 in this species. These reductions were marked after longer periods in plants submitted to severe deficit

35 irrigation, which decreased photosynthesis and could delay plant recovery and cause permanent damage.

36 Differences between stem and leaf water potential values have seen to be a good indicator of

37 instantaneous shoot transpiration. Water consumption was influenced by the active periods of growth and

38 inflorescence formation. It is concluded that moderate deficit irrigation can be used successfully in

39 *Callistemon citrinus* plant production to reduce water consumption while maintaining good overall

40 quality.

41

42 **Key words:** Deficit irrigation, Evapotranspiration; Gas exchange; Ornamental potted plant; Root

43 system; Water relations.

44

45 **Abbreviations:** C, control; C*, chroma; DI, deficit irrigation; DW, dry weight; ET,

46 evapotranspiration; g_s , stomatal conductance; h° , hue angle; L^* , lightness; MDI, moderate deficit

47 irrigation; P, significance; PAR, photosynthetic active radiation; P_n , net photosynthesis; RDD, relative

48 chlorophyll content; RGR, relative growth rate; RH, relative humidity; SDI, severe deficit irrigation;

49 SWC, substrate water content; VPD, vapour pressure deficit; WUE, Water use efficiency of production;

50 Ψ_l , leaf water potential; Ψ_s , stem water potential

51

52

53 **1. Introduction**

54 An efficient use of limited water resources and better growth under a limited water supply are
55 desirable traits for plants in drought environments (Jaleel et al., 2008). For many crops, it is now possible
56 to accurately schedule irrigation to match the loss of water through evapotranspiration from the crop
57 canopy and the soil, but there has been little quantification of the irrigation requirements of ornamental
58 nursery plants (Mee et al., 2003; Henson et al., 2006). The quantity and frequency of irrigation applied in
59 most nurseries is based on personal experience and is rarely modified to match the requirements of the
60 crop water needs (Grant et al., 2012). However, plant water use varies greatly from day to day, through
61 the course of a season, and with growth. Therefore, regardless of the engineered efficiency of water
62 management, there is a potential to improve the physiological efficiency of water use by the crop. Such
63 physiological approaches, based on the biology of plant water use, provide opportunities to manage both
64 water use and quality in terms of an economic balance (Mahan et al., 2012). Thus, if water productivity is
65 defined as the ratio of yield, measured as biological or economic output to crop evapotranspiration,
66 ornamental crops have high water productivity compared to agronomic crops or even fruit crops.

67 Deficit irrigation (DI) is the application of water at a rate and volume lower than the
68 evapotranspiration rate and may be used in potted ornamental plants to improve plant quality, by reducing
69 excessive vigour and promoting a more compact habit (Cameron et al., 2006), but the degree and duration
70 of the water stress imposed in each species in field conditions is also critical to reach this purpose
71 (Álvarez et al., 2009). Some shrubs can be maintained with minimal irrigation level if plant selection is
72 appropriate for site conditions (Sachs et al., 1975). Therefore, deficit irrigation requires precise
73 scheduling to minimise the risk of excessive drying of the substrate, even, taking into account that
74 ornamental crops are largely grown in containers with a small water capacity. In this sense, numerous
75 works in ornamental plants have demonstrated that the plant quality decreases as the severity of DI
76 increases (Hansen and Petersen, 2004; Henson et al., 2006; Katsoulas et al., 2006; Chyliński et al., 2007;
77 Silber et al., 2007; De Lucia, 2009; Álvarez et al., 2009; Sánchez-Blanco et al., 2009; Bolla et al., 2010;
78 Andersson, 2011; Bernal et al., 2011).

79 Adaptation to reduced water supply is achieved primarily through a reduction in stomatal conductance
80 and, over the longer term, a reduction in the new leaves synthesised. The dynamics of root growth under
81 drought conditions might be a key factor to understanding the contribution of roots to drought tolerance.
82 The effect of drought stress is usually greater on shoot growth than it is on root growth, but also other root

83 characteristics may be changed by drought, aspects that could promote the more rapid establishment of
84 ornamental plants in the garden, particularly in dry regions (Franco et al., 2006).
85 Among Australian ornamental plants, one of the most important genus is *Callistemon*, which includes
86 several species showing interesting ornamental features (Mitchen, 1993). *Callistemon laevis* and
87 *Callistemon citrinus* appeared to be particularly resistant to both water stress and root restriction
88 conditions (Vernieri et al., 2006; Mugnai et al., 2009). Although the general effects of drought on plant
89 growth of *Callistemon* have been studied (Mugnai et al., 2009; Álvarez et al., 2011), further work is
90 required to quantify responses of these plants to different levels of deficit irrigation as regards growth,
91 plant quality and physiological parameters, such knowledge will help optimize water reduction levels. For
92 this reason, the present research aims to investigate the effects of two levels of deficit irrigation on gas
93 exchange parameters, water consumption, quality, growth rates and water use efficiency of *Callistemon*
94 *citrinus* during the growing season.

95

96 **2. Materials and methods**

97

98 *2.1. Plant material and experimental conditions*

99 Rooted cuttings of 2 year-old *Callistemon citrinus* (Curtis) Skeels, cv 'Firebrand' (Crimson
100 Bottlebrush) grown in 14 cm x12 cm pots by a specialized nursery were transplanted into 5 L plastic pots
101 (20 cm x16 cm) filled with an 8:7:1 (v/v/v) mixture of coconut fibre : black + sphagnum peat: perlite,
102 amended with 2 g L⁻¹ of Osmocote Plus (14:13:13 N,P,K plus microelements). Plants were placed inside a
103 plastic greenhouse equipped with a cooling system, located at Santomera (Murcia, Spain). The
104 temperature and relative humidity were recorded by a Hoboware Lite Data Logger (Escort Data Loggers,
105 Inc., Buchanan, Virginia, USA). The micro-climatic conditions registered to the total experimental period
106 were 14.8 °C (mean minimum), 25.6 °C (mean maximum), and 19.3 °C (average) temperature; and 1.61
107 (mean maximum) and 0.80 (average) vapour pressure deficit.

108 All of the plants were watered daily for three weeks to container capacity prior to starting the
109 treatments.

110

111 *2.2. Treatments and experimental design*

112 *Callistemon citrinus* plants were grown in nursery conditions and subjected to three irrigation
113 treatments (25 plants per treatment) using a computer-controlled drip irrigation system from March 2009
114 to April 2010. The irrigation treatments consisted of a control (C), when substrate moisture was
115 maintained close to container capacity, it was watered so that 15% (v/v) of the applied water was leached,
116 and two deficit irrigation treatments: applying 50% of the amount of water supplied in the control
117 treatment, (moderate deficit irrigation; MDI) and 25% of the control irrigation water, (severe deficit
118 irrigation; SDI). All plants were irrigated daily. The electrical conductivity of the water applied was 0.8
119 dS m⁻¹. One drip nozzle, delivering 2 L h⁻¹ per pot, was connected to two spaghetti tubes (one on each
120 side of every pot) and the duration of each irrigation episode was used to vary the amount of water
121 applied. The volume of water varied between 200 and 500 ml per pot and irrigation episode for the
122 controls and was determined by noting when the leaching fraction reached 15-20%.

123

124 2.3. Growth and plant water measurements

125

126 At the beginning and at the end of the experimental period ten plants per treatment were harvested
127 and separated into shoots (i. e. leaves and stems) and roots. These were then oven-dried at 80 °C until
128 they reached a constant weight to measure the respective dry weights (DW). Stem diameter (mm),
129 succulence, leaf number and leaf area (cm²), using a leaf area meter (Delta-T; Devices Ltd., Cambridge,
130 UK), were determined in the same plants. The relative growth rate of biomass accumulation was
131 calculated as the rate of increase of dry weight or leaf area per unit of initial biomass.

132 The roots were cleaned by low pressure water applied through a flat nozzle. The cleaned root systems
133 were then placed in a metacrylate tray coupled to a double scanner connected to a computer with a root
134 system analyser (Winrhizo LA 1600 Regent Inc., USA). The root systems were put in an oven to dry
135 immediately after the root length and root volume measurements. Roots were classified into three
136 diameter classes: fine (<0.5 mm), medium (0.5–2.0 mm) and coarse (>2 mm). Root density was
137 determined by dividing the dry weight by root volume.

138 Throughout the experiment, plant height and number of inflorescences per plant were measured in 25
139 plants per treatment every 1-2 weeks. The relative growth rate was calculated as the rate of increase of
140 height per unit of initial plant height.

141 Leaf colour was measured at the end of the experimental period with a Minolta CR-10 colorimeter,
142 which provided the colour coordinates lightness (L^*), chroma (C^*) and hue angle (h°) (McGuire, 1992),
143 using three leaves for each plant and ten plants per treatment. The relative chlorophyll content (RCC) was
144 measured using a Minolta SPAD-502 chlorophyll meter (Konica Minolta Sensing Inc., Osaka, Japan)
145 using the same leaves as were used for the colorimetric measurements.

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

156 To determine the maximum water holding capacity of the substrate, seven samples were uniformly
157 mixed and packed to a similar bulk density. The pot surfaces were covered with aluminium foil to prevent
158 water evaporation and the lower parts were submerged, to half the pot's height, in a water bath and then
159 were left to equilibrate overnight. The next day, the pots were removed and left to drain freely until
160 drainage became negligible. The fresh weight was then recorded for each individual pot and considered as
161 the weight at field capacity. At the end of the experiment, the substrate was dried in an oven at 105 °C
162 until constant weight in order to obtain the dry weight and calculate the volumetric water content. Later,
163 the difference between the weight at field capacity and oven-dry weight was measured and volumetric
164 water content calculated (65%), which was considered as the substrate field capacity.

165 Volumetric water content was periodically calculated in seven pots per treatment throughout the
166 experiment, obtaining their weight before and after irrigation, using a balance (Analytical Sartorius,
167 Model 5201; capacity 5.2 kg and accuracy of 0.01 g). Then, the difference between the fresh weight and
168 oven-dry weight was measured, giving the volumetric water content of these monitored pots.

169 Moreover, one pot of each treatment was placed on a balance with a MITRA programmer that
170 recorded the weight every half an hour, giving the evapotranspiration (ET) throughout the experimental

171 period. Daily ET was measured using the difference in weights (weight after irrigation and weight before
172 irrigating again) and hourly ET was measured using the difference in weights between two consecutive
173 measurements.

174 During the experiment, leaf water potential (Ψ_l) and stem water potential (Ψ_s) were measured in eight
175 plants per treatment in mature leaves at midday. Ψ_l was estimated according to the method described by
176 Scholander et al. (1965), using a pressure chamber (Soil Moisture Equipment Co, Santa Barbara, CA,
177 USA), for which leaves were placed in the chamber within 20 s of collection and pressurised at a rate of
178 0.02 MPa s^{-1} (Turner, 1988). Ψ_s was measured in non-transpiring leaves that had been bagged with both a
179 plastic sheet and aluminium foil for at least 1 h before measurement in order to prevent leaf transpiration;
180 in this way leaf water potential equalled stem water potential (Begg and Turner, 1970).

181 Leaf stomatal conductance (g_s) and the net photosynthetic rate (P_n) were periodically determined in
182 eight plants at midday using a gas exchange system (LI-6400, LI-COR Inc., Lincoln, NE, USA). While
183 the P_n/g_s ratio was used as an estimation of the intrinsic water use efficiency. Water use efficiency of
184 production (WUE) was calculated at the end of the experiment by dividing the increment in dry weight by
185 the water used.

186

187 *2.4 Statistical analyses of data*

188

189 The data were analysed by one-way ANOVA using Statgraphics Plus for Windows 5.1 software.
190 Ratio and percentage data were subjected to an arcsine square-root transformation before statistical
191 analysis to ensure homogeneity of variance. Treatment means were separated with Duncan's Multiple
192 Range Test ($P \leq 0.05$).

193

194 **3. Results**

195

196 *3.1. Plant growth and ornamental parameters*

197

198 *C. citrinus* plants submitted to SDI showed a reduction in the relative growth rate (RGR) for all
199 biomass production parameters, while plants submitted to MDI treatment only decreased the RGR of leaf
200 DW and leaf area compared with control treatment (Table 1). At the end of the experiment deficit

201 irrigation reduced the number of leaves as the deficit increased, while leaf blade area, stem diameter and
202 succulence were similarly reduced in both deficit irrigation treatments (Table 2). The root to shoot ratio
203 increased proportionally to the imposed drought level (Table 3).

204 Control plants reached the greatest height, while SDI plants showed a significant reduction from 3
205 weeks after beginning of treatments, leading to the smallest plants (Fig. 1A). At the beginning of the
206 experiment plant height was similar in both the control and MDI treatment, but was inhibited by the latter
207 23 weeks after application onwards, (September), (Fig. 1A). At the end of the experiment the reductions
208 were around 11 and 22% for MDI and SDI, respectively, compared with the control. Water deficit also
209 had a significant effect on RGR as a function of plant height (Fig. 1B). Three growth periods were
210 evident during the growing season (first week of May, August and March) in all plants, although the
211 stressed plants showed a certain delay in reaching the maximum compared with the control (Fig. 1B).

212 Deficit irrigation decreased total root length proportionally to the imposed drought level and a
213 reduction in all root sizes was observed (Table 3). MDI increased the percentage of roots with a diameter
214 between 0.5 and 2 mm and decreased those with a diameter lower than 0.5 mm, compared with the
215 control. All stressed plants showed a reduced root volume, although root dry weight was not modified by
216 MDI, with the result that root density increased in these plants.

217 *C. citrinus* plants had flowers during the most of the experiment, although the greatest intensity of
218 flowering occurred at the end of the experiment (Fig. 2). SDI reduced the number of inflorescences per
219 plant compared with control 54 weeks after application of the deficit irrigation. However MDI had no
220 such effect and flowering intensity was even higher than in control plants at some moments. No changes
221 were observed in the leaf colour of MDI plants compared with the control (Table 4). The higher h° and
222 CRC values and lower C* values recorded in the leaves of the plants submitted to SDI confirmed the
223 darker and less vivid green colour of the foliage compared with control plants (Table 4). Membrane
224 damage, assessed by ion leakage was not affected by the water availability conditions of the substrate.

225

226 3.2 Substrate water content (SWC) and evapotranspiration (ET)

227

228 The water added to each pot during the whole experimental period was 164 L for the control and 82
229 and 47 L for MDI and SDI plants, respectively (50 and 25% of the amount of water compared with
230 control treatment). After irrigation, the substrate water content (SWC) in the control plants remained on

231 average at about $0.55\text{-}0.60\text{ m}^3\text{ m}^{-3}$, above and close to container capacity (Fig. 3A). SWC in MDI was
232 lower than in the control, with average values of around 40% throughout the experiment and slightly
233 lower in summer and at the end of the experiment. In the SDI treatment SWC remained on average
234 around 20%.

235 The greatest values for water consumption were recorded in summer and the lowest in winter (Fig.
236 3B), which was closely related to temperature and VPD (Fig. 3C), although daily evapotranspiration (ET)
237 values were also affected by the growth periods and flowering. ET was higher in control plants than in
238 both deficit irrigation plants due to the greater amount of available water in the substrate (Fig. 3B). ET
239 increased during the morning and the highest value was reached at midday in all plants coinciding with
240 the highest VPD, after which, ET decreased (Fig. 4). Despite similar VPD values, ET levels were higher
241 during active growth periods (Fig. 4B) than during non-growth periods (Fig. 4A). Also, even with similar
242 VPD and RGR, ET values were much higher at the end of the experiment (Fig. 4D), (April), coinciding
243 with inflorescences formation and the opening period, than during a period with less inflorescences per
244 plant (Fig. 4C). When plants showed the highest ET values (in growth and flowering periods), the
245 differences between the ET values of the control and the deficit irrigation plants were also the highest
246 (Fig. 4B and D). In contrast, when plants showed the lowest ET values, the water consumption of SDI
247 plants was practically similar to that of control plants, despite the lower levels of water in the substrate in
248 the SDI plants (Fig. 4A and C).

249

250 *3.3 Water relations and water use efficiency*

251

252 Leaf water potential at midday (Ψ_l) showed maximum values in December and minimum values in
253 August, when temperature and DPV values were highest (Fig. 5). In general, Ψ_l values were highest in
254 the control plants, although no significant differences in Ψ_l levels were noted between treatments during
255 the first five months, even though the water content of the substrates was clearly different. Stem water
256 potential (Ψ_s) values decreased proportionally to the level deficit imposed. The standard error was much
257 lower for Ψ_s than for Ψ_l , meaning that Ψ_s identified greater significant differences between treatments.
258 The maximum and minimum differences between Ψ_s and Ψ_l measured simultaneously in the same plant
259 coincided with the maximum and minimum ET, respectively (Fig. 5).

260 Both deficit irrigation treatments produced a reduction in stomatal conductance, which was more
261 marked under SDI (Fig. 6A). The seasonal pattern of g_s consisted of a minor summer depression in all
262 treatments, particularly in SDI, and a major winter depression regardless of the amount of irrigation water
263 applied (Fig. 6A).

264 In general, the plants of both water deficit treatments showed higher P_n/g_s ratios (intrinsic water use
265 efficiency) than control plants throughout the experimental period (Fig. 6B). The net photosynthetic rates
266 (P_n) increased as stomatal opening (g_s) increased, although this effect was much more evident when g_s
267 was below $150 \text{ mmol m}^{-2} \text{ s}^{-1}$, (Fig. 6C). Furthermore, P_n/g_s increased when g_s decreased from maximum
268 to approximately $100 \text{ mmol m}^{-2} \text{ s}^{-1}$, which indicates a predominantly stomatal control over P_n , but when
269 $g_s < 100 \text{ mmol m}^{-2} \text{ s}^{-1}$, P_n/g_s decreased sharply, suggesting that non-stomatal limitations to P_n were
270 predominant under these conditions (Fig. 6D). All plants had g_s values below $100 \text{ mmol m}^{-2} \text{ s}^{-1}$ during
271 winter, although plants of the SDI treatment also reached g_s values below $100 \text{ mmol m}^{-2} \text{ s}^{-1}$ during some
272 other days of the experiment. At the end of the experiment, plants subjected to deficit irrigation showed
273 that they had increased the water use efficiency of production (WUE) in each part of the plant (leaves,
274 stem and root), regardless of the reduction level applied (Fig. 7).

275

276 **4. Discussion**

277

278 The effect of water stress on plant growth and dry matter accumulation has been described in several
279 crops species (Sánchez-Blanco et al., 2002; Rodríguez et al., 2005; Vernieri et al., 2006; Álvarez et al.,
280 2012). According to Mugnai et al. (2009), in *C. citrinus* plants, deficit irrigation reduces plant height, dry
281 weight and leaf area. In our conditions, plant height was significantly inhibited by both water deficit
282 treatments. Also, these plants had fewer and smaller leaves.

283 The different water stress levels applied in our experiment induced different growth responses in *C.*
284 *citrinus*, meaning that the severity of the water stress must be considered an important aspect when is
285 used as an irrigation strategy for saving water without reducing quality in ornamental species. The
286 differential provision of water to plants influences their dry matter partitioning between roots and shoots.
287 In our conditions, exposure to deficit irrigation had a less pronounced effect on root mass, indicating that
288 shoots and roots react differently to drought (Bacelar et al., 2007; Franco et al. 2006; Chyliński et al.,
289 2007). This was confirmed by the root/shoot ratio which increased in plants under water deficit conditions

290 (Sánchez-Blanco et al., 2002; Navarro et al., 2009), probably as a result of the plants needing to maintain
291 the root surface area under drought conditions in order to absorb water from the substrate (Bradford and
292 Hsiao, 1982) and to reduce the evaporative surface area (De Herralde et al., 1998; Alarcón et al., 2006).

293 The root system is an important factor for successful transplanting and establishment in the field, and
294 root anatomy and structure may be decisive for plant survival (Steudle and Peterson, 1998; Bañón et al.,
295 2004). The shift in root diameters in moderately stressed plants was due to both the greater production of
296 medium sized roots and the lower production of fine roots. Furthermore, the greater root density of these
297 plants suggests greater robustness and, presumably, a higher accumulation of reserves (Cameron et al.,
298 2006; Franco et al., 2006; Álvarez et al., 2011). This may also be interpreted as an accumulation of
299 solutes by the plants in order to maintain the water gradient necessary for absorbing water even when in
300 short supply in the soil. Processes of osmotic adjustment in terms of the roots realised through the active
301 accumulation of organic solutes from the aerial part would alter the physical support, strengthen the roots
302 and lessen the possibility of breaking during transplanting (Bañón et al., 2006). In this sense moderate
303 deficit irrigation in nursery conditions would improve *C. citrinus* plant resistance to water deficit
304 situations when plants grow in field conditions after transplanting (Franco et al., 2001).

305 Water deficit may reduce flowering intensity, bring forward or delay flowering and shorten the same
306 (Cuevas et al., 2009; Bernal et al., 2011). Moderate deficit irrigation did not reduce the number of flower
307 in bottlebrush plants and no differences in the colour space coordinate values were observed, suggesting
308 that colour is not modified by this level of deficit irrigation and meaning that plants can cope with water
309 shortage without losing their ornamental value (Henson et al., 2006). Plant quality was affected by the
310 severe deficit irrigation treatment (lower number of inflorescences, lower RCC values and colour).

311 The water consumption of the plants was closely related with environmental factors (Bakker, 1991;
312 Montero et al., 2001; Bañón et al., 2009; Grant et al., 2012). However, the active periods of growth and
313 the inflorescence formation and opening phases clearly affected this parameter, which agrees with
314 previous studies in *Callistemon laevis* and geranium plants under controlled conditions (Álvarez et al.,
315 2011), when climatic conditions were constant throughout the experiment. In general, ornamental species
316 respond to water stress by reducing the daily ET (Jaleel et al., 2008; Lenzi et al., 2009; Bolla et al., 2010)
317 and ET decreases as the stress severity increases (less irrigation water applied or less frequent irrigation)
318 (French et al., 2009; Eiasu et al., 2012) although the intensity of this response depends on the species and
319 variety studied and varies during an experiment (García-Navarro et al., 2004; Lenzi et al., 2009).

320 Differences between treatments were not constant during the experiment and were greater as the ET
321 values increased. In this sense, Liu et al. (2006) noticed that transpiration and g_s might be partly regulated
322 by VPD, the lower the VPD, the smaller the differences in g_s and T between treatments. In our conditions,
323 water consumption (ET/d) reached the highest values in August and April, leading to the lowest leaf
324 water potential values at midday, and the lowest values in December, when Ψ_1 values were the highest.
325 As regards the behaviour of ET along the day, it began to increase at dawn due to stomatal opening, and
326 was the highest at midday, when temperature and VPD were also the highest and the stomata presented
327 their maximum opening. After midday, stomata closing began, producing a decrease in the ET. At night,
328 ET was at its lowest as a result of nocturnal stomata closing and non-evaporation from the substrate
329 (Alarcón et al., 2000).

330 The stem water potential measured at midday is a good indicator of water stress resulting from the
331 effect of irrigation in *C. citrinus* plants due to the small variability between bagged leaves (Choné et al.,
332 2001). Only when the water deficit became more severe did Ψ_1 exhibit significant differences between
333 treatments. Also, the difference between Ψ_s and Ψ_1 measured simultaneously in the same plant was shown
334 to be an indicator of instantaneous shoot transpiration, which varied with soil moisture conditions and
335 VPD in the atmosphere (Choné et al., 2001).

336 The consistent decrease in g_s in deficit irrigation plants suggested an efficient adaptative stomatal
337 control of transpiration (Hessini et al., 2008). Stomatal conductance can be mostly limited by summer
338 drought or by low winter temperatures (Gulías et al., 2009). In *C. citrinus*, gas exchange was more
339 sensitive to winter than summer conditions. The relation between P_n and g_s showed two phases in all
340 treatments: phase I, with high g_s and a wide variability in P_n , and phase II, where P_n was sharply reduced
341 as the stomatal aperture decreased. Similar results were found by Costa França et al. (2000). According to
342 Colom and Vazzana (2003), Bacelar et al. (2007) and Bolla et al. (2010), non-stomatal factors, such as
343 biochemical limitations, would probably contribute to the decrease in P_n following water stress, as was
344 observed at the end of our experimental period. In addition, the greater reduction of g_s compared with P_n
345 (increased P_n/g_s) suggests that stomatal closure was the main cause for P_n depression in deficit irrigation
346 plants in *C. citrinus*, although photoinhibition may have played a role later when stress was more severe or
347 prolonged and when P_n was proportionally more reduced than g_s (decreased P_n/g_s) (Flexas et al., 2004;
348 Gallé et al., 2009; Gulías et al., 2009; Bolla et al., 2010). Previous studies in a variety of ornamental
349 species indicated that P_n/g_s increases under deficit irrigation and CO_2 assimilation remains proportionally

350 higher than water vapour loss from the stomata as an additional drought acclimatation mechanism (Raviv
351 and Blom, 2001; Jaleel et al., 2008; Shao et al., 2008; Álvarez et al., 2009; Bolla et al., 2010). However,
352 in some ornamental species the opposite effect has been found in response to deficit irrigation (Rasoul
353 Sharifi and Rundel, 1993; Mugnai et al., 2005). The exact response depends on several factors including
354 the species and variety studied, the severity of the stress, the deficit irrigation strategy or the moment of
355 the day when measurements are realized (Costa França et al., 2000; Flexas et al., 2001; Liu et al., 2006;
356 Zollinger et al., 2006; Bacelar et al., 2007; Hessini et al., 2008; Jaleel et al., 2008; Lenzi et al., 2009).

357 *C. citrinus* plants submitted to both deficit irrigation treatments increased their intrinsic water use
358 efficiency because stomatal opening was reduced with respect to the control and they had more efficient
359 g_s than the control, and not because of increased CO_2 assimilation at the same stomatal aperture as
360 control.

361 The parameter P_n/g_s allows the identification of plants that maintain CO_2 assimilation at low stomatal
362 aperture. The most efficient g_s is when values are close to $100 \text{ mmol m}^{-2} \text{ s}^{-1}$, as higher g_s values do not
363 reflect significant increases in P_n and are progressively less efficient, while lower g_s values are much less
364 efficient. Moreover, if plants show a $g_s < 100 \text{ mmol m}^{-2} \text{ s}^{-1}$ for long periods, non-stomatal limitations could
365 be partly responsible for the decrease in P_n , which could delay plant recovery at the onset of the autumn
366 or even cause permanent damages. For these reasons, the most suitable g_s values are those between the
367 100 and $200 \text{ mmol m}^{-2} \text{ s}^{-1}$, when plants maintain acceptable photosynthetic rates. This was the case with
368 MDI, which presented g_s values in this interval during the greatest part of the season and for longer than
369 plants from the other two treatments.

370 Also, WUE was higher in *C. citrinus* plants growing in deficit irrigation conditions. This response has
371 been observed in numerous ornamental plants (Cameron et al., 2006; Jaleel et al., 2008; Álvarez et al.,
372 2009; Mugnai et al., 2009). However, according to other authors this parameters is not modified under
373 deficit irrigation conditions (Andersson, 2001), or even decreases (Anyia and Herzog, 2004; Eiasu et al.,
374 2012), depending on the species and variety, genotype, irrigation frequency and stress degree (Anyia and
375 Herzog, 2004; Cameron et al., 2006; Jaleel et al., 2008; Eiasu et al., 2012).

376 Although plants exposed to deficit irrigation showed lower biomass accumulation, the water use
377 efficiency (P_n/g_s and WUE) was higher in the water stressed plants throughout the experimental period.
378 The advantage in the case of these plants is that controlled drought may lead to an accumulation of
379 carbohydrate reserves in the plants and, together with an increased root to shoot ratio and root density,

380 which could promote a more rapid establishment of ornamental plants in the garden or landscape
381 (Cameron et al., 2006; Franco et al., 2006).

382 Ion leakage was unaffected by the irrigation treatment at the end of the experiment. Álvarez et al.,
383 (2009) observed membrane damage in younger callistemon plants submitted to deficit irrigation. Drought
384 sensitivity could depend on the age of the plant, younger plants being more sensitive and less tolerant
385 than older plants.

386

387 **Conclusion**

388 Moderate deficit irrigation (reductions of 50%) decreased the relative growth rate, but flowering and
389 leaf colour were not affected. The root/shoot ratio increased, improved the root system and increased
390 WUE and P_n/g_s . Therefore, this treatment can be used successfully in *Callistemon citrinus* plant
391 production to reduce water consumption while maintaining good overall quality. However, reductions of
392 75% of the water applied (severe deficit irrigation) are not recommended, since this treatment affected the
393 quality of plants, reducing flowering and affecting leaf colour. In this treatment stomatal conductance was
394 strongly reduced for long periods, which decreased P_n and could delay their recovery and even cause
395 permanent damage.

396

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400

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Table 1

Relative growth rate (RGR) of biomass accumulation at the end of the experimental period in *C. citrinus* plants subjected to different irrigation treatments. Values are the mean of 10 plants.

Parameters	Treatments			
	C	MDI	SDI	P
Total DW (mg g ⁻¹ d ⁻¹)	45.1 ± 6.3 b	38.0 ± 0.7 b	18.1 ± 1.6 a	**
Leaf DW (mg g ⁻¹ d ⁻¹)	34.7 ± 2.8 c	27.3 ± 1.2 b	13.7 ± 1.8 a	***
Stem DW (mg g ⁻¹ d ⁻¹)	56.0 ± 3.7 b	52.9 ± 2.3 b	33.2 ± 2.7 a	***
Shoot DW (mg g ⁻¹ d ⁻¹)	44.9 ± 3.0 b	39.6 ± 1.3 b	23.0 ± 2.1 a	***
Root DW (mg g ⁻¹ d ⁻¹)	38.3 ± 0.5 b	35.7 ± 0.2 b	11.7 ± 0.5 a	**
Total eaf area (mm ² cm ⁻² d ⁻¹)	1.84 ± 0.1 c	1.37 ± 0.2 b	0.57 ± 0.1 a	***

Means within a row without a common letter are significantly different by Duncan _{0.05} test. (P; probability level, ** P ≤ 0.01, *** P ≤ 0.001).

Table 2

Growth and biomass traits at the end of the experimental period in *C. citrinus* plants subjected to different irrigation treatments. Values are the mean of 10 plants

Parameters	Treatments			
	C	MDI	SDI	P
Number of leaves	1273.6 ± 63.9 c	1030.6 ± 34.0 b	606.4 ± 54.3 a	***
Leaf blade area (mm ²)	130.68 ± 7.85 b	93.02 ± 6.87 a	82.01 ± 4.60 a	***
Stem diameter (mm)	2.26 ± 0.12 b	1.73 ± 0.07 a	1.61 ± 0.07 a	***
Succulence	2.21 ± 0.03 b	1.91 ± 0.10 a	1.81 ± 0.11 a	*

Means within a row without a common letter are significantly different by Duncan _{0.05} test. (P; probability level, *P<0.05, *** P ≤ 0.001).

Table 3

Root morphology in *C. citrinus* plants subjected to different irrigation treatments at the end of the experimental period. Values are the mean of 3 plants

Parameters	Treatments				P
	C	MDI	SDI		
Root/shoot ratio (g g^{-1})	1.98 ± 0.09 a	2.40 ± 0.21 b	2.99 ± 0.14 c		*
Total root length (m)	283.32 ± 14.53 c	186.90 ± 11.96 b	132.48 ± 12.45 a		***
$L_{\phi < 0.5 \text{ mm}}$ (m)	158.62 ± 5.94 c	97.09 ± 6.46 b	69.54 ± 5.79 a		***
$L_{0.5 < \phi < 2.0 \text{ mm}}$ (m)	100.15 ± 5.78 c	75.03 ± 2.89 b	51.62 ± 6.00 a		**
$L_{\phi > 2.0 \text{ mm}}$ (m)	24.55 ± 2.92 b	14.78 ± 2.90 a	11.32 ± 0.88 a		*
$L_{\phi < 0.5 \text{ mm}}$ (%)	56.07 ± 0.46 b	51.95 ± 0.40 a	52.62 ± 0.56 a		***
$L_{0.5 < \phi < 2.0 \text{ mm}}$ (%)	35.32 ± 0.20 a	40.28 ± 0.60 b	38.79 ± 0.71 a		***
$L_{\phi > 2.0 \text{ mm}}$ (%)	8.61 ± 0.32	7.78 ± 0.15	8.58 ± 0.15		ns
Root volume (cm^3)	189.0 ± 18.9 b	117.8 ± 7.8 a	95.8 ± 7.4 a		**
Root dry weight (g)	69.54 ± 9.30 b	65.23 ± 3.50 b	31.00 ± 4.46 a		**
Root density (g cm^{-3})	0.35 ± 0.02 a	0.52 ± 0.04 b	0.36 ± 0.03 a		*

Root length (L). Means within a row without a common letter are significantly different by Duncan $_{0.05}$ test. (P; probability level, ns; non significance, * $P < 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$).

Table 4

Colour, relative chlorophyll content and ion leakage in *C. citrinus* plants subjected to different irrigation treatments at the end of the experimental period. Values are the mean of 10 plants

Parameters	Treatments			
	C	MDI	SDI	P
L*	41.5 ± 2.9	44.5 ± 2.1	43.2 ± 1.4	ns
C*	17.9 ± 1.8 b	15.4 ± 2.5 b	13.7 ± 0.9 a	*
h°	108.9 ± 2.1 a	107.2 ± 2.0 a	118.4 ± 2.9 b	*
RCC	39.51 ± 1.14 a	39.81 ± 0.84 a	43.27 ± 0.52 b	**
Ion leakage (%)	29.44 ± 1.21	30.71 ± 1.85	28.74 ± 0.87	ns

Lightness (L*), chroma (C*), hue angle (h°) and relative chlorophyll content (RCC). Means within a row without a common letter are significantly different by Duncan _{0.05} test. (P; probability level, ns; non significance, *P<0.05, ** P ≤0.01).

Figure captions

Fig. 1. Evolution of plant height (A) and relative growth rate of height (B) in *C. citrinus* plants submitted to different irrigation treatments. Values are means \pm s.e., $n = 25$. Symbols represent the different treatments: Control (filled circles), MDI (open circles) and SDI (open triangles).

Fig. 2. Evolution of number of inflorescences in *C. citrinus* plants submitted to different irrigation treatments. Values are means \pm s.e., $n = 25$. Symbols represent the different treatments: Control (filled circles), MDI (open circles) and SDI (open triangles).

Fig. 3. Evolution of the substrate volumetric water content (SWC) (A), daily evapotranspiration (ET) (B) in *C. citrinus* plants submitted to different irrigation treatments and daily mean values of air temperature (T) and vapour pressure deficit (VPD) (C) recorded inside the greenhouse during the experimental period. Values are means \pm s.e., $n = 7$ in Fig. 3a and each point represents a single plant in Fig. 3b. Symbols represent the different treatments: Control (filled circles), MDI (open circles) and SDI (open triangles).

Fig. 4. Evolution of hourly ET throughout several representative days of the experimental period in *C. citrinus* plants submitted to different irrigation treatments. Each point represents a single plant.

Fig. 5. Evolution of the stem water potential (Ψ_s) and leave water potential (Ψ_l) in *C. citrinus* plants submitted to different irrigation treatments. Values are means \pm s.e., $n = 8$. Symbols represent the different treatments: Control (filled circles), MDI (open circles) and SDI (open triangles). Solid lines represent Ψ_l and dashed lines represent Ψ_s . For each studied day, * indicates significantly different ($P \leq 0.05$, Duncan's, multiple range test) between treatments for Ψ_s and ** indicates significantly different between treatments for Ψ_s and Ψ_l .

Fig. 6. Evolution of stomatal conductance (g_s , A) and intrinsic water use efficiency (P_n/g_s , B). Values are means \pm s.e., $n = 8$. Relationship between P_n and g_s (C) and between P_n/g_s and g_s (d) in *C. citrinus* plants submitted to different irrigation treatments. Each point represents a single plant. Symbols represent the different treatments: Control (filled circles), MDI (open circles) and SDI (open triangles).

Fig. 7. Water use efficiency of production (WUE) at the end of the experimental period in *C. citrinus* plants submitted to different irrigation treatments. Means within a part of the plant without a common letter are significantly different by Duncan $_{0.05}$ test.

Figure 1

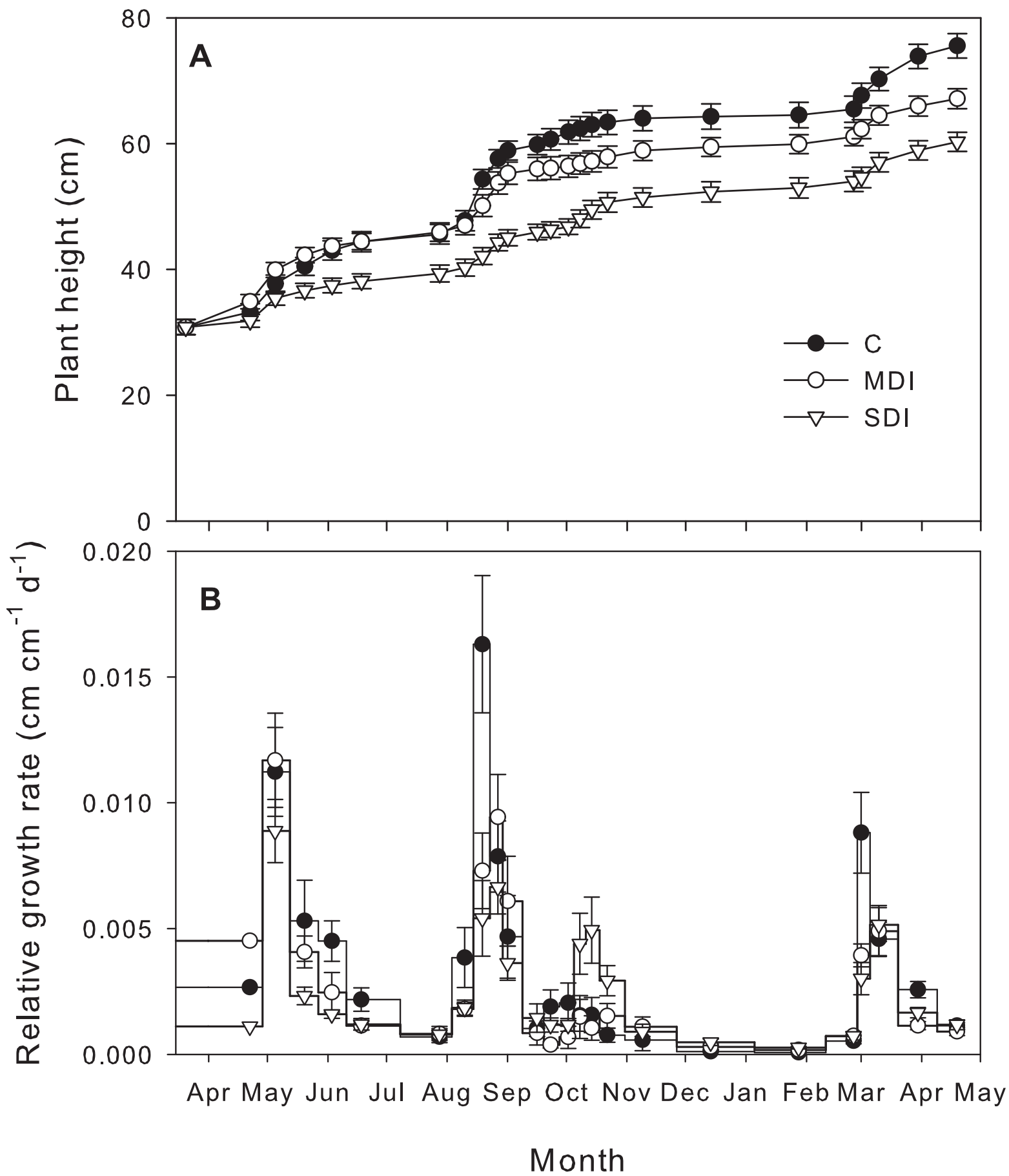


Figure 2

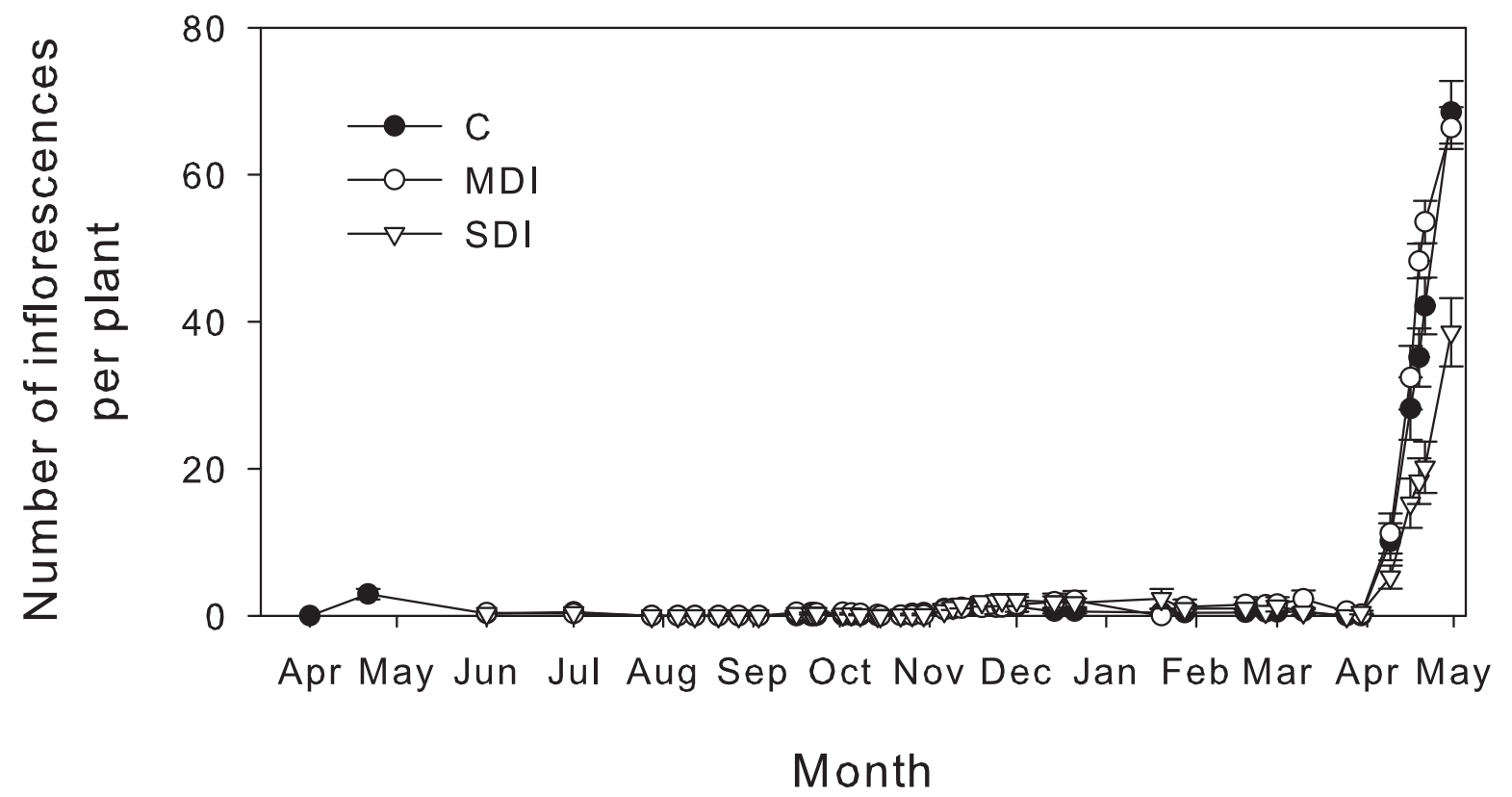


Figure 3

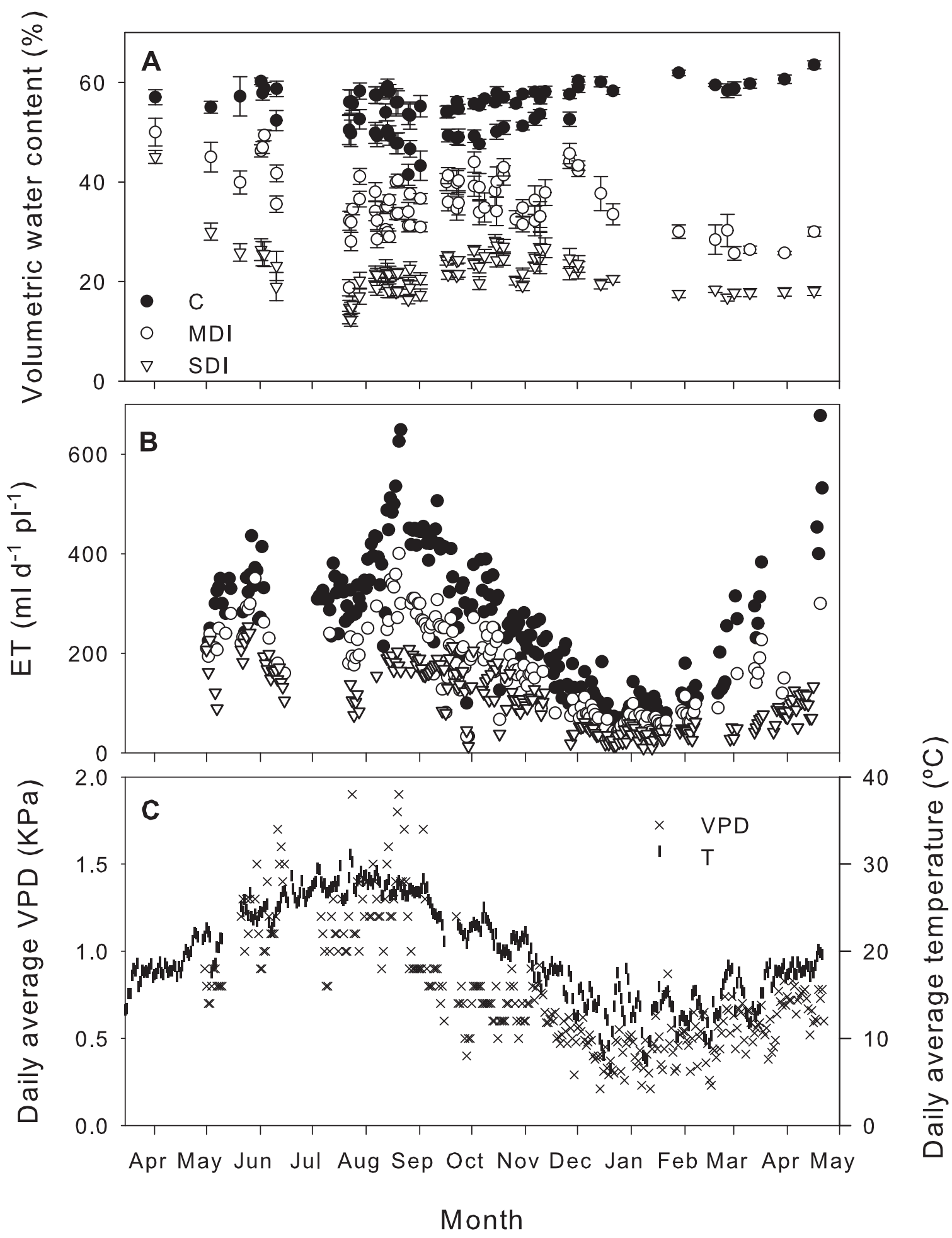


Figure 4

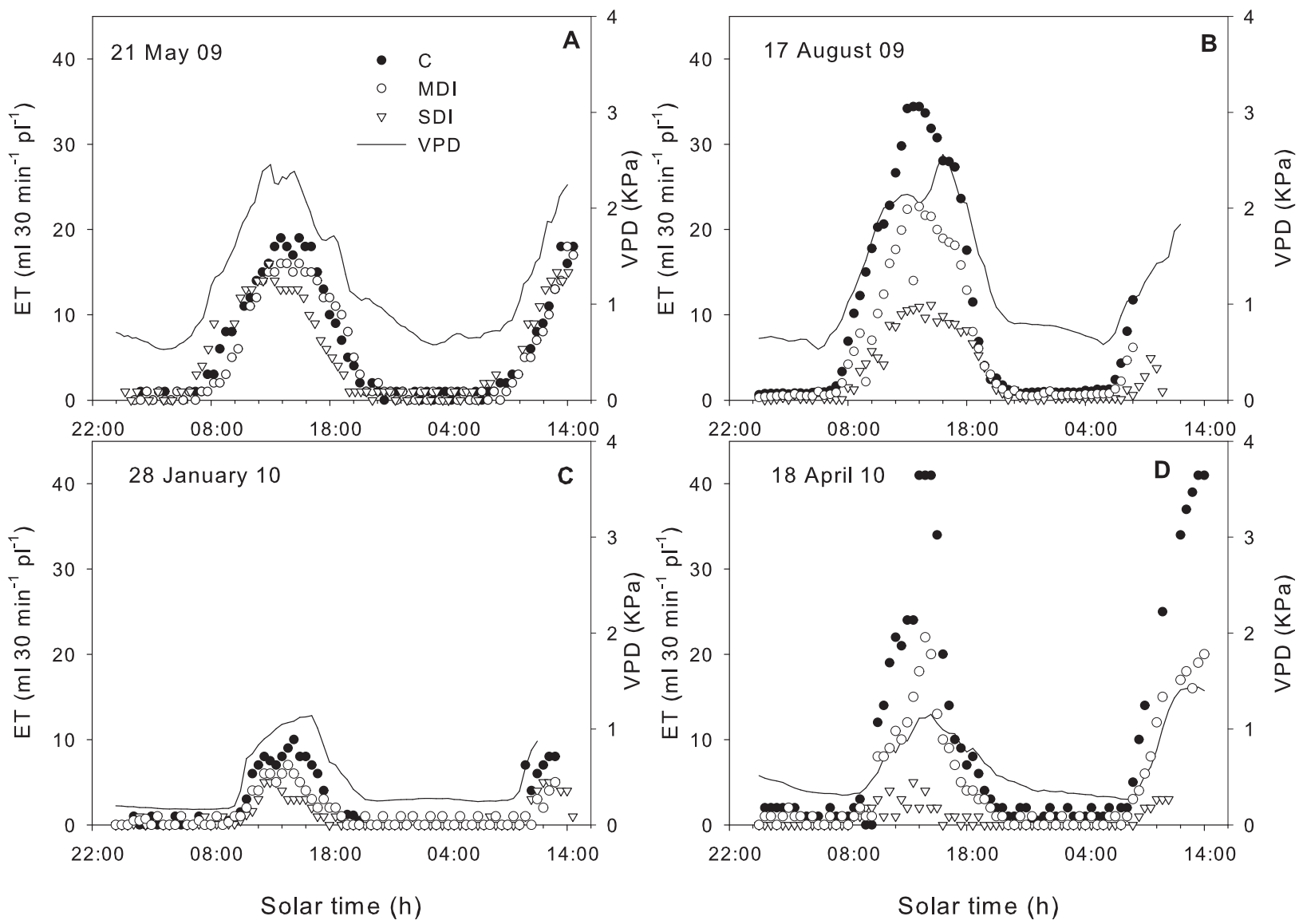


Figure 5

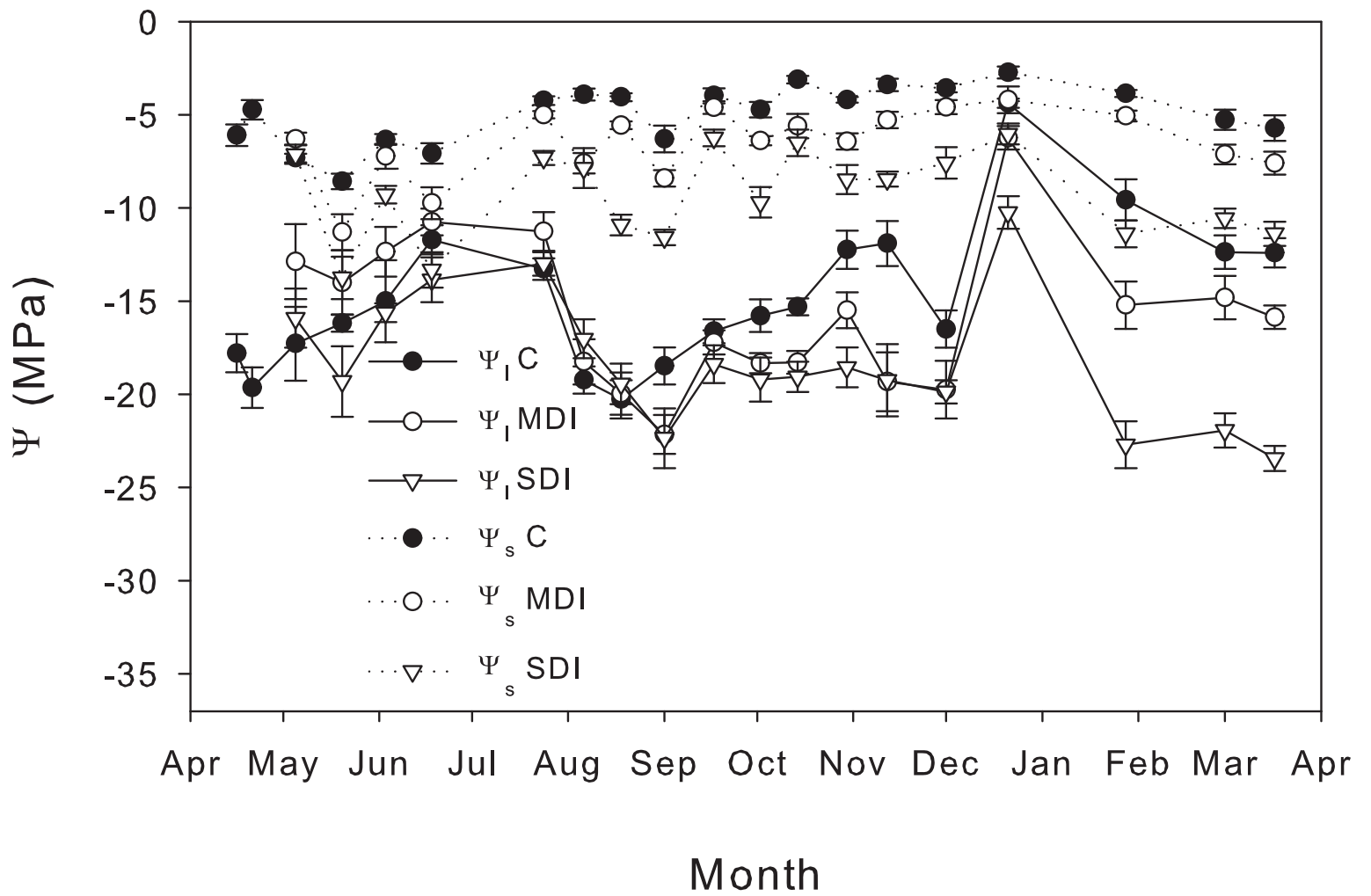


Figure 6

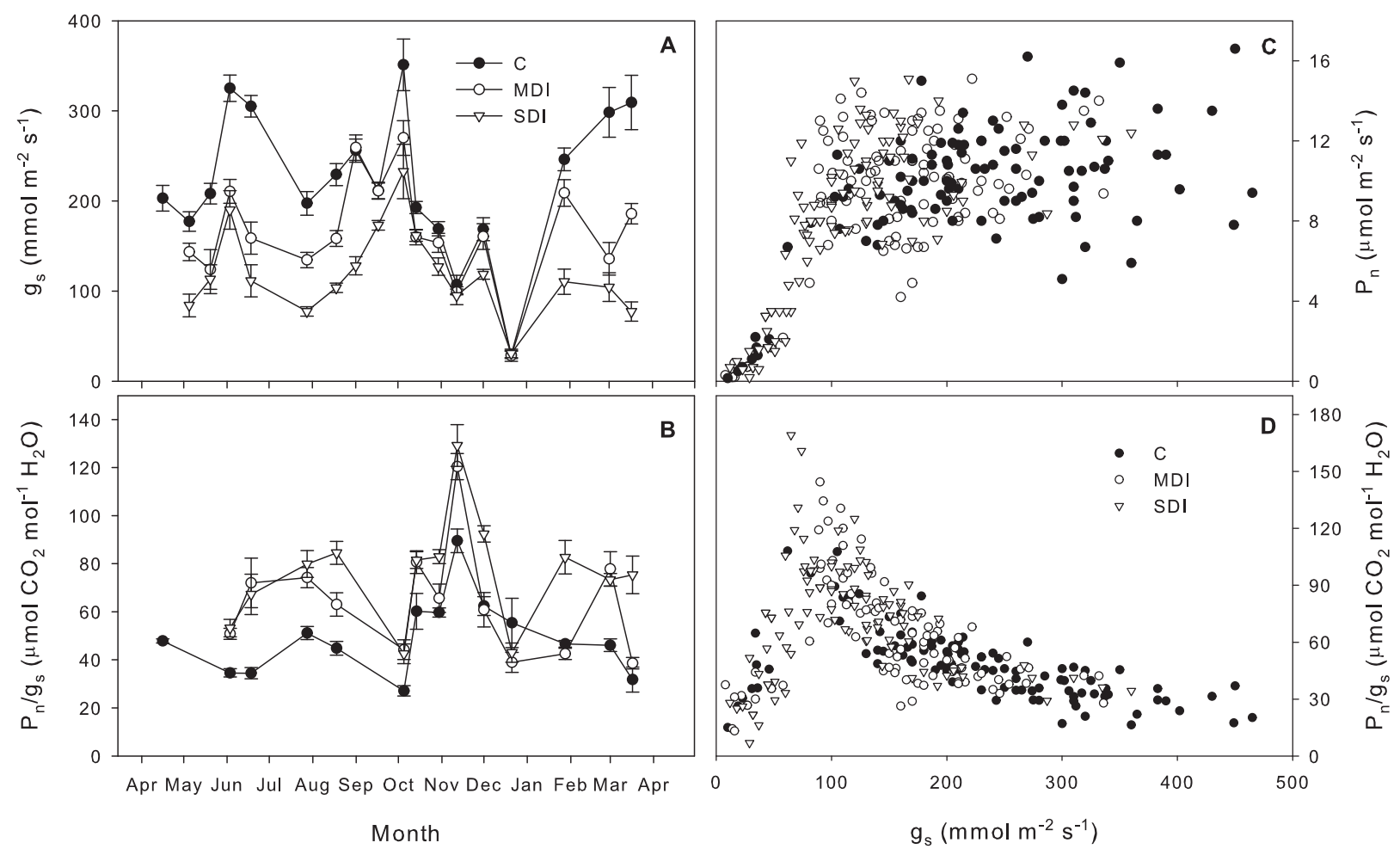


Figure 7

