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Additional Information

1 **Effects of long-term summer deficit irrigation on ‘Navelina’ citrus**  
2 **trees**

3

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15

16

17 **ABSTRACT**

18 The effects of long-term summer deficit irrigation (RDI) strategies on ‘Navelina’  
19 orange trees (*Citrus sinensis* L. Osbeck) were assessed in a drip-irrigated commercial orchard  
20 located in Senyera (Valencia, Spain). Three irrigation treatments were applied during five  
21 consecutive years (2007-2011): a control treatment, without restriction, and two RDI  
22 treatments, in which the water reduction was applied during the summer (initial fruit  
23 enlargement phase). During the first three seasons, the trees under the control treatment  
24 received 110% of the theoretically required irrigation dose (ID), and the RDI treatments  
25 received 40% and 60% of the full ID during the deficit period. During the last two years of  
the study, the control treatment was irrigated at 100% of the ID and the amount of water

26 applied in the RDI treatments was additionally decreased 20% from the reduced ID of the  
27 preceding years. The crop's response to summer deficit irrigation was analysed in relation to  
28 tree water status, which was assessed by relying on midday stem water potential ( $\Psi_{st}$ ). The  
29 lowest  $\Psi_{st}$  values were reached, as expected, at the end of the water deficit period and with the  
30 most stressed treatment. These minimum  $\Psi_{st}$  values ranged between -1.6 MPa in 2008 and -  
31 2.5 MPa in 2010. In most occasions, the trees under RDI treatments showed a fast hydric  
32 recovery and had completely re-hydrated one week after restarting irrigation. Summer RDI  
33 treatments did not cause negative effects on either the amount or on the quality of the yield if  
34 the threshold value of  $\Psi_{st}=-2.0$  MPa was not surpassed. According to the results, it can be  
35 concluded that long-term RDI strategies may be applied successfully on Navelina orange trees  
36 during summer without negatively affecting the studied parameters while allowing water  
37 savings between 12% and 27%.

38

39 *Keywords:* yield, fruit quality, regulated deficit irrigation, stem water potential, water use  
40 efficiency.

41

42

## 43 **1. Introduction**

44

45 Citrus are widely grown under diverse climatic conditions, including in semi-arid  
46 regions. Spain occupies one of the first places in the global ranking of citrus producing  
47 countries, with an average annual production exceeding 6.6 million t. Almost half of total  
48 production (about 3.5 million t) corresponds to the sweet orange group, in which the  
49 ‘Navelina’ orange is the most important cultivar, with a production of about 1.1 million t  
50 (MAGRAMA, 2015).

51 Water scarcity is an important problem in many areas of the world. It particularly  
52 affects the Mediterranean basin, with a semi-arid climate, scarce rainfall, hot summers, and a  
53 dry season that lasts for over three months. Irrigated agriculture is the sector with by far the  
54 largest water consumption. In Spain, about 72% of consumptive water is used for irrigation  
55 purposes (Frenken and Guillet, 2012).

56 Thus, increasing water scarcity demands a more efficient and optimized use of  
57 irrigation water. One of the most promising approaches for attaining this objective might be  
58 regulated deficit irrigation (RDI). RDI consists of reducing water supplies during certain  
59 stages of crop development, when yield and fruit quality might have a low sensitivity to water  
60 deficits, and providing normal irrigation doses during the rest of the season, especially during  
61 critical periods or phenological stages with a higher sensitivity to water deficits (Chalmers et  
62 al., 1986; Mitchell et al., 1984). Many works have proved the feasibility and effectiveness of  
63 this practice by reducing water use in tree crops with low or null impact on yield and fruit  
64 quality (e.g. Carr, 2012; Ruiz-Sánchez et al., 2010).

65 In order to adequately control the water stress caused by the application of RDI, it is  
66 important to monitor plant water status or soil water content appropriately. In this sense, stem

67 water potential ( $\Psi_{st}$ ) seems to be a sensitive measure of plant water status (Choné et al., 2001;  
68 Ortuño et al., 2006). However,  $\Psi_{st}$  is not easily measurable, and it is not suited for an  
69 automated irrigation scheduling. As an alternative or complement to tree water status  
70 monitoring, there are different techniques that allow for the continuous measurement of soil  
71 water content. Among others, the frequency domain reflectometry (FDR) probe, with multiple  
72 depth capacitance sensors (Fares and Polyakov, 2006; Paltineanu and Starr, 1997), has shown  
73 excellent performance so far. It is currently widely used for field applications as a decision-  
74 making tool for irrigation scheduling (Martí et al., 2013).

75         González-Altozano and Castel (2003a, 2003b, 2000, 1999) carried out several RDI  
76 tests on an experimental orchard of ‘*Clementina de Nules*’ citrus trees (*Citrus clementina* Hort  
77 ex Tan). Different levels of water restriction were compared in the main phenological periods  
78 of crop development, and the effects of water restriction on yield, fruit quality, and water use  
79 efficiency were assessed. They concluded that the effects of RDI treatments depend, among  
80 others, on the phenological period in which the water restriction is applied as well as on the  
81 degree of restriction applied. Specifically, they stated that moderate water reduction during  
82 the initial fruit enlargement phase, after the June fruitlet drop (July and August in the northern  
83 hemisphere), did not affect yield, fruit size, or quality, which allowed for significant water  
84 savings (8-22%). These experiments also defined different pre-dawn leaf water potential ( $\Psi_{pd}$ )  
85 threshold values to avoid negative effects during the phenological period considered. Thus,  
86 summer  $\Psi_{pd}$  should not surpass -1.2 MPa, which corresponds to values of  $\Psi_{st}$  around -1.9  
87 MPa.

88         Most of the RDI studies carried out on citrus provide evidence of the advantages and  
89 benefits of reducing water application during summer. Citrus fruit has the capacity to  
90 accelerate growth after a water deficit period and thus be able to reach their potential size.  
91 This capacity, named compensatory fruit growth, is essential for the successful application of

92 summer RDI strategies. However, Ballester et al. (2013) found that summer RDI treatments  
93 applied to Navel Lane Late citrus trees might prevent compensatory fruit growth after  
94 returning to irrigation at full dosage, depending on the duration and degree of severity of the  
95 plant water deficit. The latter study highlights the differences between cultivars in response to  
96 RDI, as well as the need for frequent monitoring of plant water status to avoid an excessive  
97 reduction of fruit weight that may affect yield.

98         The majority of RDI studies consider the effects of deficit irrigation treatments during  
99 two or three consecutive growing seasons. Other researches deal with the viability of long-  
100 term RDI strategies. These long-term RDI strategies may negatively affect yield capacity  
101 (Girona et al., 2005; Intrigliolo et al., 2013; Romero et al., 2004); however, some studies have  
102 reported substantial water savings without any reduction in yield or fruit size (Hueso and  
103 Cuevas, 2010; Johnson et al., 1992). It should be noted that, although there are several studies  
104 addressing the application of RDI to ‘Navelina’ citrus trees (García-Tejero et al., 2010;  
105 Aguado et al., 2012), to our knowledge, no investigation has considered the effects of long-  
106 term summer deficit irrigation with this cultivar.

107         The aim of this study is to analyse the feasibility of long-term summer RDI strategies  
108 in ‘Navelina’ citrus trees and the effects on yield, fruit quality, and vegetative growth during  
109 five consecutive years (2007-2011).

110

## 111 **2. Material and Methods**

112

### 113 *2.1 Soil and climate conditions*

114         The experiment was carried out during five consecutive growing seasons (2007-2011)  
115 on a commercial drip-irrigated plot of 1 ha in Senyera, Valencia (39°3’ N, 0°30’ W, 23 m  
116 a.s.l.), which was planted in 1982 with ‘Navelina’ orange trees (*Citrus sinensis* L. Osbeck)

117 grafted on ‘Cleopatra’ mandarin trees (*Citrus reshni* Hort.) at a spacing of 5 x 5 m, with an  
118 average ground cover of about 52%.

119 The soil was deep sandy-loam with pebbles of alluvial origin, with an average organic  
120 matter content of 1.17%, an electric conductivity (EC<sub>1-5</sub>) of 0.14 dS m<sup>-1</sup>, 39.1% of active  
121 CaCO<sub>3</sub>, and a pH in water (1/25) of 8.0. It was also poor in total nitrogen (0.06%), available  
122 potassium (0.42 meq K<sup>+</sup> 100g<sup>-1</sup>) and phosphorus (20.67 mg P kg<sup>-1</sup> Olsen). A more detailed  
123 description of the soil characteristics can be found in Martí et al. (2013).

124 The irrigation water used had an average electrical conductivity (at 25° C) of 0.82 dS  
125 m<sup>-1</sup>, with chloride content lower than 2 meq Cl L<sup>-1</sup> and a SAR value of 3.53.

126 Climatic data were provided by the meteorological station belonging to the Irrigation  
127 Technology Service (STR) of the Valencian Institute for Agricultural Research (IVIA) in  
128 Villanueva de Castellón (Spain), less than 500 m from the experimental plot. The climate is  
129 Mediterranean semi-arid. The rainfall and the corresponding evaporative demand (ET<sub>o</sub>) for  
130 each year are summarized in Table 1. The average annual rainfall in the period 2000-2012  
131 was 624 mm, and the average annual ET<sub>o</sub> was 1083 mm. The mean annual air temperature  
132 during the same period was 17.1° C.

133 Trees received fertilisation through the irrigation system at a non-limiting rate of 260-  
134 65-130 kg ha<sup>-1</sup> per year of N, P<sub>2</sub>O<sub>5</sub>, and K<sub>2</sub>O respectively, split in weekly applications from  
135 April to October. Control of plagues and other cultural practices were carried out according to  
136 the usual local criteria in that area and were identical for all treatments. Trees were pruned in  
137 2009 and 2011.

138

## 139 *2.2 Irrigation treatments*

140 Irrigation was scheduled according to crop evapotranspiration (ET<sub>c</sub>) at local irrigation  
141 conditions (ET<sub>c</sub>=ET<sub>o</sub>·K<sub>c</sub> where K<sub>c</sub> is the crop coefficient) and effective precipitation (E<sub>p</sub>).

142 Reference evapotranspiration ( $ET_o$ ) was determined by the FAO56 version of the Penman-  
143 Monteith equation relying on daily average data from the meteorological station. The crop  
144 coefficient ( $K_c$ ) was obtained for this location according to Castel (2005) based on the  
145 percentage of shaded area in the plot (40.5-54.1%). In the studied period, the different mean  
146 seasonal values of  $K_c$  values used were as follows: 0.54 in 2007, 0.61 in 2008, 0.59 in 2009,  
147 and 0.54 in 2010 and 2011. The theoretical irrigation dose (ID) to ensure full irrigation was  
148 calculated as  $ID = ET_c - E_p$ .

149 Three irrigation treatments were applied: a control treatment, irrigated without  
150 restriction throughout the whole year, and two RDI treatments (T1 and T2), which received  
151 the same amount of water as the control except during the restriction period (the initial fruit  
152 enlargement phase, from mid-July to early September). The established irrigation treatments,  
153 the duration of each one, and the dose applied throughout each growing season are  
154 summarized in Table 2.

155 During the first three years (2007, 2008, and 2009), the control treatment was irrigated  
156 at 110% of full ID. T1 and T2 received 40% and 60% of full ID during the restriction period,  
157 respectively.

158 The control treatment was irrigated at 100% of ID in the last two seasons (2010 and  
159 2011). In 2010, the amount of water applied in the RDI treatments was reduced by an  
160 additional 20%. Thus, the T1 and T2 treatments received during the restriction period 32%  
161 and 48% of ID. Finally, in 2011 the data provided by the FDR probes were used for irrigation  
162 scheduling of T2 and as an additional information source of the amount of water applied to  
163 T1. Accordingly, the T2 treatment was scheduled in order to maintain soil water content in  
164 the zone between 0-60 cm of the soil depth in the range 50-60 mm during the restriction  
165 period and between 70-80 mm during the rest of the year. The T1 treatment received 40% of  
166 ID during the restriction period and 100% of ID during the rest of the year. In this treatment,



167 the irrigation water applied was also monitored and adjusted depending on the soil water  
168 content measured with the FDR probe in the zone 0-60 cm of soil depth. Thereby, the soil  
169 water content was kept above 50 mm in the zone between 0-60 cm of soil depth during the  
170 restriction period and above 80 mm during the rest of the year.

171 Considering the five years, the irrigation treatments T1, T2, and control received,  
172 respectively, an average of 42.2%, 58.4% and 101.2% of ID during the water deficit periods.

173 The experimental design was based on a randomised complete block with three  
174 replicates per treatment. Each experimental unit consisted of a minimum of three rows with  
175 10 trees per row, using perimeter trees as guard. Thus, yield and fruit quality parameters were  
176 determined from a minimum of eight trees per experimental unit. Further details about the  
177 distribution of each replicate of the applied treatments can be found in Gasque et al. (2010).

178 The irrigation system consisted of a double line (1.8 m spaced) of drip-irrigation with  
179 eight self-regulating drippers per tree with an average flow of  $7.4 \text{ L h}^{-1}$  per dripper. Irrigation  
180 frequency, identical for all treatments, ranged between six irrigations per week during the  
181 summer and two irrigations per week during the winter. The amount of applied water was  
182 measured through weekly water-meter readings for each irrigation replicate.

183

### 184 *2.3 Tree water status measurements*

185 Midday stem water potential ( $\Psi_{st}$ ) was measured weekly around 12 h (GMT) during  
186 the growing season and less frequently during the winter using a ‘Scholander’ type (SF-Pres-  
187 35 by Solfranc Tecnologías, S.L.) pressure chamber, following the procedures described by  
188 Turner (1981).  $\Psi_{st}$  was measured for a minimum of two south oriented mature leaves per tree,  
189 which had been wrapped in bags at least two hours before, and for two trees per individual  
190 plot for each of the three replicates per treatment.

191

192 *2.4 Fruit and shoot growth*

193 Sixteen fruit per tree (four in each cardinal quadrant) were selected and tagged from  
194 three trees per treatment (one per replicate). Equatorial fruit diameter was measured weekly  
195 every year from the beginning of July until harvest. In the same trees, shoot elongation was  
196 determined during the first growth flush in the spring of 2011 on samples of 16 shoots per tree  
197 (four shoots per cardinal quadrant) from 10 March to 23 May, every 7-14 days.

198 On the other hand, fresh fruit weight (FW) and dry fruit weight (DW) were determined  
199 each season in four fruit randomly selected per replicate in three moments: before restriction  
200 (BR), at the end of the restriction (ER), and at harvest (H).

201 With the aim of verifying that RDI treatments started after the end of the ‘June drop’  
202 as well as to check the treatments’ effects on fruit abscission, the number of fruit fallen were  
203 registered weekly each season in two trees per replicate from fruit-set till harvest.

204

205 *2.5 Yield and fruit quality*

206 At the end of each season during the commercial harvest, the yield and its components  
207 were determined in at least eight trees per replicate (24 trees per treatment). The average fruit  
208 weight was evaluated by counting the number of fruit in a minimum of eight boxes per  
209 individual plot, previously weighed (about 20 kg/box). Fruit quality variables: peel, juice,  
210 sugars (Total Soluble Solids, TSS), acid content (Titratable Acidity, TA), soluble solids, pH,  
211 and vitamin C, were determined at harvest with samples of at least nine fruit per individual  
212 plot following the procedures described by González-Sicilia (1951).

213 Water use efficiency (WUE) was determined as the ratio between yield and total  
214 applied water (irrigation + rainfall).

215

216 *2.6 Vegetative growth*

217 Trunk perimeter (TP) was measured at marked sections above the graft (about 30 cm  
218 above the ground) on eight trees per replicate at the beginning and at the end of each season.  
219 Moreover, tree size, percentage of shaded area and volume of the tree top were also  
220 determined in the same trees.

221

222 *2.7 Soil water content*

223 During the last two years (2010-2011) the volumetric water content in the soil profile  
224 was monitored every 30 minutes using a multisensor capacitance probe (ENVIROSCAN,  
225 Sentek Sensor Technologies) based on FDR. Therefore, three FDR probes were installed in  
226 July 2009 on the north side of one tree per treatment, placed 25 cm from the emitter's line.  
227 The capacitance probes were properly installed within the active root system zone. Each  
228 probe presented four sensors. The first three sensors, located at 10, 30, and 50 cm of depth,  
229 covered practically 90% of the active root system (estimated during probe installation), while  
230 the fourth was outside of this zone (70, 80, and 60 cm depth in the probes for treatments T1,  
231 T2, and the control, respectively). More details about the installation of these probes can be  
232 found in Martí et al. (2013).

233

234 *2.8 Statistical analysis*

235 Statistical analyses were performed using the SPSSv16 package (SPSS Inc., Chicago  
236 IL) with a one-way analysis of variance (ANOVA), given that data fit the assumptions of the  
237 parametric tests (test K-S). Differences among treatments were studied with the Tukey test  
238 (95%).

239

### 240 3. Results and discussion

241

242 The evolution of midday stem water potential ( $\Psi_{st}$ ), fruit growth, and the fruit's  
243 relative diameter in relation to the control are depicted in Fig. 1 (period 2007-2009) and Fig. 2  
244 (period 2010-2011). Rainfall and the ETo evolution during each period are also shown in  
245 these figures. The amount of water applied in each irrigation treatment, water use efficiency  
246 values (WUE), as well as yield parameters and its components are summed up in Table 3.

247 During the period 2007-2009, the  $\Psi_{st}$  values in control trees were around -0.9 MPa.  
248 Although very low  $\Psi_{st}$  values were occasionally measured, e.g. end of 2007 (around -1.65  
249 MPa), in general, it can be accepted that the  $\Psi_{st}$  values in the control treatment were within  
250 normal midday stem water potential ranges of well-irrigated citrus trees (Ballester et al.,  
251 2014; Syvertsen and Albrigo, 1980).

252 The lowest  $\Psi_{st}$  values reached in deficit treatments occurred on the 31<sup>th</sup> August of  
253 2007 and 2008 (around -1.71 and -1.60 MPa, respectively); both values were registered  
254 during the most severe restriction treatment (T1) (Fig. 1C). These  $\Psi_{st}$  values were higher (less  
255 negative) than the suggested threshold values for citrus under summer water deficits  
256 (González-Altozano and Castel, 1999, 2003a), and they did not suggest significant water  
257 stress levels according to Domingo et al. (1996).

258 Based on the  $\Psi_{st}$  values observed during the period 2007-2009 both in the RDI and  
259 control treatments, and also on the results obtained that will be discussed later, the irrigation  
260 dose applied during the period 2010-2011 was reduced (100% of full ID) from that applied in  
261 the preceding growing seasons (110% of full ID) (Table 2).

262 Despite this reduction, the evolution of tree water status for all treatments showed a  
263 similar trend during the five years of study. The  $\Psi_{st}$  values observed for the control trees  
264 during the period 2010-2011 were around -1.0 MPa. These values indicate that the control

265 trees were always fully irrigated, although the irrigation dose that was applied was  
266 additionally reduced. Moreover, before the restriction period, hardly any differences with  
267 respect to the control in the  $\Psi_{st}$  values of the RDI treatments were detected, which maintained  
268 a high level (around -1.0 MPa), indicating a total absence of water stress.

269 The evolution of the  $\Psi_{st}$  reflected well the restriction periods as well as some  
270 unexpected water cut-offs that occurred during November and December of 2007 and  
271 September and October of 2008. In both years, these water cut-offs produced rapid and  
272 important drops of  $\Psi_{st}$  in autumn for all treatments.

273 In all seasons, after the beginning of the restriction, a slow and continuous  $\Psi_{st}$  drop  
274 was indicative of the progression of the water deficit period during the RDI treatments. The  
275 minimum potential values were reached at the end of this period every year, and, as might be  
276 expected, the higher the restriction level, the lower the values dropped. At the end of the  
277 restriction period of 2010, minimum the  $\Psi_{st}$  values for the five years were reached (-2.5 MPa  
278 in the T1 treatment and -2.1 MPa in T2) (Fig. 2C). In all seasons except for 2010, one week  
279 after restarting the full ID, trees of both RDI treatments had completely re-hydrated. In 2010,  
280 the subsequent hydric recovery of the trees under RDI treatments was faster for T2 and much  
281 slower for the more restrictive T1. This recovery rate, together with the degree of stress  
282 suffered, affected both the size and quality of the fruit, as will be discussed below. The use of  
283 data provided by the FDR probes in 2011 to schedule irrigation for T2 and to better adjust the  
284 applied dose for T1 appeared to be useful to avoid detrimental stress levels. Consequently, the  
285 obtained potential values were higher (less negative) than those reached in 2010, causing  
286 lower hydric stress.

287 During the restriction period, a slight deceleration of fruit growth was observed for the  
288 deficit treatments during all years excepting 2008 (Figs. 1B and 2B). However, fruit diameter  
289 differences in comparison to the control were only significant ( $p < 0.05$ ) in 2007, without

290 affecting the final fruit weight (Table 3). In 2009, 2010, and 2011, these differences did not  
291 reach statistical significance, but a slower growth of fruit under T1 and T2 was observed.

292 The rapid hydric recovery observed in deficit treatments after finishing the restriction  
293 was reflected in a higher relative growth of fruit under these treatments in comparison to the  
294 control (Figs. 1D and 2D). This fruit growth acceleration, known as compensatory fruit  
295 growth, is usual when irrigation at full dose restarts after a water restriction period (Chalmers  
296 et al., 1986; González-Altozano and Castel, 2003a; Mitchell and Chalmers, 1982; Ruiz-  
297 Sánchez et al., 2000). Compensatory fruit growth was not found in fruit under the RDI  
298 treatments in 2008. This was in accordance with the absence, during this year, of growth  
299 deceleration.

300 In 2011, the trees under the RDI treatments showed a rapid hydric recovery at the end  
301 of the restriction, a situation which is in accordance with the more rapid fruit growth under  
302 stressed treatments (Fig. 2D). This finding notes once again the importance of recovering and  
303 maintaining an adequate water status in the trees after the restriction and until harvest, which  
304 allows for fruit growth compensation. The minimum potential values reached by trees of the  
305 RDI treatments in 2011 ( $\Psi_{st} = -1.95$  MPa in T1 and  $\Psi_{st} = -1.6$  MPa in T2), did neither cause  
306 negative effects on the production nor on the quality of harvest (Table 3).

307 Regarding fruitlet drop, it was similar for all treatments in all of the studied years  
308 (results not shown). In all growing seasons, the 'June drop' had concluded by the end of June  
309 (DOY 182) and always before the start of the restriction treatments. The end of water  
310 restriction neither produced appreciable fruitlet drop nor flowering for any treatment or year.  
311 In addition, the irrigation restrictions neither affected the flowering process nor the growth  
312 flush after June (Fig. 2). Finally, as depicted in Fig. 2B, shoot growth of the first flush was  
313 clearly separated in time from the period of fruit growth and occurred long before the

314 restriction period started. Therefore, the required conditions for achieving successful  
315 implementation of RDI in Navelina citrus trees were fulfilled.

316         Attending to the influence of the irrigation treatments on yield and its components  
317 during the entire period (Table 3), significant differences were observed in yield between  
318 years ( $p < 0.05$ ) ranging from 134.4 kg/tree in 2008 to 43.3 kg/tree in 2009, with an average  
319 of 79.8 kg/tree for the control treatment. These differences were mainly due to the number of  
320 fruit/tree, ranging in control trees from 185 fruit/tree in the least productive year (2009) to 593  
321 fruit/tree in the most productive one (2008). Differences in average fruit weight were not so  
322 marked and ranged from 231.6 g (in 2008) to 296.0 g (in 2007). It should be noted that the  
323 aforementioned slowdown in fruit growth of stressed trees was observed during all years  
324 except 2008, while it had been expected that due to the higher crop load, the effects of water  
325 stress in 2008 should have been more evident. The reported results indicate that the effects of  
326 water deficit on the fruit growth rate were of minor importance, given that only small  
327 differences between treatments were detected in this parameter, even in 2010, when the  
328 lowest  $\Psi_{st}$  values for the five years were registered. However, there were differences in the  
329 pattern of fruit growth between years, indicating that, with moderate levels of stress (such as  
330 those suffered by trees during the present study), the number of fruit/tree has greater influence  
331 on the production and on the final fruit size than the level of stress reached.

332         The RDI treatments yielded systematically higher production than the control,  
333 although these differences were only significant ( $p < 0.05$ ) in the growing seasons 2009 and  
334 2011. Different results were reported by García-Tejero et al. (2010 and 2012) in Navelina  
335 citrus trees, in a study in which more severe water stress was applied during the flowering,  
336 fruit growth and maturity phases; in this study the number of fruit per tree was significantly  
337 lower in trees held under deficit irrigation. The response to water stress may therefore vary

338 depending on the duration and severity of treatments, as stated by other researchers (Ballester  
339 et al. 2013; Treeby et al. 2007).

340 A higher number of fruit per tree usually involves a smaller fruit size. However, this  
341 was not the general trend in the present study. Here, the trees under deficit treatments  
342 produced in some cases (e.g. T2 in 2007 and T1 in 2009) more fruit per tree than the control  
343 trees ( $p < 0.05$ ), while no differences were found in average fruit weight (Table 3). Moreover,  
344 in 2011, the fruit size under T1 and T2 was very acceptable and, at the same time, higher than  
345 the annual average (246.2 g), while these treatments yielded many more fruit per tree than the  
346 control ( $p < 0.05$ ). Both deficit treatments returned significantly higher production than the  
347 control, despite having reached, as aforementioned, a minimum potential value of  $\Psi_{st} = -1.95$   
348 MPa in the most stressed treatment.

349 The higher number of fruit per tree in trees under deficit irrigation occurred after the  
350 growing seasons in which lower  $\Psi_{st}$  values were reached, and/or after certain unscheduled  
351 water stress levels during autumn. Therefore, results seem to indicate that trees that had  
352 previously suffered from stress could be more sensitive to an occasional shortage of water in  
353 autumn, which might have favoured to some extent the fruiting-flowering-fruit-set process. It  
354 should be pointed out that no significant differences were observed between treatments with  
355 respect to the variables that define tree size (trunk perimeter, percentage of shaded area,  
356 volume of the tree top) either before the beginning of this study or any of the five years during  
357 the study. Although on average, the size of the trees under RDI was slightly higher than that  
358 of the control trees (6-12% in volume of tree top; 3-4% in trunk perimeter), this small  
359 difference does not explain the large differences found in yield.

360 The relationship of fresh to dry weight accumulated in the fruit (FW/DW) for each  
361 treatment is shown in Table 4. Before restriction (BR), it was verified that this relationship  
362 was similar between treatments each year. When the restriction period was complete (ER), the



363 relationship FW/DW was lower for the RDI treatments than for the control during all the  
364 seasons, and the differences were statistically significant ( $p < 0.05$ ) in four of the five years.  
365 These results indicate that the RDI fruit still accumulated dry matter during the restriction  
366 period, thus reducing FW in comparison to the control treatment (Table 4). As no differences  
367 in the FW/DW relationship were detected before restriction, the differences found when the  
368 restriction period was concluded could be attributed to the treatments that had been applied.  
369 Once restarting full irrigation, compensatory fruit growth under RDI treatments occurred at  
370 the expense of accumulated dry matter. Therefore, these differences tend to disappear at  
371 harvest (H).

372         Only during 2010, when the highest stress levels were reached in RDI treatments, no  
373 differences were observed in dry matter accumulation in the fruit at the end of the restriction  
374 period (ER). There was also no compensatory fruit growth, and at harvest, the fruit of these  
375 trees tended to be smaller than those under the control (Table 3). The differences were  
376 significant ( $p < 0.05$ ) in treatment T1, with the highest concentration of dry matter (lower  
377 ratio FW/DW), indicating that the harvest took place when these fruit were less hydrated; they  
378 also probably still had a certain capacity for growth.

379         Table 5 shows the influence of irrigation treatments on fruit quality. In contrast to  
380 other RDI studies (Ballester et al., 2014; García-Tejero et al., 2010; Yakushiji et al., 1996)  
381 that found a significant increase in TSS and TA under RDI strategies, in this study, no  
382 significant differences were found in comparison to the control in any of the studied fruit  
383 quality parameters with the exception of the lower juice content in the fruit under T1 in 2010.  
384 The higher concentration of sugar and soluble solids reported in other studies could be due to  
385 the longer duration of the stress period or to the higher levels of water stress suffered.

386         Regarding to the juice content, it was on average (excluding the 2010 season) 47.9%  
387 of the fruit weight, similar to the normal values for this variety (48.2% according to

388 Sanchoene Gonçalves, 1998), and very similar to those found by other authors in ‘Lane Late’  
389 citrus fruit (Pérez-Pérez et al., 2009). In 2010, the fruit showed lower juice content values  
390 than those cited in the literature and much lower values than in other seasons. In 2010, the  
391 fruit under the RDI treatments were also the smallest ones within the five years. These  
392 differences in juice content and fruit size were especially noticeable in the T1 treatment after  
393 having reached the lowest values of stem water potential ( $\Psi_{st} = -2.5$  MPa). This result  
394 provides evidence that the stress level reached in this treatment during 2010 exceeded the  
395 recommended level. However, the T2 treatment, after having reached a minimum value of  $\Psi_{st}$   
396  $= -2.1$  MPa, did not affect the production or the quality of the fruit. Even though the fruit of  
397 this treatment were smaller than those of the control, the differences were not significant,  
398 indicating the proximity to the stress level to avoid negative effects.

399 In 2011, when less water was applied, although distributed in a more suitable way than  
400 in 2010 as reflected in the  $\Psi_{st}$  evolution (Fig. 2), production under RDI was much higher than  
401 under the control due to a higher number of fruits per tree. Although fruit size was also  
402 affected, this effect was relatively minor given that the final fruit size in the more stressed  
403 treatment (T1) was higher (no significant differences) to that of the control average in the  
404 entire period. In addition, the internal quality of the fruit was not affected, indicating that the  
405 RDI strategy adopted this year was more appropriate (minimum potential  $\Psi_{st} = -1.95$  MPa  
406 under T1). All these results suggest that  $\Psi_{st} = -2.0$  MPa might be considered a potential  
407 threshold value which should not be exceeded to avoid the negative effects of applying RDI  
408 in summer.

409 The most suitable parameter for reflecting the effect of irrigation treatments on  
410 vegetative growth was the increment in trunk perimeter ( $\Delta TP$ ). The values of  $\Delta TP$  from 2007  
411 to 2011 were 0.12, 0.10, and 0.13 meters in T1, T2, and the control, respectively. No  
412 statistical differences were found between treatments, showing that water restriction did not

413 affect trunk growth. This finding is contrary to those commonly found in experiments carried  
414 out with different tree crops, specifically citrus, and could be due to the larger development  
415 and age of the trees. In the present study, the trees were 25 years old at the beginning of the  
416 experiment, whereas in other studies, trees were 8-10 years old (Clementina de Nules,  
417 Ballester et al., 2014, 2011; González-Altozano and Castel, 2000), and seven years old (Navel  
418 Lane Late, Ballester et al., 2013). In these studies with younger trees, it was verified that  
419 trunk growth under RDI was lower than under full irrigation. Probably, the lower growth  
420 potential due to the size and age of the trees in the present study prevented the RDI treatments  
421 from affecting the growth of the trunk. Hence, with the reported results, after five years of  
422 study, it is not expected that the application of long-term summer RDI strategies have  
423 significant effects on production, which is ultimately supported by trunk and roots (tree size  
424 and height).

425         Although in citrus, water use efficiency (WUE, Table 3) may be affected by the timing  
426 and fruit stage where the water restriction is applied (Carr, 2012), in this case, it is evident  
427 that summer RDI treatments provided significantly higher WUE than the control treatment, as  
428 is generally associated (Feres and Soriano, 2007), which contrasts with the results shown in  
429 other experiments with citrus trees (Ballester et al., 2014; García-Petillo and Castel, 2004).

430

#### 431 **4. Conclusions**

432

433         Based on the results derived from this study, long-term RDI strategies during the  
434 summer (initial phases of fruit enlargement) can be successfully applied in commercial  
435 Navelina citrus trees.

436          $\Psi_{st} = -2.0$  MPa is proposed as potential threshold value which should not be surpassed  
437 in order to avoid negative effects on yield or on the quality of fruit. During the whole period

438 of study, water savings between 12% and 27% resulted in only a slight reduction in average  
439 fruit weight in the RDI treatments, in the more restrictive cases, which was balanced with a  
440 higher yield.

441 FDR probes were very effective for adjusting the applied doses to avoid detrimental  
442 stress levels. In the context of this work, using FDR probes could be considered a preliminary  
443 step for achieving more accurate irrigation scheduling.

444 According to the obtained results, the application of summer RDI in Navelina is much  
445 more cost-effective than the traditional full doses under the boundaries tested. Therefore, the  
446 practical and reliable information provided in this work could be employed for optimizing  
447 water management in commercial applications.

448

449

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## LIST OF FIGURE CAPTIONS

Fig. 1. Evolution during 2007, 2008 and 2009 of: ETo and Rainfall (A), fruit diameter (B), midday stem water potential (C), and relative diameter fruit growth of RDI treatments in relation to the control (D). The vertical dotted lines show the beginning and the end of the restriction period. Harvest dates were 8<sup>th</sup> January, 17<sup>th</sup> November and 18<sup>th</sup> December for the first, second and third season, respectively.

Fig. 2. Evolution during 2010 and 2011 of: ETo and Rainfall (A), fruit growth (2010-2011) and shoot growth of the first flush (2011) (B), midday stem water potential (C), and relative diameter fruit growth of RDI treatments in relation to the control (D). The vertical dotted lines show the beginning and the end of the restriction period. Harvest dates were 26<sup>th</sup> January 2011 and 13<sup>th</sup> January 2012, respectively.



Figure 1

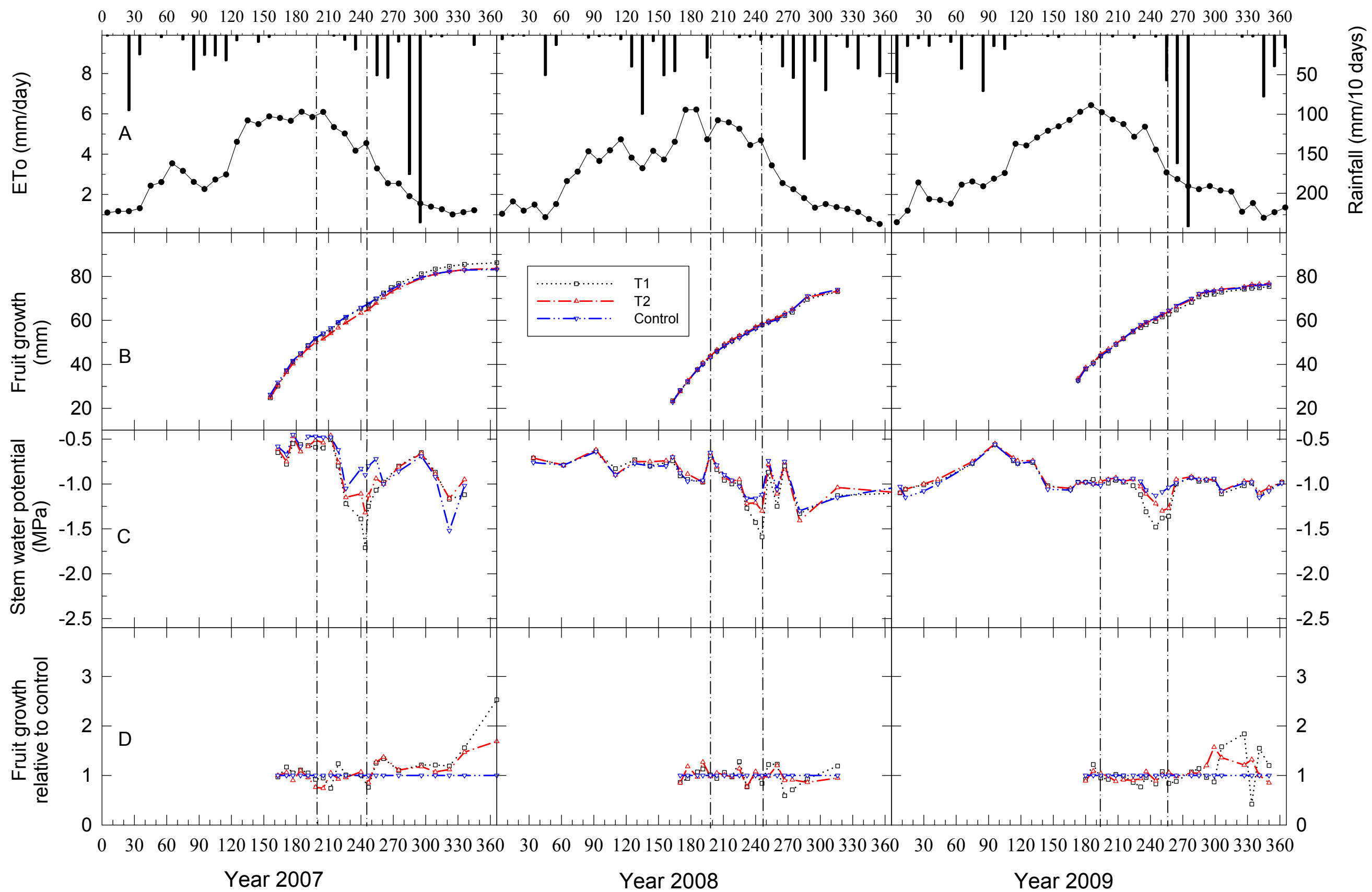
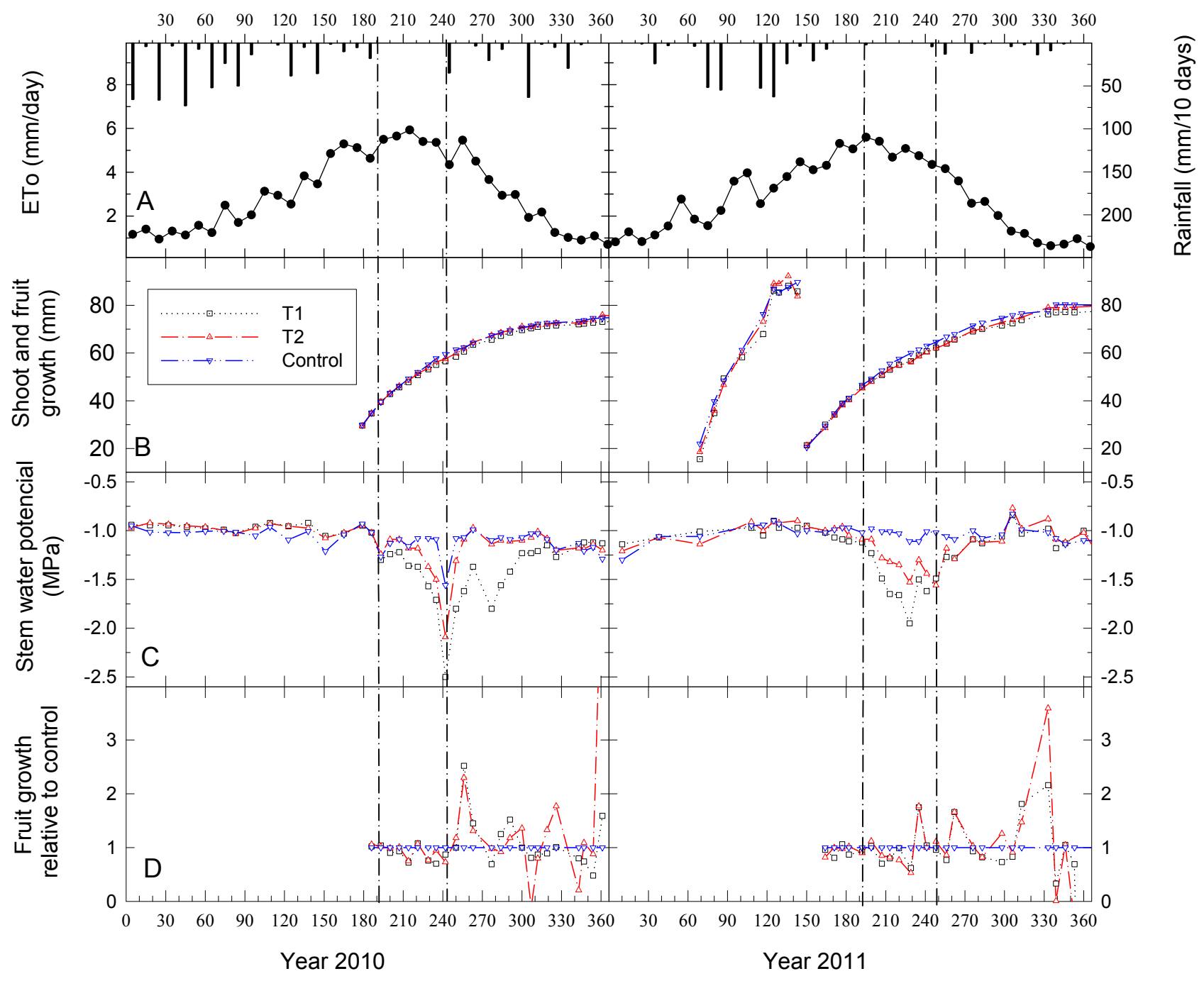


Figure 2



## LIST OF TABLE CAPTIONS

Table 1. Rainfall and evaporative demand (ET<sub>o</sub>) registered at the meteorological station nearest to the experimental plot during the studied period.

Table 2. Irrigation treatments applied during the experimental period (2007 to 2011).

Table 3. Amount of water applied in each irrigation treatment, yield and its components, and water use efficiency (WUE).

Table 4. Relation FreshWeight/DryWeight (FW/DW), before restriction (BR), at the end of the restriction (ER) and at harvest (H) in each treatment and year.

Table 5. Fruit quality parameters of 'Navelina' citrus trees per treatment and year.

1

2 **Table 1.** Rainfall and evaporative demand (ETo) registered at the meteorological station  
3 nearest to the experimental plot during the studied period.

	<b>2007</b>	<b>2008</b>	<b>2009</b>	<b>2010</b>	<b>2011</b>
<b>Rainfall (mm)</b>	869	796	840	566	616
<b>ETo (mm)</b>	1160	1124	1202	1132	1067

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2 **Table 2.** Irrigation treatments applied during the experimental period (2007 to 2011).

Treatment		Irrigation doses applied		
		2007 - 2009	2010	2011
T1	Restriction period <sup>[a]</sup>	40% ID	32% ID	40% ID <sup>[b]</sup>
	Rest of the year	110% ID	100% ID	100% ID <sup>[b]</sup>
T2	Restriction period	60% ID	48% ID	50-60 mm <sup>[c]</sup>
	Rest of the year	110% ID	100% ID	70-80 mm <sup>[c]</sup>
control	Whole year	110% ID	100% ID	100% ID

3 ID means theoretical full irrigation dose

4 <sup>[a]</sup>Restriction period: 17/07-02/09 in 2007 and 2008; 13/07-13/09 in 2009; 12/07-30/08 in 2010, 12/07-  
5 28/08 in 2011.6 <sup>[b]</sup> Theoretical irrigation dose adjusted according to the soil water content measured with the FDR  
7 probe between 0-60 mm of the soil depth. The soil water content was kept above 50 mm in the  
8 restriction period and above 80 mm during the rest of the year.9 <sup>[c]</sup> Water content (mm) between 0-60 cm of the soil depth recorded by the FDR probe.

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Table 3

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1  
2 **Table 3.** Amount of water applied in each irrigation treatment, yield and its components, and  
3 water use efficiency (WUE).

Year	Treatment	Irrigation (mm)	Water savings (%)	Yield (kg/tree)	Relative yield (%)	n° fruits/tree (-)	Average fruit weight (g)	WUE (kg/m <sup>3</sup> )
2007	T1	396	23	79.2	112.4	281	292.1	2.50*
	T2	431	16	84.1	119.4	309*	282.3	2.59*
	control	516	--	70.5	100	242	296.0	2.04
2008	T1	410	20	140.4	104.5	637	220.5	4.70
	T2	429	16	146.8	109.3	680	217.9	4.80
	control	515	--	134.4 <sup>[a]</sup>	100	593	231.6	4.11
2009	T1	371	27	72.7*	168.1	306*	241.6	2.22*
	T2	449	12	64.0*	148.0	291*	224.6 <sup>[b]</sup>	1.84*
	control	512	--	43.3 <sup>[a]</sup>	100	185	235.8	1.19
2010	T1	265	27	76.0	104.8	352*	214.7*	3.69
	T2	285	21	88.6	121.0	420*	217.2	4.16*
	control	362	-	73.2	100	311	237.6	3.15
2011	T1	248	21	108.7*	143.4	433*	256.1*	5.03*
	T2	253	20	102.9*	135.7	407*	253.6*	4.73*
	control	315	-	75.8	100	282	271.3	3.25

4 \* significant differences with respect to control treatment of each year (p<0.05).

5 <sup>[a]</sup> significant differences between years (p<0.05).

6 <sup>[b]</sup> significant differences respect to treatment T1 (p<0.05).

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**Table 4**[Click here to download Tables: Table 4\\_revised\\_clean copy.docx](#)**Table 4.** Relation Fresh Weight/Dry Weight (FW/DW), before restriction (BR), at the end of the restriction (ER) and at harvest (H) in each treatment and year.

Treatment		2007	2008	2009	2010	2011
BR	T1	3.82	-	-	3.98	4.49
BR	T2	3.83	-	-	3.87	4.65
BR	control	3.85	-	-	3.89	4.53
ER	T1	<b>5.74*</b>	<b>5.51*</b>	<b>5.68*</b>	4.77	<b>5.09*</b>
ER	T2	<b>5.68*</b>	<b>5.79*</b>	<b>6.01*</b>	4.77	<b>5.19*</b>
ER	control	6.61	6.31	6.85	4.83	5.64
H	T1	6.52	<b>6.11*</b>	9.08	<b>3.81*</b>	5.63
H	T2	6.51	6.59	9.48	<b>4.17*</b>	5.81
H	control	6.50	6.98	9.81	4.68	5.90

\* significant differences with respect to control treatment of each year ( $p < 0.05$ ).

Table 5

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1

2 **Table 5.** Fruit quality parameters of 'Navelina' citrus trees per treatment and year.

Year	Treatment	Peel %	Vitamin C (mg/100 g juice)	Juice (%)	TSS (° Brix)	TA (% Acids)	Maturity index (-)	pH juice (-)
2007	T1	26.89	65.05	50.46	10.57	1.08	9.84	3.33
	T2	27.08	68.68	51.66	10.67	1.05	10.37	3.36
	control	<b>26.44</b>	70.31	<b>50.86</b>	<b>10.63</b>	<b>0.89</b>	<b>12.08</b>	<b>3.46</b>
2008	T1	26.59	68.25	46.57	9.50	1.15	8.29	3.12
	T2	26.36	63.34	43.80	9.87	1.01	10.09	3.14
	control	<b>26.22</b>	67.61	<b>46.28</b>	<b>9.10</b>	<b>0.92</b>	<b>10.10</b>	<b>3.10</b>
2009	T1	29.47	74.57	48.53	10.73	0.74	14.51	3.41
	T2	31.63	67.97	54.10	10.80	0.68	15.84	3.29
	control	<b>26.90</b>	<b>67.27</b>	<b>51.23</b>	<b>11.00</b>	<b>0.74</b>	<b>15.27</b>	<b>3.15</b>
2010	T1	28.73	90.47	<b>35.87*</b>	13.13	1.15	11.41	3.43
	T2	27.13	94.22	38.57	13.73	1.21	11.44	3.41
	control	<b>29.07</b>	<b>95.62</b>	<b>41.47</b>	<b>13.53</b>	<b>1.04</b>	<b>12.99</b>	<b>3.51</b>
2011	T1	31.53	77.34	43.57	11.73	1.04	11.52	3.51
	T2	30.83	77.14	42.33	11.07	0.91	12.25	3.65
	control	<b>28.53</b>	<b>77.70</b>	<b>45.13</b>	<b>11.03</b>	<b>0.95</b>	<b>11.67</b>	<b>3.60</b>

3

\*significant differences with respect to control treatment of each year ( $p < 0.05$ ).

4

TSS: Total Soluble Solids content

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TA: Titratable Acidity

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