

# EFFECTS OF WATER DEFICITS ON WHOLE TREE WATER USE EFFICIENCY OF ORANGE

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## **Abstract**

To study the effects of water deficits on water use efficiency (WUE) of citrus trees, whole tree transpiration and CO<sub>2</sub> assimilation were measured in a semi-arid environment during the summer of 2012. Young orange trees “Valencia Late”, either water stressed (DI) and well-irrigated (C), were monitored in selected days using a gas exchange chamber. Tree transpiration was also measured on a continuous basis with sap flow sensors. The water restriction reduced the transpiration of the DI treatment down to 60% of the maximum potential (treatment C) during the peak of water stress. The instantaneous WUE ranged between 1.7 and 79 g CO<sub>2</sub> L<sup>-1</sup> H<sub>2</sub>O and was tightly related to the vapour pressure deficit. Differences in instantaneous WUE due to water stress were insignificant. On a daily basis, WUE ranged between 4.9 (7 August) and 8.8 (7 June) g L<sup>-1</sup> for the daytime period; and between 4.0 and 8.2 g L<sup>-1</sup> for the 24h period. As water stress was imposed on the DI treatment, a trend of increasing WUE in DI relative to C was observed, reaching, in the maximum stress period, a difference of 13%-15% (daytime) and 20-22% (24h) although not statistically significant. Partial rewatering returned the WUE to similar values in both treatments. An analysis of the differences in the diurnal patterns of transpiration suggests that the increase in WUE due to water stress in citrus is achieved indirectly by shifting the overall carbon assimilation towards the morning hours of lower evaporative demand.

### **Keywords:**

Citrus; Transpiration; CO<sub>2</sub> assimilation; Gas-exchange chamber; Sap-flow

## 1 **1. Introduction**

2  
3 Most citrus species originated in the sub-humid, subtropical areas of south-eastern Asia  
4 (Scora, 1975; Spiegel-Roy and Goldschmidt, 1996) where drought episodes are  
5 infrequent but, over the centuries, the crop has adapted to the arid and semi-arid zones of  
6 the world. In these areas, citrus are normally grown under irrigation and water scarcity is  
7 often the norm, thus requiring that water is used as efficiently as possible. Assessing the  
8 efficiency of water use in crop production has been based on quantifying the water use  
9 efficiency (WUE), broadly defined in agronomy as the ratio between crop production and  
10 water used (Hsiao et al., 2007). It has long been known that for a given crop and climate,  
11 WUE is relatively constant, either under ample or deficient water supply (De Wit, 1958).  
12 From the physiological standpoint, WUE is defined as the ratio of CO<sub>2</sub> assimilation to  
13 transpiration and is often termed transpiration efficiency (TE). For methodological  
14 reasons, measurements of TE are carried out at the single leaf scale and studies  
15 characterizing the TE of citrus are no exception (e.g. García-Sánchez et al., 2007;  
16 Habermann et al., 2003; Nebauer et al., 2013; Ribeiro et al., 2009; Syvertsen et al., 2003;  
17 Syvertsen et al., 1997). Scaling up TE measurements obtained on single leaves to  
18 determine the WUE of whole trees or canopies carries a high degree of uncertainty due to  
19 the variations in radiation levels and in the microclimate around trees. If whole tree  
20 measurements could be performed, they should be much more meaningful for scaling up  
21 to the behaviour of canopies. The use of gas-exchange chambers or canopy bags (Corelli-  
22 Grappadelli and Magnanini, 1993) to measure carbon assimilation, transpiration or both  
23 simultaneously in whole plants has been expanding progressively in different species: for

24 example apple (Dragoni et al., 2005; Lakso et al., 1996), grapevine (Dragoni et al., 2006;  
25 Intrigliolo et al., 2009) and olive (Pérez-Priego et al., 2010; Villalobos et al., 2012).

26 The strong theoretical and experimental evidence of the relative constancy of WUE under  
27 water deficits (De Wit, 1958; Steduto et al., 2007; Tanner and Sinclair, 1983) contrasts  
28 with the reports at the physiological level indicating that TE in leaves increases under  
29 water deficits. Such an increase is based on an analysis of the resistance analogues to CO<sub>2</sub>  
30 and H<sub>2</sub>O fluxes (Jones, 1993), and on experimental evidence at the leaf level in various  
31 species (reviewed by Chaves and Oliveira, 2004). In citrus, a review of the early literature  
32 (Kriedemann and Barrs, 1981) indicated that there were conflicting reports about the  
33 effects of soil water deficits on TE (with some increase or decrease under water stress  
34 relative to what was observed under ample supply), and concluded that further work was  
35 desirable to clarify this issue.

36 Deficit irrigation is a practice that has been recommended for citrus (Ballester et al.,  
37 2012; González-Altozano and Castel, 2000) but the tolerance mechanisms that operate  
38 during the water deficits periods have not been uncovered. If the WUE of whole trees  
39 increase under water deficits, this could be one of the mechanisms contributing to the  
40 positive response that deficit irrigation elicits in several citrus species, such as orange  
41 (Castel and Buj, 1990) and mandarin (Ballester et al., 2011). We conducted an  
42 experiment to measure the whole-tree transpiration water use efficiency (henceforth  
43 WUE) of whole young orange trees under ample and deficient water supply, to ascertain  
44 the fate of tree WUE as it undergoes water stress and during recovery, at different time  
45 scales

46

47

## 48 **2. Materials and Methods**

49

### 50 **2.1 Site description and experimental setup**

51 The experiment was conducted at Cordoba (south-western Spain; 37.8° N, 4.8° W, 110 m  
52 altitude), at the Institute for Sustainable Agriculture of the Spanish Research Council  
53 (CSIC) during summer 2012. The soil is a Typic Xerofluvent of sandy loam texture easily  
54 penetrable by roots beyond 2 m. The upper (field capacity) and lower (wilting point)  
55 limits of available water are 0.23 and 0.09 m<sup>3</sup> m<sup>-3</sup>, respectively.

56 The experimental plot consisted of a small orchard (nine rows with five trees per row) of  
57 4-year old orange [*Citrus sinensis* (L.) Osbeck ‘Valencia Late IVIA126’] trees, grafted on  
58 ‘Volkamer’ lemon (*Citrus volkameriana* Tan. and Pasq.), planted at a distance of 4 x 4 m.  
59 The plot was fertilized with 75 kg ha<sup>-1</sup> of N, 50 kg ha<sup>-1</sup> of P, and 50 kg ha<sup>-1</sup> of K in spring.  
60 Trees were irrigated by the drip method, with a different combination of emitter number  
61 and discharge rate to match the target irrigation treatment. Control trees had four emitters  
62 per tree placed on a single drip line near the tree. Two 4 L/h emitters were located 0.5 m  
63 away from the trunk, and two 2 L/h emitters were placed one m apart from the other two,  
64 on each side of the tree. The deficit treatment was irrigated with two, 2 L/h emitters  
65 placed 0.5 m on each side of the tree trunk, that were sided by other two 4L/h emitters  
66 from 28 August to ensure the recover the deficit irrigated trees.. Prior to planting the  
67 orchard, vertical plastic sheets of 150 µm thickness were placed between the trees down  
68 to 1.2 m depth to isolate their root systems. This allowed the use of individual trees as  
69 experimental units without the need for adjacent trees acting as borders.  
70 Six trees in two different water regimes were used for the experiment:

71 - three individual trees received a control treatment (C), where irrigation fully replenished  
72 the estimated crop evapotranspiration (ET). No water deficits were allowed throughout  
73 the experiment. The irrigation season lasted from 16 May to 25 September.

74 - three individual trees received a deficit irrigation treatment (DI), with the same start and  
75 ending dates as C, received the following amount of irrigation:

76 - 33% of the full water requirements, from 16 May until 15 July;

77 - 16.5% from 16 July to 27 August;

78 - 100% from 28 August to 6 September, to study the recovery from stress;

79 - 33% from 8 September to 25 September (end of the season).

80 During the irrigation season the two irrigation treatments supplied 2221 L tree<sup>-1</sup> (138  
81 mm) to the Control trees and 804 L tree<sup>-1</sup> to the DI treatment (50.3 mm).

82 For irrigation scheduling, the full water requirements were calculated as ET-rainfall,  
83 where ET was calculated using crop coefficients for citrus trees corrected for canopy size  
84 and the Penman–Monteith mean monthly reference evapotranspiration or ET<sub>0</sub> (Allen et  
85 al., 1998) for Cordoba, obtained from 30-year historical data series.

86

## 87 ***2.2 Gas-exchange chamber***

88 A transitory-state chamber was designed and built at IAS-CSIC for simultaneous  
89 measurements of CO<sub>2</sub> and water vapour exchange. The chamber was based on previous  
90 prototypes (see Pérez-Priego et al., 2010 for description and drawing), and consisted of a  
91 rectangular prism with a base of 1.2 x 1 m, and a height of 2 m, giving an overall volume  
92 of 2.16 m<sup>3</sup>. The walls were made with 4 sections of rigid aluminium frames and were fit  
93 together with simple screws. This particular prototype has no moving windows and is

94 placed as a cap over the tree; it fits on fixed stainless steel frames acting as a base, firmly  
95 anchored in the soil around the base of each experimental tree. The bottoms of the base  
96 frames are sealed with a thick polyethylene panel, thus fluxes from the soil are excluded  
97 from the measurements. Leaks are avoided by placing a soft rubber gasket along the  
98 junctions between the chamber and the support frame. The top and the sides of the  
99 chamber are covered with clear bi-axially-oriented polyethylene terephthalate (BoPET)  
100 film of 75µm thickness, stretched and fixed to the aluminium frames. The chamber  
101 contains four 10 W fans of 15cm diameter, mixing the air inside the chamber while it is  
102 closed (which under normal operating conditions lasts around three minutes). A vacuum  
103 pump circulates the air through the sampling circuit: the air is taken from inside the  
104 chamber through many intake points, spatially distributed along the chamber and is then  
105 returned to it. A sample of 1 L min<sup>-1</sup> of this airflow is sniffed by a small pump and  
106 diverted to a CO<sub>2</sub>/H<sub>2</sub>O infrared gas analyser (IRGA) (model LI-COR LI-840, Lincoln,  
107 NE, USA) which measures CO<sub>2</sub> and water vapour concentrations simultaneously at 1Hz  
108 sampling rate; the output is recorded by a datalogger (model CR23X, Campbell  
109 Scientific, Logan, UT, USA). The IRGA, datalogger and small pump were powered by  
110 12V batteries. The vacuum pump and the fans are powered using a small portable A/C  
111 generator. An infrared thermometer (model IRR-P, Apogee, Logan, UT, USA) is  
112 mounted on the centre of the chamber top, facing downwards, to measure the temperature  
113 of the canopy. The sensor has a 44° field of view, and can be aimed and adjusted in  
114 height for optimal foliage targeting. The canopy temperature variation is checked at post-  
115 processing time to ensure that the disturbance in foliage thermal conditions is negligible.  
116 The air temperature and relative humidity were also measured inside the chamber with a

117 combined probe (model HMP45AC, Vaisala, Helsinki, Finland) placed near the top of the  
118 chamber into a radiation shield.

119 Two operators are needed for the opening and closing action. At the time of measuring,  
120 the chamber is put on the steel frame, closed and the fans are turned on. The CO<sub>2</sub> and  
121 water vapour concentrations, measured by the IRGA, change steadily after a short lag  
122 time. The concentrations of both gases are recorded at a 1 Hz sampling frequency. After  
123 completing a measurement, the chamber is lifted up to the open position away from the  
124 tree, with the fans off.

125 The IRGA was operated in the range of 0-2000  $\mu\text{mol mol}^{-1}$  for CO<sub>2</sub> and 0-80  $\text{mmol mol}^{-1}$   
126 for water vapour. A two-point calibration procedure was carried out in the laboratory  
127 before measurement operations.

128 The fluxes were calculated by fitting a second order polynomial model to the gas  
129 concentration time series, after removing the lag time (equivalent to 15s in our chamber  
130 arrangement) then obtaining the undisturbed gas concentration rate of change as the first  
131 derivative at time zero to avoid errors due to the drift from the original gradients (Wagner  
132 et al., 1997). The gas fluxes were transformed to mass basis per surface and time unit,  
133 simultaneously, following Jarvis et al. (1971), and included the correction for air  
134 temperature and atmospheric pressure inside the chamber (Reicosky et al., 1990).

135 The daily time course of net CO<sub>2</sub> above-ground assimilation (A) and transpiration (E<sub>p</sub>)  
136 were measured on Julian days 144, 152, 159, 193, 206, 220, 242, 249 (May to September  
137 2012), with repeated measurements over the whole day (starting before sunrise and  
138 ending after sunset), each one lasting three minutes of chamber closure. On some days  
139 (144, 152 and 193) the measurements ended before sunset due to accidental events



140 (mainly unexpected cloudiness). The Water Use Efficiency (WUE) was calculated as  
141  $A/E_p$  for the relevant time scale; WUE and transpiration efficiency (TE) are thus  
142 synonyms in the context of this work. Chamber measurements began before sunrise and  
143 ended after sunset, thus measurements in complete darkness and contrasting temperatures  
144 were available in each measurement date. These measurements allowed to compute  
145 canopy respiration ( $R_c$ ) with a simple model of the form  $R_c = R_0 e^{kT}$  where  $R_0$  is the  
146 measured respiration,  $T$  is air temperature, and  $k$ , a constant. After being calibrated for  
147 each individual tree the model was used to estimate the respiration during night-time  
148 hours preceding and following the diurnal measurement curve and thus calculate  $A$  and  
149 WUE for a 24 hour period.

150 The canopy conductance of the whole tree ( $G_c$ ) was calculated by inverting the Penman-  
151 Monteith equation assuming a negligible aerodynamic resistance (imposed evaporation).  
152 This assumption is reasonable for highly-coupled surfaces such as isolated trees (Raupach  
153 and Finnigan, 1988), and has been successfully used in the past (Orgaz et al., 2007;  
154 Villalobos et al., 2009).

155

### 156 *2.3 Sap flow*

157 Chamber measurements are necessarily discontinuous in time; to have a continuous  
158 record of transpiration in both treatments tree sap flow was measured by the compensated  
159 heat pulse technique. The system was installed in the summer (when the difference in  
160 water status begun to be appreciable) and run until the end of the experiment. The sap  
161 flow system used in this experiment was designed and manufactured at the IAS-CSIC  
162 laboratory in Cordoba (Testi and Villalobos, 2009). Each sensor consists of a 4.8W

163 stainless steel heater of 2 mm diameter and two four-point temperature probes, also in  
164 stainless steel needle casings of the same diameter. The temperature probes were installed  
165 10 and 5 mm down- and up-stream of the heater, respectively. Each temperature probe  
166 has four embedded Type E (chromel-constantan) thermocouple junctions, spaced 10 mm  
167 along the needle, sampled separately to obtain heat pulse velocities at different depths in  
168 the xylem. The probes were connected to multiplexers (AM16/32, Campbell Scientific  
169 Inc., Logan, UT, USA); each one allows the measurements of 8 probes. The system is  
170 controlled by a datalogger (CR1000, Campbell Scientific Inc., Logan, UT, USA) that, at  
171 given intervals, executes a measurement cycle consisting of: (a) measuring  $\Delta T$  (the  
172 temperature difference between down- and upstream measurement points with respect to  
173 the heater) every second during 10 s; (b) firing a 2-s heat pulse; and (c) measuring the  $\Delta T$   
174 every second during the following 180 s after each heat pulse. All the  $\Delta T$  measurements  
175 were stored, thus allowing post-processing and assessment of data quality for each  $\Delta T$   
176 curve following a heat pulse. The heat-pulse velocities and corresponding sap flows were  
177 calculated according to Testi and Villalobos (2009), where more details about this system  
178 can be found.

179 The same six trees used for the chamber measurements (three trees per treatment) were  
180 equipped with one probe each. Probes were individually calibrated versus the  
181 transpiration measured by the chamber, allowing the continuous recording of  
182 transpiration for each tree until the end of the experiment.

183

184 ***2.4 Other measurements***

185 Volumetric soil water content was measured with a neutron probe (503 DR Hydroprobe  
186 Moisture Gauge, Campbell Pacific Nuclear, Martinez, CA, USA). The probe was  
187 calibrated versus gravimetric measurements of water content; for this reason soil bulk  
188 density was determined in spring 2012, with soil samplings in 4 layers down to 1 m  
189 depth. Separate calibrations were performed for the upper (0–30 cm) and lower (below 30  
190 cm) layers. Four of the six trees selected for the chamber and sap-flow measurements  
191 (two trees per treatment) were also instrumented with five access tubes each, three  
192 located in the wet zone and two in the dry area outside the zone wetted by the emitters.  
193 Measurements were taken every 15 days in four layers (0-15, 15-30, 30-60 and 60-90 cm  
194 depth).

195 Tree leaf area was measured by counting all leaves of each tree and calculating tree leaf  
196 area based on the average individual leaf area measured in a subsample of 150 leaves,  
197 which were measured with an electronic planimeter (model LI-3100, LI-COR, Lincoln,  
198 NE (USA). The canopy volume and ground cover was estimated by photographic image  
199 analysis of the crown from four orthogonal directions.

200 Stem water potential ( $\Psi$ ) measurements were taken in three leaves per tree at mid-day on  
201 different dates throughout the experiment to evaluate tree water status. Fully expanded  
202 leaves located on branches near the main trunk were covered with aluminium foil for at  
203 least 30 min before excision; afterwards,  $\Psi$  was immediately measured with a pressure  
204 chamber (Soil Moisture Equip., Santa Barbara, CA, USA).

205 A weather station, located 700m from the experimental plot provided additional  
206 meteorological data measured every 10 min. The seasonal evolution of  $ET_0$ , rainfall and  
207 vapour pressure deficit (VPD) over the measurement period is shown in Fig. 1. The total

208  $ET_0$  for 2012 was 1322 mm, which is considered very close to the long-term average of  
209 the area; total annual rainfall was 650 mm, value that exceeds the long term average by  
210 about 15%.

211

212

### 213 **3. Results**

214

215 At the start of measurements, average canopy volume was  $0.82 \text{ m}^3$  and the trees covered  
216 9% of the ground. The average number of leaves per tree was 3457 (min. 2877, max.  
217 3706) and the average leaf area per tree was  $8.81 \text{ m}^2$  (min.  $7.78 \text{ m}^2$ , max.  $9.75 \text{ m}^2$ ). The  
218 leaf area index (LAI) of the plot was  $0.55 \text{ (m}^2 \text{ m}^{-2}\text{)}$  while the average leaf area density  
219 was around  $11 \text{ m}^2 \text{ m}^{-3}$ .

220 Even though the trees were small and the winter rainfall had filled the soil profile, the  
221 irrigation treatments imposed a clear-cut influence on soil and plant water status; the  
222 deficit treatment underwent mild to moderate water stress conditions - a water status  
223 representative of commercial orchards managed under deficit irrigation. The soil water  
224 content measured inside and outside the soil area influenced by the emitters is shown in  
225 Fig. 2a and b for a C and a DI tree, respectively. In the C treatment, the soil water content  
226 in the wet zone always exceeded  $0.22 \text{ m}^3 \text{ m}^{-3}$ , being always close or above field capacity.  
227 Outside the emitter influence, in the C treatment, the soil water declined slowly down to  
228  $0.17 \text{ m}^3 \text{ m}^{-3}$  at the end of September (Fig. 2a), a level which corresponds to 60% of the  
229 soil available water. In the DI treatment, the soil dried from the start of the differential  
230 irrigation in June, reaching minimum values of  $0.13 \text{ m}^3 \text{ m}^{-3}$  at the end of August, and

231  $0.10 \text{ m}^3 \text{ m}^{-3}$  (practically the soil lower limit) at the end of September (Fig. 2b). The  
232 decline in soil water content in the wetted zone of the RDI treatment during the  
233 experimental period indicated a clearly insufficient irrigation supply; only when the rate  
234 of irrigation was increased in September to study the recovery the soil water content  
235 increased. Following the heavy rainfall event of 26-27 September, the soil water content  
236 recovered in all cases. As expected, tree water status reflected the soil water content  
237 trends. The C treatment (Fig. 2a) maintained stem  $\psi$  values between -0.4 and -0.8 MPa,  
238 with a slight overall declining trend from June to September, probably due to the gradual  
239 increase in evaporative demand (see Fig. 1).

240 On the contrary, the stem  $\psi$  in DI decreased quickly from values around -0.5 MPa  
241 (similar to C) at the end of June, to values close to -1.5 MPa one month later. The DI  
242 stem  $\psi$  was always consistently lower than that of C until the end of the deficit irrigation  
243 period, when the increased water supply to DI caused the water status of the stressed trees  
244 to approach that of C.

245 Representative observations of the diurnal courses of tree gas exchange are shown in Fig.  
246 3, displaying three complete measurement days taken at; a) the beginning; b) at the  
247 maximum stress; and, c) after partial relief of the water restriction. On 7 June, DOY 159  
248 (Fig. 3a) the soil water content was over  $0.2 \text{ m}^3 \text{ m}^{-3}$  in both treatments; assimilation (A)  
249 and transpiration ( $E_p$ ) of the two treatments were similar, although with considerable  
250 fluctuations, also evident in the canopy conductance. The daily course of WUE had its  
251 maximum in the early morning after sunrise, and decreased continuously until after 1600,  
252 when a small rise in WUE occurs. Little or no difference in WUE can be found between  
253 the two treatments, as expected; ANOVA found no significant difference ( $p=0.05$ )

254 between the means at any given time of the day. Fig. 3a shows the diurnal gas exchange  
255 curve taken on 7 August, when the two treatments already showed significant differences  
256 in tree water status. On this date, the DI treatment had already depleted over 70% of the  
257 available soil water (see Fig. 2b) and  $\psi$  was below -1 MPa. The transpiration of the C  
258 treatment exceeded that of the DI during the whole day; assimilation in C was also  
259 slightly higher than in DI, especially during the early morning. Water deficits affected  
260 canopy conductance which was always higher in the C treatment relative to DI. Water use  
261 efficiency was very similar in both treatments, although DI had somewhat higher values,  
262 especially in the morning, (930) and in the afternoon (1730); this last measurement was  
263 the only one that showed statistically significant WUE between the treatments (at  
264  $p=0.05$ ).

265 Fig. 3c depicts the diurnal course of gas exchange on 5 September, one week after the  
266 irrigation rate in DI was increased to recover the DI treatment. The recovery in DI was far  
267 from complete (cf. Fig. 2b) and both  $A$  and  $E_p$  are still clearly higher in C, as well as the  
268 canopy conductance curves which are also clearly different. The two curves of WUE are  
269 almost undistinguishable; no statistical differences in WUE were found at any time of the  
270 day.

271 The daily integrated values of  $A$  and  $E_p$  measured along the season are shown in Table 1,  
272 with the calculated WUE for the day, both for daytime and for 24h. The carbon  
273 assimilation peaked on 7 June, both for daytime (44.9 and 40.2 g CO<sub>2</sub> day<sup>-1</sup> in the C and  
274 DI respectively) and for 24h periods (41.7 and 37.9g CO<sub>2</sub> day<sup>-1</sup> in the C and DI  
275 respectively). The maximum transpiration is reached on 7 August in C (6.2 L day<sup>-1</sup>) and  
276 on 24 July in DI (5.3 L day<sup>-1</sup>). The water use efficiency of the DI (excluding incomplete

277 days) always showed a slightly higher WUE than the C treatment except on 7 June and 5  
278 September. The daily values of WUE for daytime ranged between 4.9 and 8.8 g CO<sub>2</sub> L<sup>-1</sup>  
279 in C and between 5.6 and 8.6 g CO<sub>2</sub> L<sup>-1</sup> in DI; for 24h periods WUE ranged between 4  
280 and 8.2 g CO<sub>2</sub> L<sup>-1</sup> in C and between 4.9 and 8.1 g CO<sub>2</sub> L<sup>-1</sup> in DI. In both treatments and  
281 both for daytime and for 24h periods the minimum is reached on 7 August and the  
282 maximum on 7 June. The measurements taken on 7 and on 29 August showed clear  
283 differences: daytime WUE of DI was 15% and 13% (22 and 20% for WUE in 24h) higher  
284 than that of C, respectively. The difference in WUE between treatments disappears again  
285 on 5 September, when the DI has already recovered for the most part from water stress  
286 (Fig. 2) and was transpiring at 85% of its potential rate (see below, Fig. 4).

287 The continuous transpiration measurements (obtained from sap flow after calibration with  
288 the chamber), are shown in the upper plot of Fig. 4. In the lower part of the figure, the  
289 DI/C transpiration ratio is plotted over the same abscissa. Tree transpiration decreased in  
290 both treatments from mid-July onwards, due to the decrease in evaporative demand; the  
291 maximum was reached at the beginning of July, with a value of 9.7 L tree<sup>-1</sup> day<sup>-1</sup>. At that  
292 time Ψ of DI is beginning to drop (see Fig. 2), but the transpiration rates of C and DI are  
293 still very similar (Fig. 4). Subsequently, at the beginning of August, the DI transpiration  
294 rate dropped quite rapidly to around 60% of the potential rate due to the irrigation  
295 shortage (from 33 to 16.5%) applied at the end of July. At the end of August, the milder  
296 evaporative demand and an increase in irrigation of DI (from 16% to 100% of crop  
297 maximum ET) allowed to recover the relative transpiration rate up to 0.9. The recovery  
298 proved temporary, and the transpiration of DI declined again, first steadily and then more  
299 abruptly until reaching once more the value of 60% of the maximum in the second part of

300 September. The heavy rainfalls of the last week of September (110 mm in 3 days) first  
301 drove the transpiration of both treatments to zero due to negligible evaporative demand  
302 (see Fig. 1), and then allowed the transpiration of DI to recover to C values in six days  
303 (Fig. 4b; see also soil water content in Fig. 2b).

304 The strong inverse relation between instantaneous values of WUE and VPD in orange is  
305 shown in Fig. 5a, where all the chamber measurements taken in each treatment are pooled  
306 together. The same empirical model was fitted to each treatment dataset and the  
307 difference between the two treatment lines is difficult to discern. Some outliers (indicated  
308 by arrows) are associated to very low fluxes (both A and  $E_p$ ) which make their ratio drift  
309 unpredictably; all of them are measurements taken at dawn or dusk with light close to the  
310 compensation point. These outliers have been removed from the model fitting.

311 Although the difference between the two curves is barely perceivable, we computed the  
312 percent gain in WUE of the DI treatment (the difference between the two curves  
313 expressed in percent of the WUE of the control). While the gain at very low VPD is  
314 possibly an artefact (the adopted model yields infinite WUE at  $VPD = 0$ ), there are hardly  
315 any differences at VPD values between 1 and 2 kPa, and an increase of about 10% at  
316  $VPD=5$  kPa (Fig. 5a).

317

## 318 **4. Discussion**

319

### 320 *4.1 Effects on carbon assimilation and transpiration*

321 The gas exchange measurements taken after water stress became detectable in DI showed  
322 that transpiration, canopy conductance and assimilation (although in variable magnitude)



323 were higher in C from dawn to dusk. Assimilation was also affected, although in variable  
324 magnitude (Fig. 3b and c). The instantaneous WUE showed a clear time pattern: high in  
325 the early morning then declining to a plateau in the afternoon, without fluctuations. Only  
326 when the differences in water status were greatest (Fig. 2) was the instantaneous WUE  
327 slightly higher in DI relative to C (Fig. 3b, 2-5% depending on the hour but without  
328 statistical significance - data not presented).

329 The average daily transpiration measured with the sap-flow probes never exceeded 10 L  
330 tree<sup>-1</sup> day<sup>-1</sup>, corresponding to slightly more than 0.6 mm day<sup>-1</sup> at orchard level. This small  
331 transpiration flux is not surprising, as the trees were very young and covered only the 9%  
332 of the ground. As a comparison, Fig. 4 also shows the transpiration calculated trees with a  
333 simple transpiration model for unstressed citrus canopies proposed by Villalobos et al.  
334 (2009)., which compares well with the C treatment. Consumptive transpiration in the  
335 period of sap-flow measurements (11 July to 22 November) was 643 and 565 L tree<sup>-1</sup> for  
336 the C and DI treatments respectively; the simple transpiration model predicted 669 L tree<sup>-1</sup>  
337 for the C treatment in the same period. The reported DI/C transpiration ratio (Fig. 4b) is  
338 equivalent to the relative transpiration rate (actual over potential transpiration) of DI. The  
339 relative transpiration trends of DI in September exemplify the problems of managing  
340 deficit irrigation in areas of high evaporative demand. The partial recovery in  
341 transpiration exhibited in September was followed by a sharp drop during the second part  
342 of the month (Figure 4), even though ET<sub>0</sub> was much less than in mid-summer (Figure 1).  
343 This decline in relative transpiration in DI was caused by the low level of stored soil  
344 water near the end of the irrigation season (Fig. 2), in turn generated by the deficit  
345 irrigation practice.

346 .

#### 347 *4.2 Behaviour of instantaneous WUE*

348 Differences in WUE between the C and the DI trees were almost impossible to discern  
349 (Fig. 3a, b and c). As the instantaneous WUE varies widely along the day, it is difficult,  
350 due to its strong dependence on VPD, to clearly detect differences between treatments  
351 given the measuring technique used (a single, portable chamber), in particular at times of  
352 low VPD and when temperatures are changing rapidly. Fitting a relationship to the WUE  
353 data against VPD (Fig. 5a) allows the comparison of instantaneous WUE with other  
354 species or the same species in other conditions. For example in Fig. 5b the WUE curve of  
355 this experiment is compared with some of the limited data available in the literature for  
356 citrus trees. The fitted model with the parameters optimised for C fits perfectly the data of  
357 orange WUE presented by Ribeiro et al. (2009) from an experiment conducted in Brazil  
358 (measured range 7 to 30 g CO<sub>2</sub> L<sup>-1</sup>H<sub>2</sub>O) , and very well (especially for unshaded leaves)  
359 against those of Syvertsen et al. (2003), also in orange in Florida (measured range 7.5 to  
360 11.3 g CO<sub>2</sub> L<sup>-1</sup>H<sub>2</sub>O). The agreement of our results with the two reports above is  
361 remarkable, given that the WUE data were obtained at the leaf level rather than at canopy  
362 level as in this experiment.

363 Olive is another evergreen that often shares the same growing areas of citrus orchards,  
364 especially in the Mediterranean region. In Fig. 5a, the two lines with squares and full  
365 rounded symbols represent two WUE-VPD relationships for olive obtained at different  
366 spatial scales, measured respectively by Testi et al. (2008) using eddy covariance (plot  
367 scale) and by Villalobos et al. (2012) using gas exchange chambers (plant scale). The  
368 difference between the two curves for olive is probably the result of the evaporation from

369 the soil, which is included when using eddy covariance and was excluded from the tree  
370 chambers of Villalobos et al. (2012). Although the patterns are very similar, orange WUE  
371 was always somewhat higher than that of olive, in particular in the low VPD range; this  
372 could be a consequence of citrus having evolved in more humid climates than the olive,  
373 where less stomatal control at low VPD (a behaviour which enhances WUE) would not  
374 be detrimental, as it may be in the semi-arid environments where olive evolved.

375

#### 376 *4.3 Effects on WUE at different time scales*

377 The two empirical models of the type  $Y = a + b X^c$ , fitted to the C and DI datasets  
378 generated very similar curves (Fig. 5a) which are not statistically different; thus, we  
379 cannot conclude that instantaneous WUE in orange was affected by the level of water  
380 stress tested in this experiment. Nevertheless, the relative differences between the two  
381 curves plotted in a separate line (DI gain, Fig. 5a) makes it perceivable that the DI curve  
382 showed always a slightly higher WUE than that fitted on C measurements, and the  
383 difference was greater under high VPD (around 10%) . This behaviour is in partial  
384 accordance with the output of photosynthesis-transpiration models (e.g. Dewar, 2002;  
385 Tuzet et al., 2003). These models predict that the stomata closure caused by water stress  
386 increases the instantaneous WUE, but to a greater extent compared to our measurements:  
387 up to 30% under ranges of VPD of 4-5 KPa (Villalobos, unpublished data).

388 When the time scale changed from instantaneous to daily, the DI trees exhibited higher  
389 WUE than the C trees (Table 1). Villalobos et al (2012) demonstrated for the olive that  
390 the WUE of stressed trees was higher than that of well watered ones on longer time scales  
391 (day or month), even though the instantaneous WUE was the same. The phenomenon can

392 be explained through a different diurnal pattern of canopy conductance. The more  
393 pronounced stomata closure during the afternoon that occurs in a water-stressed plant  
394 shifts a greater fraction of the total daily water transpired towards the morning - a time of  
395 lower evaporative demand - which enhances the overall daily WUE even with  
396 instantaneous WUE similar to that of a well watered one at any time of the day. This  
397 increased asymmetry in the diurnal course of transpiration has been described several  
398 times in stressed plants in the past (e.g. Fereres, 1984; Olioso et al., 1996; Schulze and  
399 Hall, 1982), although its impact on the WUE were not quantified. It should be highlighted  
400 that every increase in the asymmetrical behaviour of stomatal conductance (with lower  
401 values in the afternoon) will necessarily increase the daily WUE in semi-arid  
402 environments, although at the price of decreasing the total assimilation.

403 The link between canopy conductance (or transpiration) with WUE is often subtle and it  
404 is difficult to detect asymmetrical distortions between diurnal curves under different  
405 water status when the magnitude of fluxes or conductances changes as well. Besides, the  
406 differences in daily WUE detected in this experiment are relatively small. The diurnal  
407 curves of whole-tree canopy conductance (Fig. 3), although different in magnitude, are  
408 similar in shape for C and DI. Given that the discrete number of chamber measurements  
409 prevents the appraisal of small differences in the curve shape, the continuous records of  
410 sap flow could help evaluating the hypothesis of the displacement of the pattern of water  
411 use in DI trees towards the morning hours. Figure 6 shows the fraction of the total  $E_p$   
412 daily flux that is transpired before 1200 (noon) in different dates, corresponding to  
413 different levels of water stress in the DI. By comparing the data of Fig. 6 with Fig. 2 and  
414 Table 1, as the water status of the DI declines (see also the relative transpiration rate of

415 Fig. 4), the fraction of water transpired in the morning by DI relative to that of C  
416 increases, and the overall WUE for the day (either considering daytime or 24h) is also  
417 increased. On 24 July (DOY 205) the DI treatment - even though it had a lower  $\Psi_{\square}$  than  
418 C (Fig. 2) - was still transpiring at about 95% of C (Fig. 4) and both treatments transpired  
419 in the morning the same fraction of the total (38%; Fig. 6) leading to daily WUE that  
420 were practically the same (Table 1). On the contrary, after the 11<sup>th</sup> of August, when water  
421 stress became more noticeable in DI, the fraction of water transpired in the morning hours  
422 in DI always exceeded those of C. At the beginning of September the relative  
423 transpiration (Fig. 4) and its diurnal pattern matched again that of C (Fig. 6) due to the  
424 partial recovery of DI. The WUE of DI at the beginning of September was again the same  
425 as C (Table 1).

426 In conclusion, the WUE of whole orange trees ranged - on a daily basis - between 4.9 and  
427 8.8 g CO<sub>2</sub> L<sup>-1</sup>H<sub>2</sub>O considering the daytime hours, and between 4.0 and 8.2 CO<sub>2</sub> L<sup>-1</sup>H<sub>2</sub>O  
428 over 24 hours. Water stress had a minor effect on instantaneous WUE, but showed  
429 greater values than for the control trees when computed over a 24 hour period. This was  
430 caused by a shift in the pattern of transpiration towards the morning hours, a behaviour  
431 already documented in the olive, where the evidence is stronger than what was found in  
432 this study.

433

434

## 435 **5. Acknowledgements**

436

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548

549

## Tables

**Table 1**

Daytime totals of transpiration ( $E_p$ ;  $L \text{ day}^{-1} \text{ tree}^{-1}$ ), net above-ground  $\text{CO}_2$  assimilation (A;  $g \text{ CO}_2 \text{ day}^{-1} \text{ tree}^{-1}$ ) and derived water use efficiency (WUE,  $g \text{ CO}_2 L^{-1}$ ) measured with the gas exchange chamber. Values for 24 hours include the night time tree respiration, estimated by a simple model calibrated with the chamber measurements obtained before dawn and after dusk. The asterisks denote days with incomplete diurnal curves (measurements suspended before sunset due to cloudiness or equipment failure). The measured variables are presented as averages; within brackets is the standard deviation. Statistical significance at  $p=0.05$  is not reached in any measurement date for WUE.

Date	DOY	C					DI				
		$E_p$ ( $L \text{ d}^{-1} \text{ tree}^{-1}$ )	A ( $g \text{ CO}_2 \text{ d}^{-1} \text{ tree}^{-1}$ )		WUE ( $g \text{ CO}_2 L^{-1}$ )		$E_p$ ( $L \text{ d}^{-1} \text{ tree}^{-1}$ )	A ( $g \text{ CO}_2 \text{ d}^{-1} \text{ tree}^{-1}$ )		WUE ( $g \text{ CO}_2 L^{-1}$ )	
			daytime	24h	daytime	24h		daytime	24h	daytime	24h
* 23-May	144	5.0 (0.1)	35.1 (0.3)	33.0 (0.7)	7.0 (0.0)	6.6 (0.1)	4.8 (0.1)	35.0 (2.1)	33.5 (1.5)	7.3 (0.5)	7.0 (0.4)
* 31-May	152	6.8 (1.8)	47.8 (0.7)	44.2 (0.4)	7.1 (0.3)	6.6 (0.2)	7.0 (1.3)	50.4 (6.8)	47.9 (6.1)	7.2 (0.5)	6.9 (0.6)
7-Jun	159	5.1 (0.3)	44.9 (2.6)	41.7 (2.6)	8.8 (0.1)	8.2 (0.1)	4.7 (0.4)	40.2 (2.2)	37.9 (1.4)	8.6 (0.4)	8.1 (0.4)
* 11-Jul	193	4.5 (0.2)	32.7 (1.4)	29.4 (1.4)	7.3 (0.7)	6.6 (0.7)	3.6 (0.8)	29.8 (3.7)	27.4 (2.8)	8.3 (0.8)	7.9 (0.9)
24-Jul	206	6.0 (0.2)	40.5 (2.1)	35.2 (2.1)	6.8 (0.2)	5.9 (0.2)	5.3 (0.3)	36.1 (2.4)	32.6 (1.8)	6.9 (0.8)	6.2 (0.1)
7-Aug	220	6.2 (0.1)	30.0 (1.1)	25.0 (1.1)	4.9 (0.1)	4.0 (0.1)	5.2 (0.2)	28.9 (1.1)	25.4 (0.9)	5.6 (0.3)	4.9 (0.3)
29-Aug	242	5.4 (0.3)	32.5 (2.8)	27.1 (2.8)	6.0 (0.2)	5.0 (0.2)	4.4 (0.1)	30.0 (1.1)	26.3 (1.9)	6.8 (0.3)	6.0 (0.5)
5-Sep	249	5.9 (0.3)	40.2 (3.2)	35.8 (3.2)	6.8 (0.2)	6.1 (0.2)	5.0 (0.1)	34.1 (1.1)	31.0 (1.9)	6.8 (0.3)	6.2 (0.5)

## List of figures

Fig. 1 Meteorological conditions (daily values) during the experiment. Plots of reference evapotranspiration ( $ET_0$ , mm, straight line), vapour pressure deficit (VPD KPa, dotted line) and rainfall events (mm, vertical bars) for the part of the year 2012 relevant for this experiment, measured at the weather station located near the experimental plot.

Fig. 2 Seasonal evolution of the soil water content and water status of two single trees in different treatments. a: Control treatment; b: Deficit Irrigation treatment. The volumetric soil water content in the wet and dry zones (solid and dashed line respectively) is displayed separately. Also shown are the measurements of mid-day stem water potential  $\Psi$  (MPa; white dots).

Fig. 3 Diurnal time courses of plant variables measured with chambers. Are presented: net above-ground  $CO_2$  assimilation ( $A$ ,  $g\ CO_2\ h^{-1}$ ), tree transpiration ( $E_p$ ,  $L\ h^{-1}$ ), canopy conductance ( $G_c$ ,  $mmol\ m^{-2}\ s^{-1}$ ) and water use efficiency (WUE,  $g\ CO_2\ L^{-1}$ ), measured on: a) 7 June (DOY 159); b) 7 August (DOY 220) and c) 5 September (DOY 249). All the values reported are averages of three replicates. No significant difference ( $p=0.05$ ) between the WUE of the two treatments was found in any of these measurements, except at 1740 of 7 August.

Fig. 4. a): seasonal course of transpiration ( $L\ day^{-1}\ tree^{-1}$ ) of the two treatments. The transpiration estimated by the model of Villalobos et al. (2009) for a Citrus orchard of the same ground cover is also shown. b): ratio between the water stressed (DI) and the unstressed (C) transpiration. All the values reported are

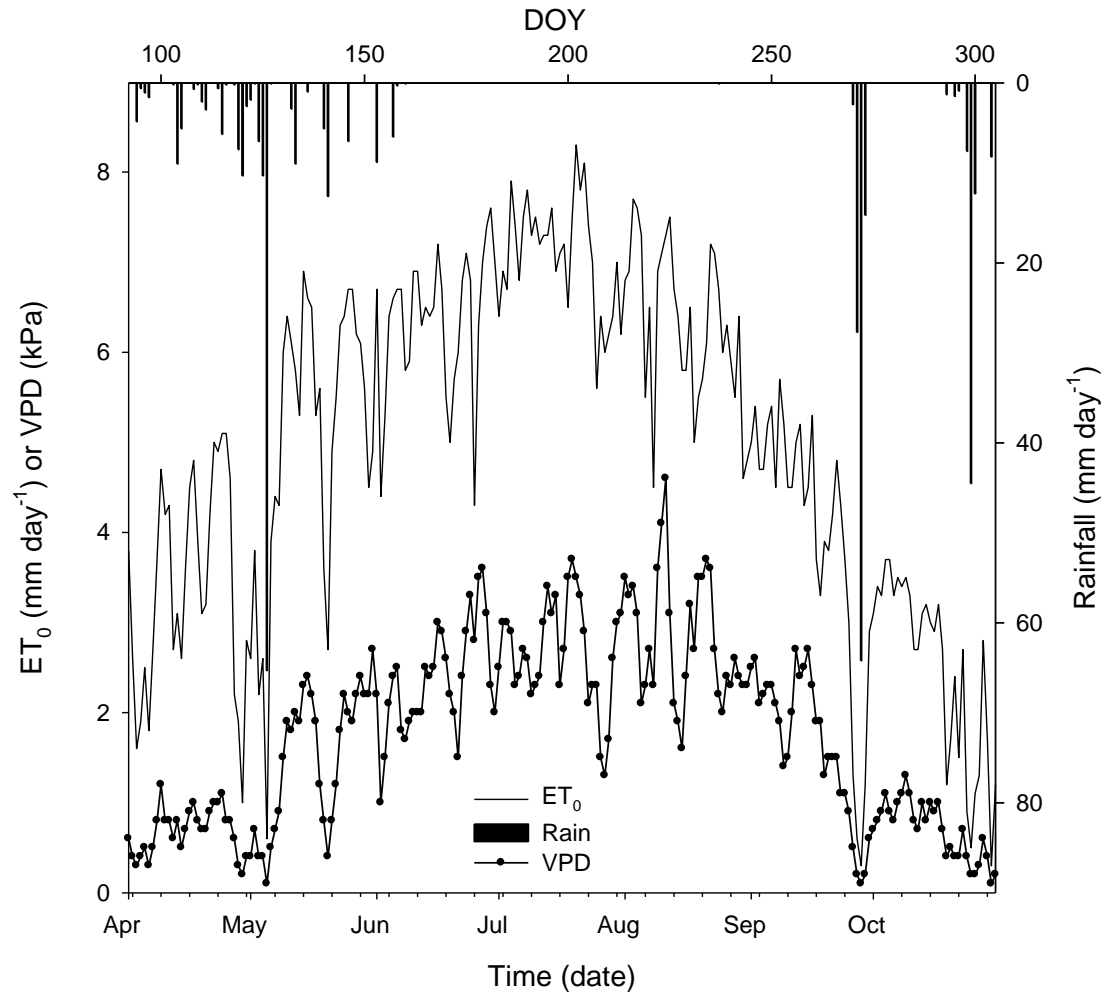
averages of three replicates. The average standard deviation of the transpiration of a day between trees was 0.7 and 0.4 L day<sup>-1</sup> respectively for C and DI.

Fig. 5. Relationship between WUE and instantaneous Vapour Pressure Deficit a): experimental data of well irrigated (C, circles) and water stressed (DI, crosses) treatments. The curves represent the empirical models fitted to the data: C (solid line, n=231), DI (dashed line, n=236). The fitted curve for both treatment data pooled together (not shown in the picture) has the equation  $2.65 + 20.1 \text{ VPD}^{-1.47}$ . The relative difference between the two models (DI gain) is plotted as dotted line. Also shown are the empirical fits of WUE obtained in olive by Testi et al. (2008) by eddy covariance and by Villalobos et al. (2012) by chambers. b): WUE measurements reported in unshaded leaves by Syvertsen (2003) and Ribeiro (2009) are plotted over the curve fitted to the Control in this experiment.

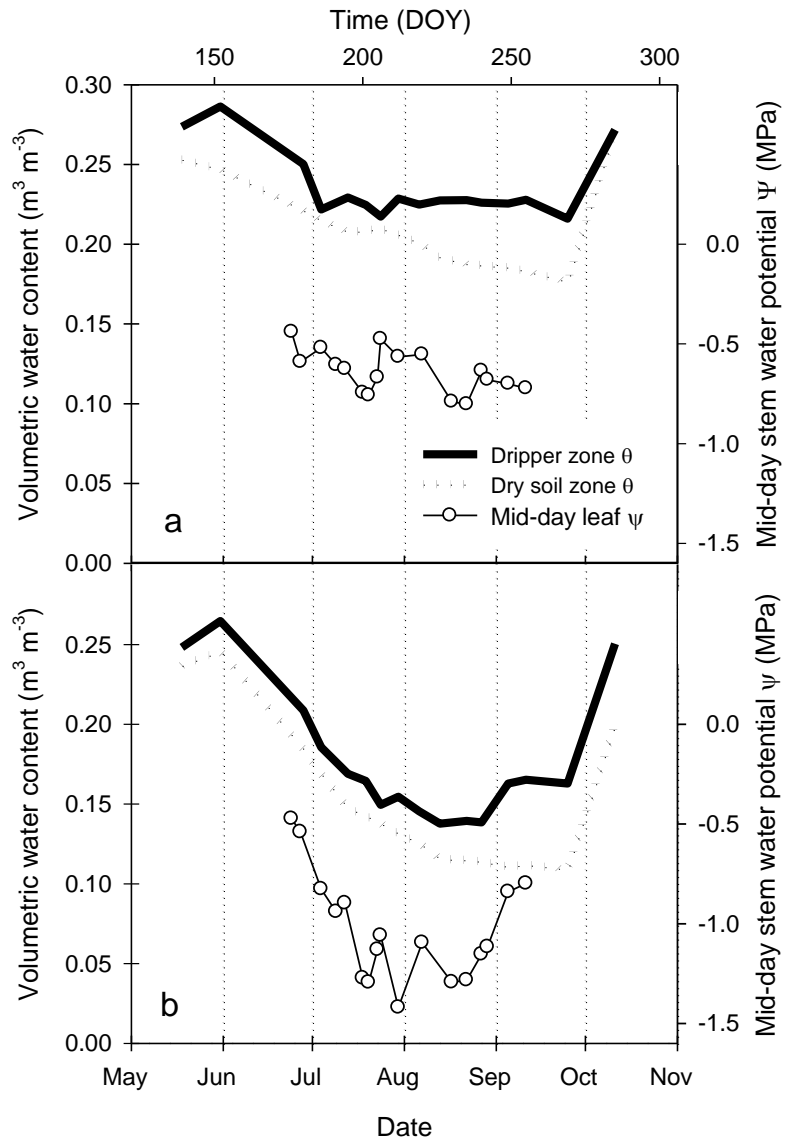
Fig. 6. Fraction of the total water transpired before noon by the two treatments (averages) in days with different water status of the DI. The relative transpiration for each day ( $E_p \text{ DI} / E_p \text{ C}$ ) is also reported.



# Figures

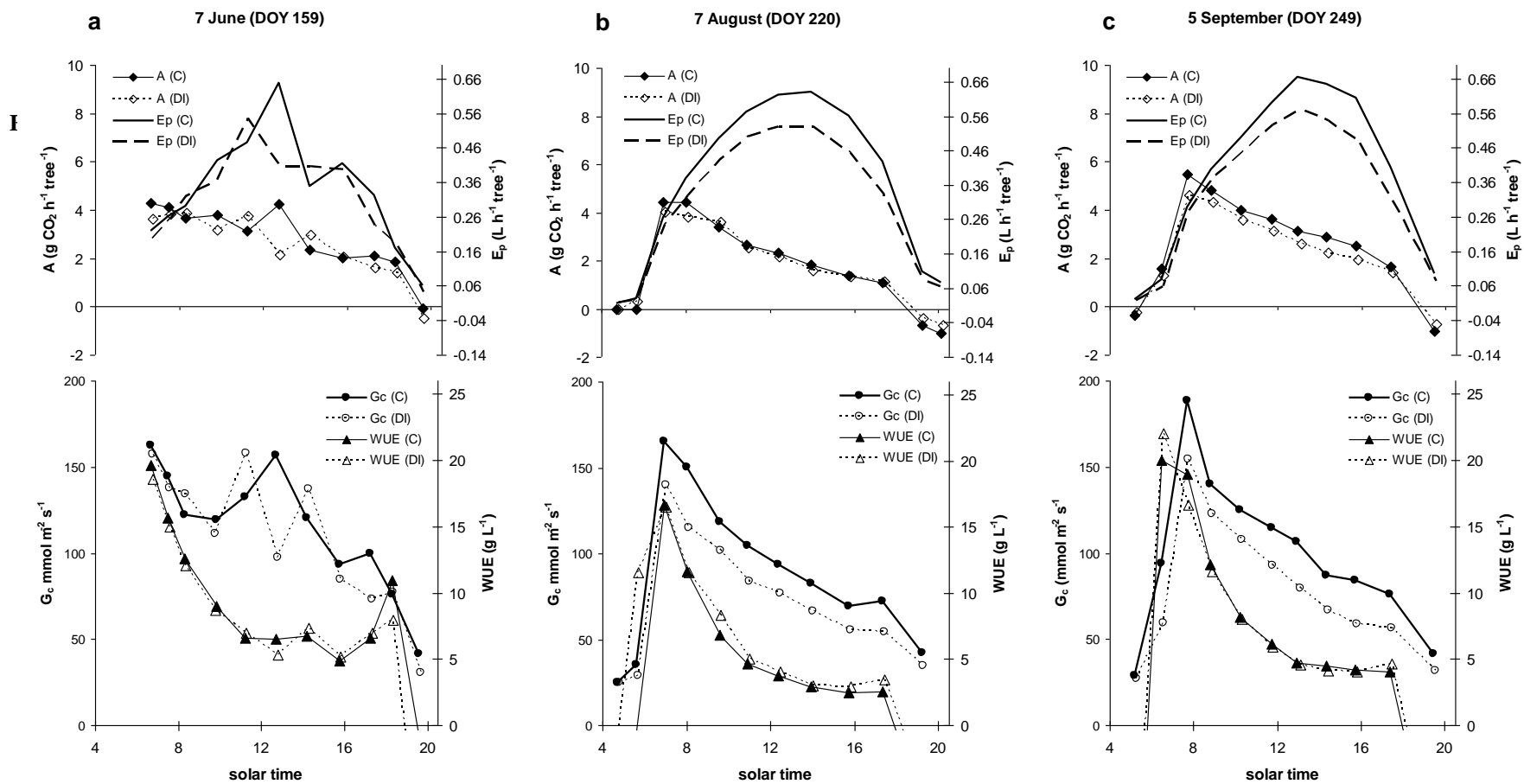


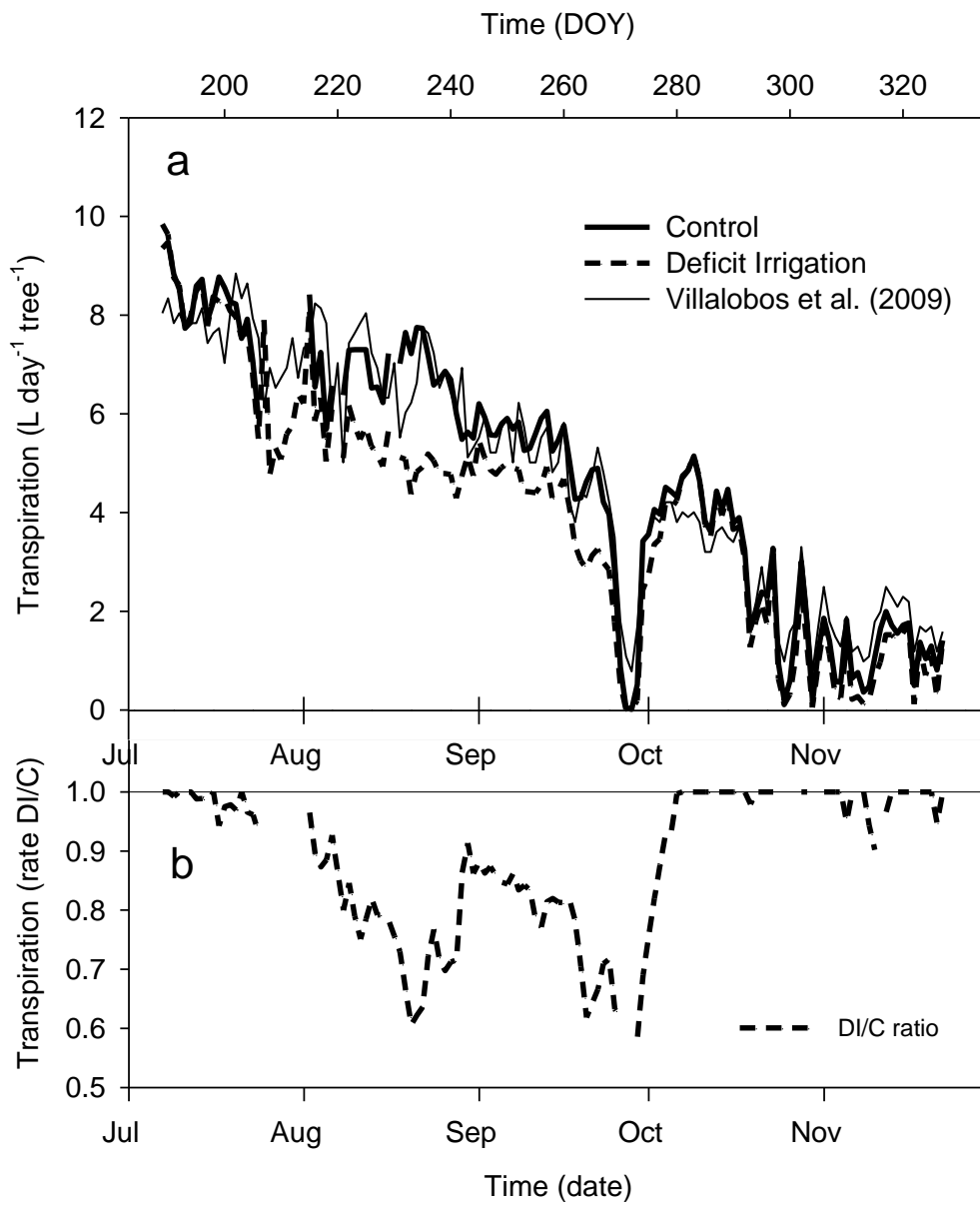
**Fig. 1**



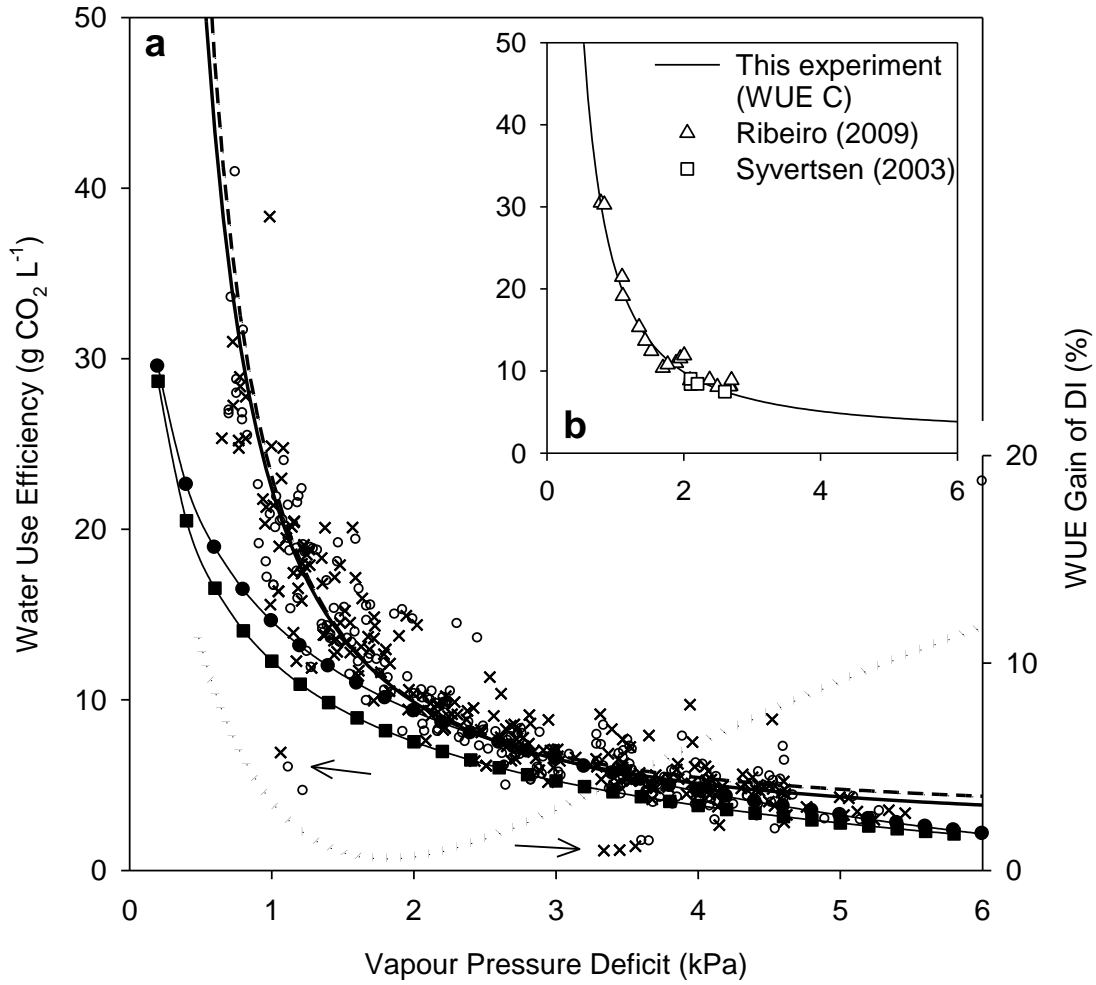
**Fig. 2**





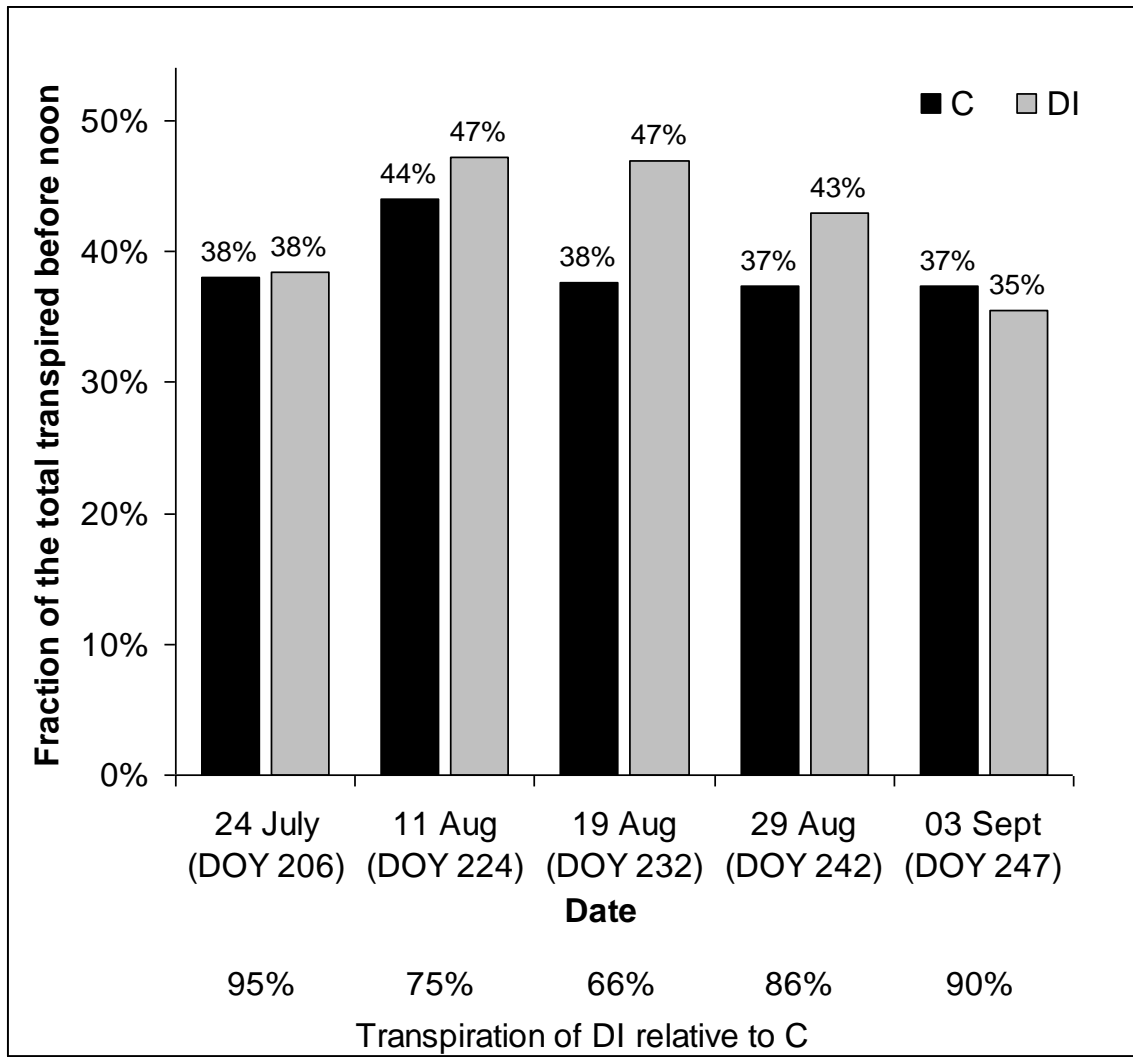


**Fig. 4**



- $\circ$  Control (C)
- $\times$  Deficit Irrigation (DI)
- $\text{WUE (C)} = 2.16 + 20.21 \text{ VPD}^{-1.39}$
- - -  $\text{WUE (DI)} = 3.10 + 20.03 \text{ VPD}^{-1.55}$
- ⋯⋯⋯ WUE Gain of DI
- Olive (well-watered, Testi *et al.*, 2008)
- Olive (well-watered, Villalobos *et al.*, 2012)

**Fig. 5**



**Fig. 6**