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Title: LOW WATER STRESS CONDITIONS IN TABLE OLIVE TREES (OLEA EUROPAEA L.) DURING PIT HARDENING PRODUCED A DIFFERENT RESPONSE OF FRUIT AND LEAF WATER RELATIONS.

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Abstract: The scarcity of water for agricultural use is producing a generalization of deficit irrigations in most of the fruit trees. Regulated deficit irrigation in olive trees is scheduled with a period of water stress during the pit hardening phase with low or, even, no decrease in yield. During this phenological stage, fruit is a great sink of assimilates and competes with vegetative growth, producing a significant change in the water relation of the tree. The aim of this work is to study the water relations in leaves and fruits in a period of drought during the phenological stage of pit hardening in a mature (43 year-old) table olive orchard. Water relations of leaves and fruits were compared between a Control of fully irrigated trees and Stressed trees (with a period of drought from 1 week after the beginning of pit hardening until 1 week before harvest). The water stress conditions were considered as low level, according with the stem water potential data. Leaf water relations were quickly affected with a reduction of midday stem water potential and turgor pressure at 14 days after the beginning of the drought (DABD). Leaf osmotic adjustment was measured only at the end of the drought cycle (63 DABD). On the other hand, fruit water relations were affected slowly and only osmotic potential was reduced at 14 DABD. Such variations produced a change in the source of water flow from xylem to phloem according to the variations in leaf-fruit water potential. The pattern of adaptation of leaves and fruit during the drought cycle and the relationship between them is discussed.

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Dear Dr.Clothier:

We should be grateful if you would consider the attached manuscript entitled “**LOW WATER STRESS CONDITIONS IN TABLE OLIVE TREES (OLEA EUROPAEA L.) DURING PIT HARDENING PRODUCED A DIFFERENT RESPONSE OF FRUIT AND LEAF WATER RELATIONS.**” for publication in the Special Issue RH Sevilla 2012 of Journal Agricultural Water Management.

All the authors have read the manuscript and approved it for publication.

Sincerely yours

Alfonso Moriana

Highlights

Water stress produced changes in the water relations of fruit and leaves.

Leaf turgor pressure decreased earlier than stomata closure and osmotic adjustment.

Fruit turgor pressure was less affected than leaf turgor pressure.

1 **LOW WATER STRESS CONDITIONS IN TABLE OLIVE TREES (*OLEA***
2 ***EUROPAEA* L.) DURING PIT HARDENING PRODUCED A DIFFERENT**
3 **RESPONSE OF FRUIT AND LEAF WATER RELATIONS.**

4

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23 **Abstract**

24 The scarcity of water for agricultural use is producing a generalization of deficit
25 irrigations in most of the fruit trees. Regulated deficit irrigation in olive trees is
26 scheduled with a period of water stress during the pit hardening phase with low or,
27 even, no decrease in yield. During this phenological stage, fruit is a great sink of
28 assimilates and competes with vegetative growth, producing a significant change in the
29 water relation of the tree. The aim of this work is to study the water relations in leaves
30 and fruits in a period of drought during the phenological stage of pit hardening in a
31 mature (43 year-old) table olive orchard. Water relations of leaves and fruits were
32 compared between a Control of fully irrigated trees and Stressed trees (with a period of
33 drought from 1 week after the beginning of pit hardening until 1 week before harvest).
34 The water stress conditions were considered as low level, according with the stem water
35 potential data. Leaf water relations were quickly affected with a reduction of midday
36 stem water potential and turgor pressure at 14 days after the beginning of the drought
37 (DABD). Leaf osmotic adjustment was measured only at the end of the drought cycle
38 (63 DABD). On the other hand, fruit water relations were affected slowly and only
39 osmotic potential was reduced at 14 DABD. Such variations produced a change in the
40 source of water flow from xylem to phloem according to the variations in leaf-fruit
41 water potential. The pattern of adaptation of leaves and fruit during the drought cycle
42 and the relationship between them is discussed.

43

44 **Keywords:** Leaf conductance, osmotic adjustment, regulated deficit irrigation, water
45 potential.

46

47 **1. Introduction**

48 Water is a scarce natural resource which is very important in agricultural practices.
49 Although irrigated lands are around 17% of the total agricultural surface, they provide
50 more than 40% of the total production (Fereres and Evans, 2006). However, the increase
51 of water scarcity in arid and semi-arid zones, the competition with other social uses
52 (such as sanitary, landscape uses) and the general feeling that irrigated agriculture is an
53 over-exploited system, are producing a decrease in the availability of water resources
54 for agricultural use. Regulated deficit irrigation (RDI) is a practice which was suggested
55 around the early 80's in peach trees (Chalmer et al., 1981) and consists of a reduction of
56 water applied during the most drought resistant phenological stages without a yield
57 penalty. From the first work in peach orchards, RDI has been a common research line in
58 most fruit trees (Bebohudian and Mills, 1997). Therefore, in most of the species the
59 drought sensitivity to water stress has been well described (Bebohudian and and Mills,
60 1997).

61 The water deficit schedule in olive trees is traditionally based on severe water
62 withdrawal around the beginning of massive pit hardening (Goldhamer 1999; Moriana
63 et al 2003). During this period of time, the fruit development alters all the water
64 relations of the tree in conditions of high fruit load (Martín-Vertedor et al., 2011). The
65 effect of water stress in leaf water relations has been widely described for olive trees
66 (Bongi and Long, 1987; Angelopoulos et al., 1996; Fernández et al., 1997; Dichio et al.,
67 1997, 2003 and 2006; Moriana et al., 2002) but little is known about fruit. The olive is a
68 very drought resistant fruit tree, in which water stress produces leaf osmotic adjustment
69 (Dichio et al., 1997, 2003 and 2006), strong stomatal control (Angelopoulos et al., 1996,
70 Moriana et al., 2002) and a high level of dehydration (Moriana et al., 2002).

71 Expansion of fruit requires, among other factors, an adequate flow of water to
72 the organ and sufficient turgor to drive cell enlargement. Berges and Selles (1993) in
73 peach fruit suggested that the water flow into the fruit was the sum of xylem and
74 phloem water transport. Therefore, changes in phenological stages and/or water status
75 of the tree may change the water pathway, in addition to fruit transpiration. Greenspan
76 et al (1994 and 1996) reported a change in the water transport to the grape berry with
77 the phenological stage of the fruit. During pre-veraison the net inflow corresponds to
78 xylem, while in post-veraison it changes to the phloem (Greenspan et al., 1994 and
79 1996). In olive trees, Proietti et al. (1999) reported a decrease in the photosynthesis
80 activity of fruits from the beginning of fruit development until 6-8 weeks after
81 polinisation. Drought sensibility of fruit changes with different phenological stages
82 (grape, Greespan et al., 1996; strawberry, Pomper and Breede, 1997). Water stress
83 conditions increase the maximum daily shrinkage at the beginning of fruit growth (pre-
84 veraison in grape and green-white stage in strawberry) but do not change around
85 ripening (post-veraison in grape and red stage in strawberry). A significant osmotic
86 adjustment of strawberry fruits during the green-white phenological stage is reported,
87 but not in the red fruit stage (Pomper and Breen, 1997).

88 The aim of this work is to study the water relations of fruit and leaves at pit
89 hardening in adult olive trees, under field conditions. Since this is the period of time
90 when water deficit restrictions are common, the response of water relation to a cycle of
91 water stress during this phenological stage was described. RDI works report that
92 moderate water stress conditions during pit hardening do not reduce yield. Therefore,
93 our hypothesis is that fruits would present higher drought resistance than leaves.

94

95 **2. Material and Methods**

96 **2.1. Description of the experiment**

97 Experiments were conducted at La Hampa, the experimental farm of the Instituto de
98 Recursos Naturales y Agrobiología (CSIC). This orchard is located at Coria del Río
99 near Seville (Spain) (37°17'N, 6°3'W, 30 m altitude). The sandy loam soil (about 2 m
100 deep) of the experimental site was characterized by a volumetric water content of 0.33
101 $\text{m}^3 \text{m}^{-3}$ at saturation, 0.21 $\text{m}^3 \text{m}^{-3}$ at field capacity and 0.1 $\text{m}^3 \text{m}^{-3}$ at permanent wilting
102 point, and 1.30 (0-10cm) and 1.50 (10-120 cm) g cm^{-3} bulk density. The experiment
103 was performed on 43-year-old table olive trees (*Olea europaea* L cv Manzanillo) during
104 2011. Tree spacing followed a 5 m x 5 m square pattern. Pest control and fertilization
105 practices were those commonly used by the growers and no weeds were allowed to
106 develop within the orchard. Irrigation was carried out during the night by drip using one
107 lateral pipe per tree row and five emitters per plant, delivering 8 L h⁻¹ each. Irrigation
108 requirements were determined according to daily reference evapotranspiration (ET_o)
109 and a crop factor based on the time of year and the percentage of ground area shaded by
110 the tree canopy (Fernández et al., 1998).

111 Trees were irrigated with 100% of ET_c in order to obtain non-limiting soil water
112 conditions until the beginning of pit hardening. The beginning of the pit hardening was
113 estimated according to Gijón et al. (2010) around day of the year (DOY) 157. One week
114 later (DOY 165) irrigation was withdrawn to three lines of olives. Measurements were
115 made in 4 olives irrigated at 100% ET_c during all the experiment (Control trees) and 4
116 olives in the central line of the plot where irrigation was withdrawn (Stressed trees). All
117 the measurements were made in these 4 trees per treatment. The drought cycle was
118 performed for 63 days and then trees were irrigated with the same amount of water than

119 Control trees. The experiment was stopped 7 days after the recovery because the harvest
120 had taken place.

121 **2.2 Measurements**

122 Micrometeorological 30 min data, namely air temperature, solar radiation, relative
123 humidity of air and wind speed at 2 m above the soil surface were collected by an
124 automatic weather station located some 40 m from the experimental site. Daily
125 reference evapotranspiration (ET_o) was calculated using the Penman-Monteith equation
126 (Allen et al., 1998). The meteorological data in the period of the experiment is presented
127 in Figure 1. Maximum temperatures varied from 26.8 °C (19 days after the beginning of
128 the drought period (DABD)) to 38.5 °C (66 DABD) (Figure 1a). Mean and minimum
129 temperatures were parallel to the maximum data. Minimum temperatures varied from
130 15.3 °C (24 DABD) to 24.4 °C (67 DABD) (Figure 1a). The values of the potential
131 evapotranspiration varied from 7.2 mm day⁻¹ (2 DBAD) to 3.5 mm day⁻¹ (68 DABD),
132 though most of the data were between 5.5 to 7 mm day⁻¹ (Figure 1b). Only one event of
133 rain was measured during the experiment at 48 DABD (2 mm).

134 The daily pattern of the leaf stomatal conductance in olive trees is characterized
135 with a maximum during the morning with a decrease after that until midday when the
136 minimum value is measured (Xiloyanis et al., 1996). This maximum leaf conductance is
137 more sensitive to water stress (Moriani et al., 2002). The drought cycle was
138 characterized by weekly measurements of maximum leaf conductance (g) and midday
139 stem water potential (Ψ_{stem}). Abaxial leaf conductance was measured in two full
140 expanded and well illuminated leaves per tree in each treatment with a steady state
141 porometer (LICOR-1600, LICOR, UK) around 10:00 GMT, when maximum values are
142 expected. Midday stem water potential in one leaf per tree was measured with a

143 pressure chamber (Model 1000, PMS, USA) around 13:00 GMT. Leaves were covered
144 with aluminium foil two hours before measuring.

145 The water relations of the leaves and fruits were measured around the time of
146 maximum leaf conductance. Two fully expanded and well illuminated leaves per tree
147 were selected. Leaf water potential (Ψ_{leaf}) was measured with the pressure chamber
148 (Model 1000, PMS, USA) in one of them. Then, this leaf was covered with aluminium
149 foil and immediately frozen in liquid nitrogen and stored at -80°C . These samples were
150 used to measure actual osmotic potential ($\Psi_{\pi \text{ leaf}}$). The second leaf was put in a test tube
151 with distilled water, in which only the petiole was in contact with the water. The test
152 tube was covered with aluminium foil and put into a portable freezer until arrival at the
153 laboratory. Then the test tubes were kept in the dark for 24 hours at $6-8^{\circ}\text{C}$ and then
154 frozen in liquid nitrogen and stored at -80°C . These samples were used to measure leaf
155 saturated osmotic potential ($^{100}\Psi_{\pi \text{ leaf}}$). Fruit water potential (Ψ_{fruit}) was measured with
156 the pressure chamber (Model 1000, PMS, USA) in one fruit per tree. Then, the fruit was
157 covered with aluminum foil and immediately frozen in liquid nitrogen and stored at -
158 80°C . These samples were used to measure actual fruit osmotic potential ($\Psi_{\pi \text{ fruit}}$). All
159 frozen tissues (leaf and fruit) were equilibrated at 20°C for 15 min before determination
160 of osmotic potentials. In the leaf samples, the central nerve was separated from the rest
161 of tissue. Then the tissue was used for determination of osmotic potential. Cell contents
162 in fruit were extracted by centrifugation of samples (10,000 rpm during 3 min). The
163 osmotic potential of samples (leaf and fruit) was determined using a psychrometer TRU
164 PSI Model WP3 calibrated against a salt solution. Values of turgor pressure (Ψ_p) were
165 calculated as:

166
$$\Psi_p = \Psi - \Psi_{\pi} \quad (1)$$

167 Where:

168 Ψ_p is the turgor pressure

169 Ψ is the water potential

170 Ψ_π is the osmotic potential

171 In order to describe the effect of the different irrigation strategies, the water stress

172 integral (Ψ_{int}) was calculated from the Ψ_{stem} data as defined by Myers (1988) and

173 modified for García-Tejero et al. (2010):

174

$$\Psi_{int} = \sum_{i=1}^{i=t} \left| \Psi_{i+1}(n_{i+1} - n_i) + \frac{1}{2}(\Psi_i - \Psi_{i+1})(n_{i+1} - n_i) \right| \quad (2)$$

175

176 Where:

177 Ψ_{int} is the integral of stress in a period of t days

178 Ψ_{i+1} is the value of midday stem water potential at day i+1

179 Ψ_i is the value of midday stem water potential at day i

180 n_{i+1} is the day i+1

181 n_i is day i

182

183 In a similar way, the integral of leaf conductance (g_{int}) was also calculated:

$$g_{int} = \sum_{i=1}^{i=t} \left| g_{i+1}(n_{i+1} - n_i) + \frac{1}{2}(g_i - g_{i+1})(n_{i+1} - n_i) \right| \quad (3)$$

184

185

186 Where:

187 g_{int} is the integral of stress in a period of t days

188 g_{i+1} is the value of midday stem water potential at day i+1

189 g_i is the value of midday stem water potential at day i

190 n_{i+1} is the day i+1

191 n_i is day i

192

193 Finally two fruit surveys were made at 49 and 63 days after the beginning of
194 drought (DABD) with 10 fruits per tree and treatment. The longitudinal and transversal
195 (at equatorial point) diameters of the fruit were measured and volume was estimated.
196 The data were subjected to one-way ANOVA and means were compared using the
197 Tukey test (Significance was set at $P < 0.05$) with SPSS 10.0.

198

199 **3. Results**

200 The level of water stress was measured with the midday stem water potential (Ψ_{stem})
201 and maximum leaf conductance (Figure 2). Ψ_{stem} in Control trees decreased from -1.0
202 MPa at the beginning of the experiment to -1.4 MPa at 29 DABD, when it was almost
203 constant until the beginning of the recovery period (Figure 2a). The pattern of the
204 Stressed trees was similar with almost the same value at the beginning of the
205 experiment, but with a sharp decrease. Significant differences in Ψ_{stem} were measured
206 from 14 DABD, and the minimum value was around -1.8 MPa in the Stressed trees (42
207 DABD). The recovery was completed in 7 days after the beginning of the irrigation of
208 the Stressed trees.

209 The pattern of maximum leaf conductance (g) is shown in Figure 2b. From the
210 beginning of the experiment g of Stressed trees was systematically and significantly
211 lower than in Control trees. The effect of water stress imposed was more patent from 29
212 DABD, and particularly in the period between 49 and 63 DABD. In both treatments, a
213 continuous increase in g was measured from the beginning of the experiment until 14

214 DABD with a maximum around $350 \text{ mmol m}^{-2} \text{ s}^{-1}$, with a sharp decrease from 29
215 DABD when it was around $150 \text{ mmol m}^{-2} \text{ s}^{-1}$. Stressed trees presented lower values than
216 Control throughout the experiment with significant differences at 0, 14, 29, 35, 56, 63
217 and even at 7 days after the recovery.

218 The pattern of leaf (Ψ_{leaf}) and fruit (Ψ_{fruit}) water potential, measured at the time
219 of maximum leaf conductance, and the differences between both is presented in Figure
220 3. The values of Ψ_{leaf} in Control trees varied during the experiment between -1.18 to -
221 2.05 MPa (Figure 3a). Significant differences in Ψ_{leaf} between Stressed and Control
222 trees were measured from 14 days after the beginning of the drought cycle (DABD)
223 until the recovery period. Only 56 DABD Ψ_{leaf} values were not significantly different
224 and this was likely related to a problem in the irrigation of Control trees. The minimum
225 Ψ_{leaf} values in Stressed trees reached -2.4 MPa at 35 DABD. The increase of Ψ_{leaf} , at 49
226 DABD, in both treatments was related to a reduction in the vapor pressure deficit
227 (VPD). No significant differences in Ψ_{leaf} were observed 7 days after the beginning of
228 the irrigation in Stressed trees.

229 The values of Ψ_{fruit} in Control trees varied between -1.4 MPa and -2.0 MPa
230 (Figure 3b). The Ψ_{fruit} in the Stressed trees presented a similar pattern as the Control
231 treatment. The values of Ψ_{fruit} varied from -1.5 MPa to -2.2 MPa. Significant differences
232 between treatments were observed at 14, 35, 42, 56 and 63 DABD, with clear trend to
233 decrease from 35 DABD (except for the date 49 DABD, in which a decrease of VPD
234 was observed). The recovery of Ψ_{fruit} values were almost completed 7 days after the
235 beginning of the irrigation in the Stressed trees.

236 The difference between Ψ_{leaf} and Ψ_{fruit} ($\Delta\Psi$) presented a different pattern
237 between treatments (Fig. 3c). Values of $\Delta\Psi$ in Control trees tended to be positive
238 during the first part of the experiment (until 29 DABD) with a maximum of 0.4 MPa.
239 From this date the $\Delta\Psi$ decreased and showed values between 0.2 and -0.1 MPa until
240 the end of the experiment. In Stressed trees, however, though the two first data are
241 similar to Control, $\Delta\Psi$ tended to lower values than Control from 14 DABD. Such
242 differences were significant at 21 and 29 DABD and negative values were observed
243 from 21 DABD until 42 DABD, with a minimum value of -0.4 MPa. After 35 DABD, a
244 sharp increase in $\Delta\Psi$ is produced and a similar pattern to Control is presented with
245 oscillation between ± 0.1 MPa.

246 The measurements of $\Psi_{\pi \text{ leaf}}$, $\Psi_{\pi \text{ fruit}}$ y $^{100}\Psi_{\pi \text{ leaf}}$ are shown in Figure 4. The
247 pattern of $\Psi_{\pi \text{ leaf}}$ (Figure 4a) was near a constant value in both treatments. In Control
248 trees, $\Psi_{\pi \text{ leaf}}$ varied between -2.1 and -3.4 MPa, though most of the values were around -
249 3.2 MPa. The pattern of Stressed trees was very similar to Control but more constant
250 and tended to produce lower values. Significant differences were found at 0, 14 and 35
251 DABD. The $\Psi_{\pi \text{ leaf}}$ in Stressed trees varied between -2.5 and -3.5 MPa. In both
252 treatments sharp increases were measured at 35 and 63 DABD. After 7 days of
253 recovery, the $\Psi_{\pi \text{ leaf}}$ were still significantly different and the values measured were
254 around -3.0 MPa for Control and -3.4 MPa for Stressed trees.

255 The pattern of $\Psi_{\pi \text{ fruit}}$ was very similar between treatments (Figure 4b). $\Psi_{\pi \text{ fruit}}$
256 values slightly increased, in both treatments, from -2.5 MPa, at the beginning, until -2.0
257 MPa at the end of the recovery period. There was a sharp increase at 42 DABD in both
258 treatments. The differences between treatments were lower than 0.5 MPa on all dates,

259 but Stressed trees tended to produce lower values than Control which were significant at
260 14, 21, 29, 35, 56 DABD. After 7 days of recovery, the $\Psi_{\pi \text{ fruit}}$ were still significantly
261 different.

262 The pattern of $^{100}\Psi_{\pi \text{ leaf}}$ was almost constant throughout the experiment and the
263 differences were lower than 0.4 MPa between treatments (Fig 4c). $^{100}\Psi_{\pi \text{ leaf}}$ varied
264 between -2.34 to -3.16 MPa, with a slight tendency to decrease along the experiment.
265 Only at 63 DABD were significantly lower values in Stressed trees found, but the
266 differences were lower than 0.4 MPa. After 7 days of recovery no significant differences
267 were found, though Stressed trees tended to produce lower values than Control.

268 The turgor pressure in the leaves and fruits is presented in Figure 5. The pattern
269 of turgor pressure in leaves ($\Psi_{p \text{ leaf}}$) throughout the experiment in both treatments was
270 almost constant around 1.2 MPa in Control and slightly lower in Stressed trees (Fig. 5a).
271 Only at 35 and 63 DABD were sharp decreases measured in both treatments. Significant
272 differences were found only at 29 and 49 DABD, though Stressed trees tended to
273 produce lower values from 14 DABD. In recovery $\Psi_{p \text{ leaf}}$ of Stressed trees was
274 significantly higher than Control. Fruit turgor pressure ($\Psi_{p \text{ fruit}}$) presented an erratic
275 pattern in both treatments with a trend to a continuous decrease from 1 MPa until 0.4
276 MPa (Fig. 5b). Negative values were estimated in both treatments and are likely related
277 to errors in the fruit osmotic pressure, probably related to an overestimation due to the
278 process of freezing used for the measurement of osmotic potential. Significant
279 differences were found only at 21, 29 and 36 DABD, with higher values in Stressed
280 trees on the two first dates and the opposite on the third.

281 The stress integrals for water potential (Ψ_{Int}) and leaf conductance (g_{Int}) are
282 shown in Figure 6. In both parameters Stressed trees are significantly higher values than

283 Control trees (around 15% and 17%). However, such differences did not affect the fruit
284 volume (Table 1). Fruits in Stressed trees were bigger than Control in volume in the two
285 samples (at 49 and 63 DABD). The differences in volume were of 6% at 49 DABD and
286 11% at 63 DABD.

287

288 **4. Discussion**

289 The period of pit hardening in olives is very important for the physiology of the tree.
290 Fruit is a very important sink of nutrient and water from this date (Rallo and Suarez,
291 1989). Fruit development in conditions of high fruit load produced an increase in leaf
292 conductance and a decrease in water potential (Martin-Vertedor et al., 2011) and an
293 important decrease in vegetative growth even in fully irrigated conditions (Rallo and
294 Suarez, 1989). However, in our conditions, there were not clear differences between
295 Ψ_{leaf} and Ψ_{fruit} in Control trees. Therefore, there was not a preferential water pathway
296 during pit hardening from root to fruit in conditions of low fruit load. The osmotic
297 potential was lower and the turgor pressure higher in leaves than in fruits throughout the
298 experiment in Control trees. These conditions would be related to a preferential
299 vegetative growth respect to fruit growth, which consistent with the low fruit load
300 conditions of the experiment.

301 The water stress level obtained after 63 days of the drought period was low, due
302 to the high spring rainfalls (140 mm from April to June, last rains 30 mm at the
303 beginning of June). Although, midday stem water potential (Ψ_{stem}) was significantly
304 lower in Stressed than Control trees, the minimum values only reached at -1.8 MPa
305 from 42 DABD. This minimum Ψ_{stem} value is considered a low water stress level in

306 comparison to the values reported in the literature in this phase of fruit development
307 (i.e. potted olive, Dichio et al 1997, 2003 and 2006; field olive, Moriana et al., 2002;
308 Moriana et al., 2003; Iniesta et al., 2009). According to our results, at this level of water
309 stress the leaf osmotic adjustment was small, because significant differences in leaf
310 osmotic potential at full turgor ($^{100}\Psi_{\pi \text{ leaf}}$) were only found at the end of the experiment.
311 On that date (63 DABD), Stressed trees presented an osmotic adjustment of 0.33 MPa,
312 which was slightly decreased after 7 days of recovery to 0.17 MPa. This result in
313 osmotic adjustment is slightly lower than the ones reported by Dichio et al (2003) in
314 low water stress potted trees, which was 0.45 MPa, but is higher than ones reported with
315 P-V curves in this work, which was 0.11 MPa. The value of osmotic adjustment after
316 the recovery (0.17 MPa) was very similar to the ones reported by Dichio et al (2006) in
317 the recovery period of potted olive trees (0.14 MPa). The delay in the recovery of $^{100}\Psi_{\pi}$
318 $_{\text{leaf}}$ is also consistent with the data reported by Dichio et al. (2006) who measured a
319 significant osmotic adjustment even 30 days after the beginning of the recovery of
320 potted olive trees. This residual osmotic adjustment may be related to an uncompleted
321 rehydration of the trees. In our results, though there were no significant differences in
322 Ψ_{stem} , leaf conductance was slightly, but significantly, lower. The conditions of
323 completed and fast rehydration are strongly related to a high wet surface in the recovery
324 period (Pérez-López et al., 2008) that usually is not provided in field conditions.

325 This low and slow period of water stress produced a different response in leaf
326 and fruit physiology. In leaves, water potential (Ψ_{leaf}) was more clearly reduced than in
327 fruits (Ψ_{fruit}). Such changes meant that from 14 DABD, when a significant water stress
328 was detected (Ψ_{stem} , was significantly lower), the difference between Ψ_{leaf} and Ψ_{fruit}
329 ($\Delta\Psi$) was clearly negative. Therefore, Ψ_{leaf} was lower than Ψ_{fruit} . Nobel and de la

330 Barrera (2000) in platyopuntias plants suggested that such differences indicated that the
331 water entered the fruit via the phloem rather than the xylem. Several authors reported a
332 decrease in $\Delta\Psi$, in daily cycles, due to the effects of water stress (vines, Greenspan et
333 al., 1996; strawberries, Pomper and Breen, 1997), though only in
334 strawberries, $\Delta\Psi$ changes from positive to negative (Pomper and Breen, 1997).
335 However, these decreases were steady until 42 DABD when a sharp increase meant that
336 $\Delta\Psi$ were similar to Control trees. On the date (42 DABD), minimum Ψ_{stem} occurred and
337 probably a stomatal closure began (though the main differences in g occurred at 56
338 DABD). Therefore, the main ways for water flow in the fruit may be changed at the
339 beginning of water stress and reversible if water stress progressed. Greenspan et al.
340 (1994 and 1996) suggested that the bulk of vascular water flow changes from xylem in
341 pre-veraison to phloem in post-veraison in full irrigated grape berry. Mathews and
342 Shackel (2005) suggested that in fully irrigated prunes the relative importance of xylem
343 and phloem in the water flow to the fruit may be reversible.

344 The drought conditions, in addition, affected the components of water potential.
345 The fruit osmotic potential ($\Psi_{\pi \text{ fruit}}$) was significantly reduced from 14 DABD and the
346 leaf turgor pressure ($\Psi_{\text{p leaf}}$) from 29 DABD (though it tended produce lower values
347 from 14 DABD). On the other hand, fruit turgor pressure ($\Psi_{\text{p fruit}}$) and leaf osmotic
348 pressure ($\Psi_{\pi \text{ leaf}}$) were not clearly affected during the experiment. These responses
349 suggest that vegetative growth is more sensitive to water stress than fruit growth in
350 olive trees. Such drought resistance of the fruit is likely related to a fruit osmotic
351 adjustment which may be produced by an increase of the phloem flow in the fruit.
352 Pomper and Breen (1997) reported an osmotic adjustment of strawberry fruits in
353 conditions of water stress during green-white stage. In addition, these results are

354 consistent with the conclusion of the regulated deficit irrigation works which reported a
355 decrease in the vegetative growth with low impact on the fruit yield (i.e. Goldhamer,
356 1999; Alegre et al., 2002; Moriana et al., 2003; Lavee et al., 2007; Tognetti et al., 2006;
357 Iniesta et al., 2009).

358 The slow progress of low level of water stress permits the description of several
359 mechanisms in the water relations of leaves and fruit. Water stress induced, probably
360 first, a significant leaf dehydration (Ψ_{stem} and Ψ_{leaf}). Such a response likely reduced the
361 water transport to the fruit from xylem (decrease of $\Delta\Psi$) which produced a decrease of
362 the fruit osmotic potential ($\Psi_{\pi \text{ fruit}}$) and likely fruit osmotic adjustment. Then, the fruit
363 delayed the decrease of fruit turgor pressure compared to the leaf. Because of the
364 progression of water stress, fruit water potential was affected (Ψ_{fruit}) and also the fruit
365 turgor pressure ($\Psi_{\text{p fruit}}$). This alone, or with the permanent decrease of leaf turgor
366 pressure, produced the stomatal closure. The reduction of leaf transpiration induced a
367 change in the water flow into the fruit with an increase of xylem flow and a recovery in
368 the fruit turgor pressure. Finally, a leaf osmotic adjustment at the end of the experiment
369 is likely related to the improvement of leaf turgor pressure. In the mechanism proposed
370 the level of water stress is as important as the duration, as Hsiao (1990) suggests. Olive
371 trees are considered species tolerant to high internal dehydration (Moriana et al., 2002).
372 Therefore, the decrease of leaf water potential (Ψ_{stem} and Ψ_{leaf}) is one of the most
373 important signals that likely produce changes in the water relations of the tree. Moriana
374 and Fereres (2002) in field olive trees, reported that gas exchange is less sensitive to
375 water stress than water potential at the beginning of a drought cycle. This delay between
376 the beginning of leaf dehydration and stomatal closure would provide the trees with the
377 capacity to maintain the assimilation. The closure of stomata would be produced by an

378 increase of the loss of hydraulic conductivity (Lo Gullo et al., 1988) from a threshold
379 water potential (as in conifers (Froux et al., 2005)). The resistance of fruits to these
380 initial conditions of water stress is consistent with their important role in the water
381 relations of the tree (Martín-Vertedor et al., 2011).

382

383 **5. Conclusions**

384 The water flow in the fruit during pit hardening in fully irrigated conditions is produced
385 from xylem and phloem. In conditions of low fruit load, there were clear differences
386 between the component of water potential between leaves and fruits. Osmotic potential
387 at the time of maximum leaf conductance was lower in leaves than in fruits. Turgor
388 pressure at the same time was higher in leaves than in fruits. Such differences may be
389 related to a higher vegetative growth produced by the low fruit load.

390 Low water stress conditions produced significant changes in the water relations
391 of fruit and leaves. There was a clear delay in the stomatal closure and leaf osmotic
392 adjustment that produced a decrease in the leaf turgor pressure. On the other hand, there
393 was a change in the leaf-fruit water potential that likely benefited fruit growth, with no
394 decrease in fruit turgor pressure, in comparison with leaves. This process is consistent
395 with a higher drought sensitivity of vegetative growth than fruit growth, which permits
396 the reduction of irrigation with no effect on yield. The midday stem water potential of -
397 1.8 MPa is a reference of water stress levels for deficit irrigation.

398

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404

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511 **Figure Captions**

512 Figure 1. Pattern of temperature (maximum, mean and minimum, °C) (a) and potential
513 evapotranspiration (ET_o, mm day⁻¹) (b) during the experiment. White circle in figure b
514 represented the days when the measurements were made. Time is presented as days
515 after the beginning of drought (DABD)

516 Figure 2. Pattern of midday stem water potential (Ψ_{stem} , a) and maximum leaf
517 conductance (b) along the experiment. Each point is the average of 4 data. Asterisk
518 represents the date when significant differences were found. Time is presented as days
519 after the beginning of drought (DABD)

520 Figure 3. Pattern of leaf (a) and fruit (b) water potential and the different between them
521 ($\Delta\Psi$, c). Each point is the average of 4 data. Asterisk represents the date when
522 significant differences were found. Time is presented as days after the beginning of
523 drought (DABD).

524 Figure 4. Pattern of leaf osmotic potential (a), fruit osmotic potential (b) and saturated
525 leaf osmotic potential (c). Each point is the average of 4 data. Asterisk represents the
526 date when significant differences were found. Time is presented as days after the
527 beginning of drought (DABD).

528 Figure 5. Pattern of leaf (a) and fruit (b) turgor pressure during the experiment. Each
529 point is the average of 4 data. Asterisk represents the date when significant differences
530 were found. Time is presented as days after the beginning of drought (DABD).

531 Figure 6. Stress integral of midday stem water potential (a) and maximum leaf
532 conductance (b). Each bar is the average of 4 data.

533

Fig1

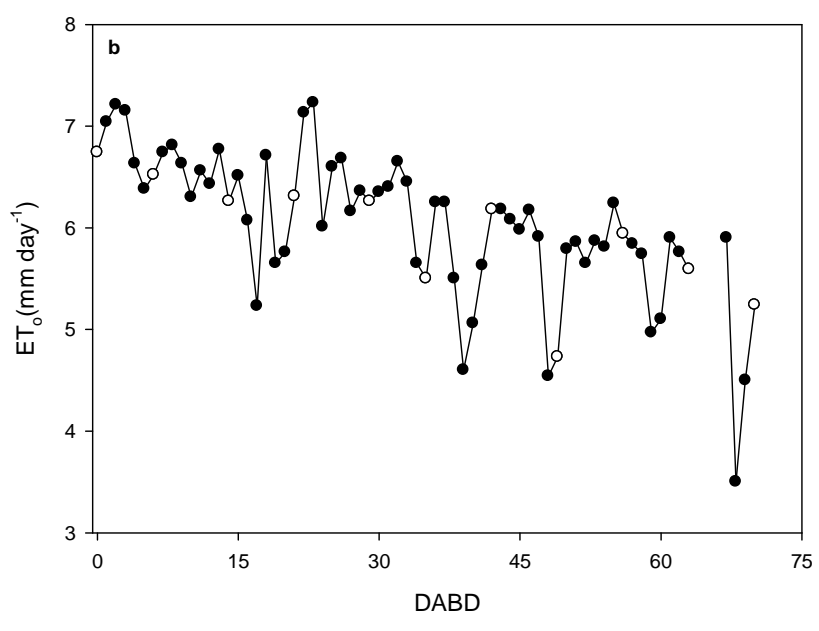
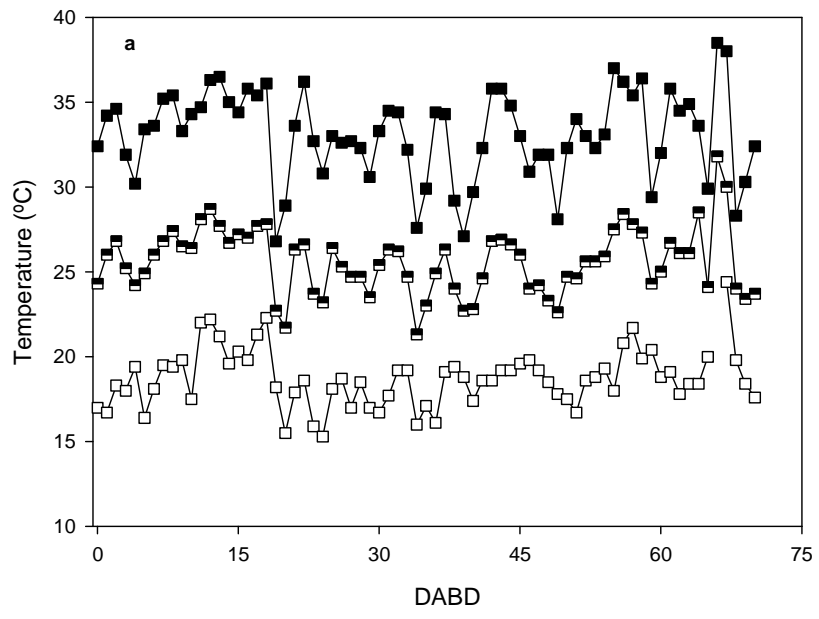


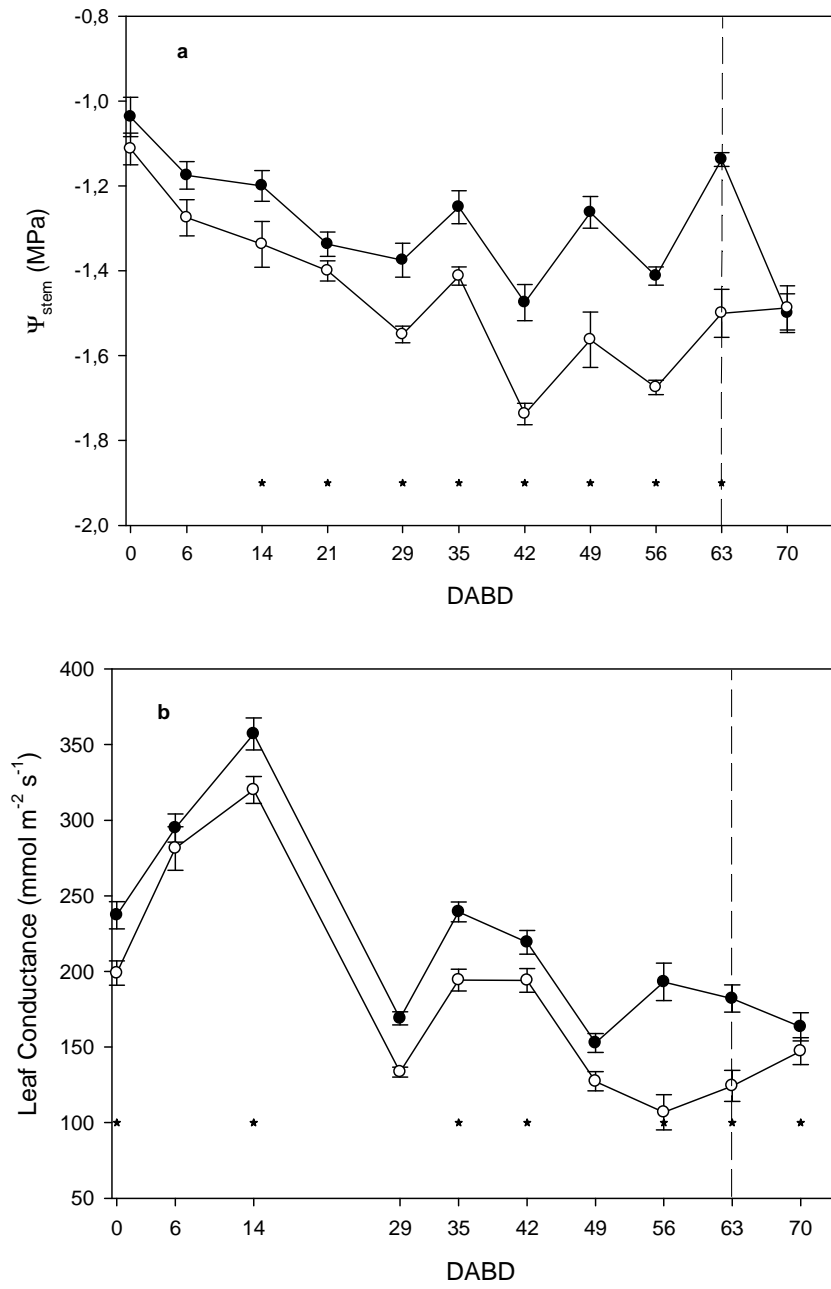
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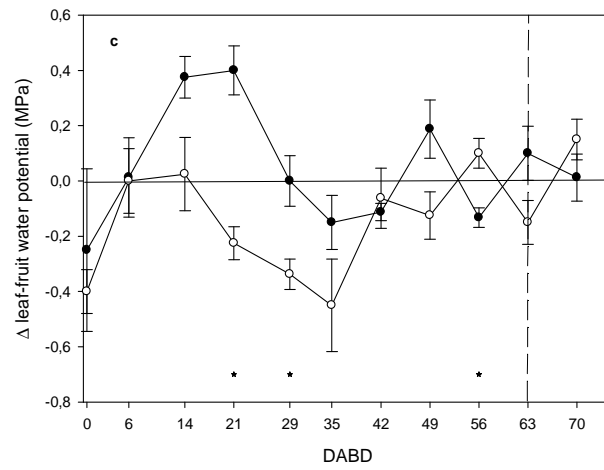
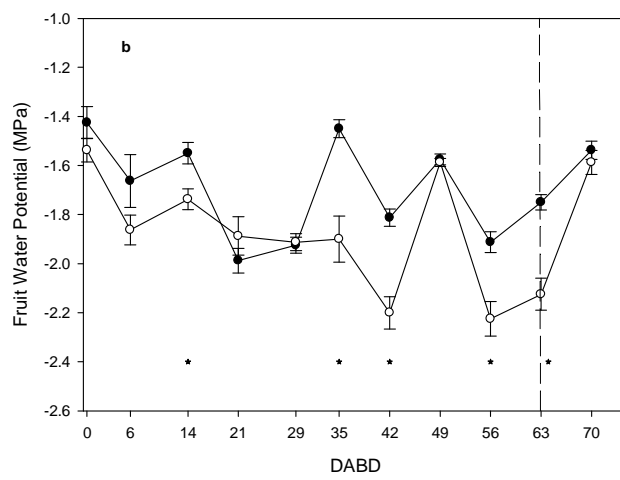
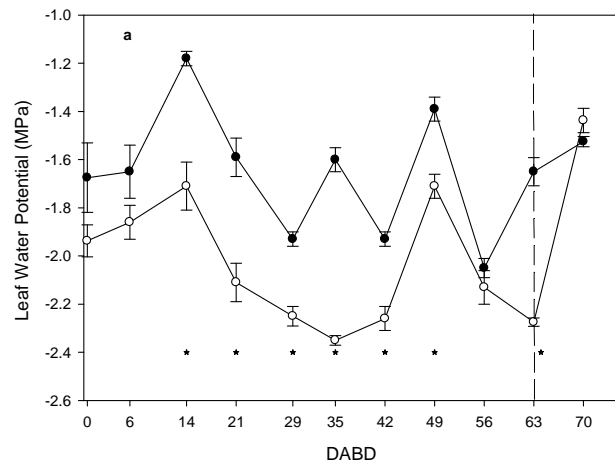
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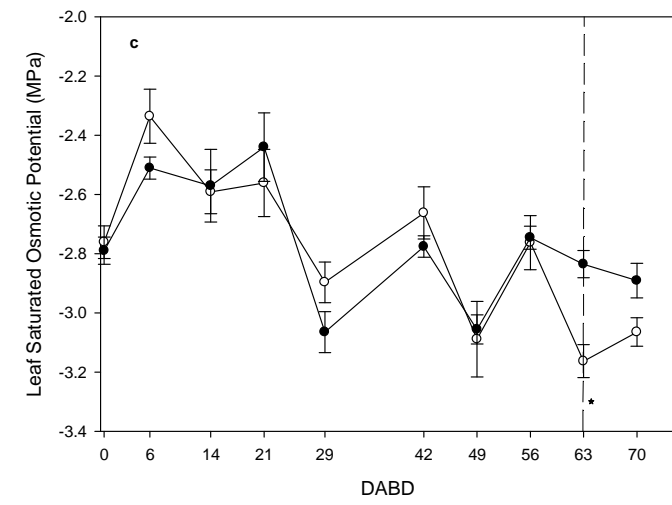
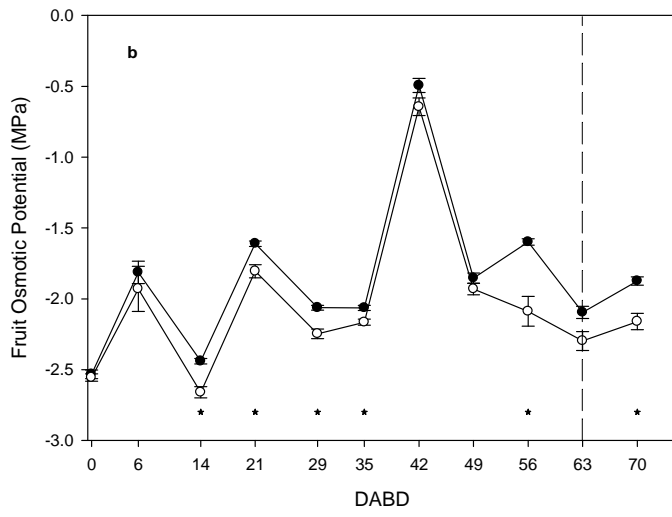
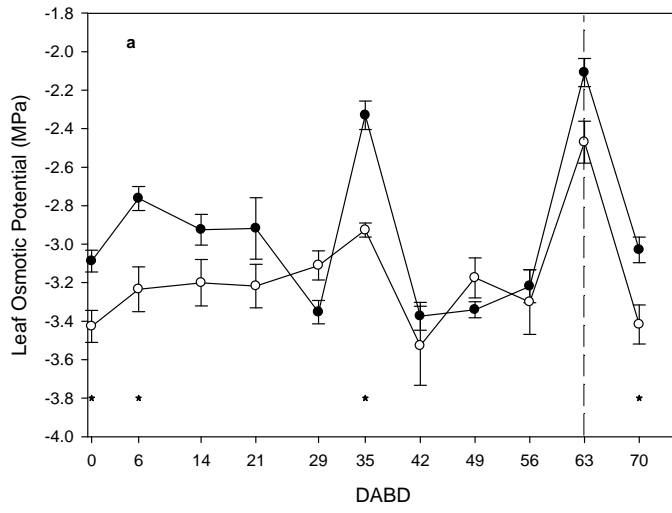
Fig4

Fig5

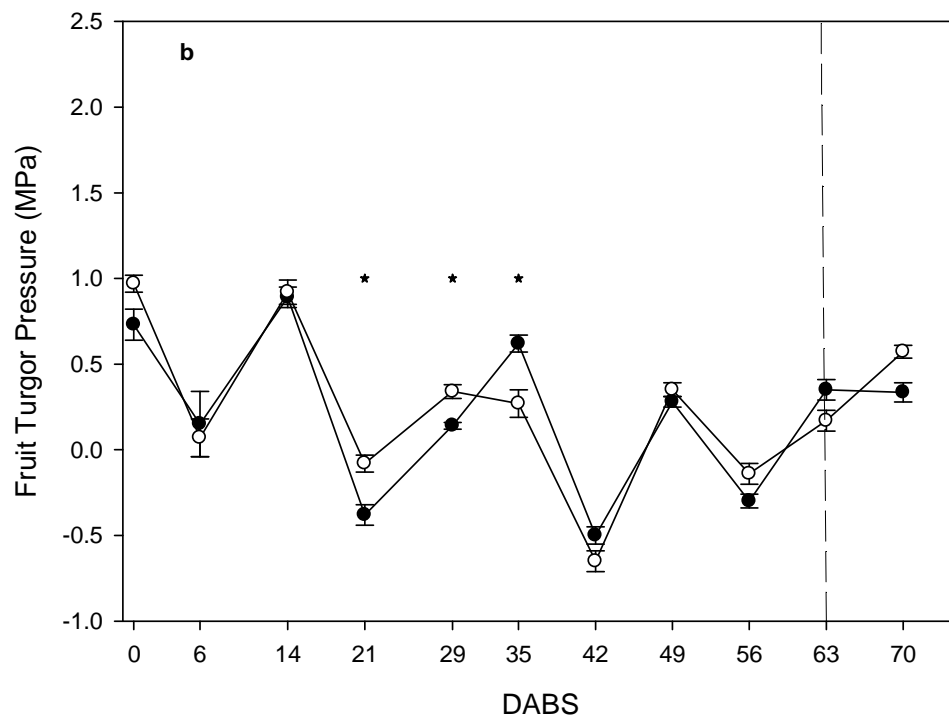
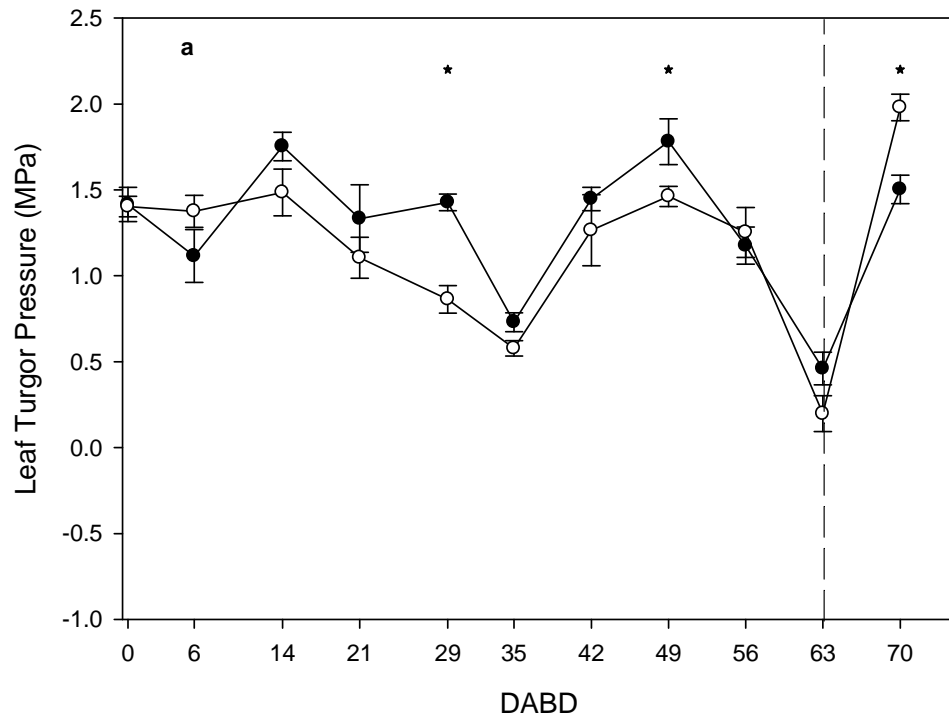


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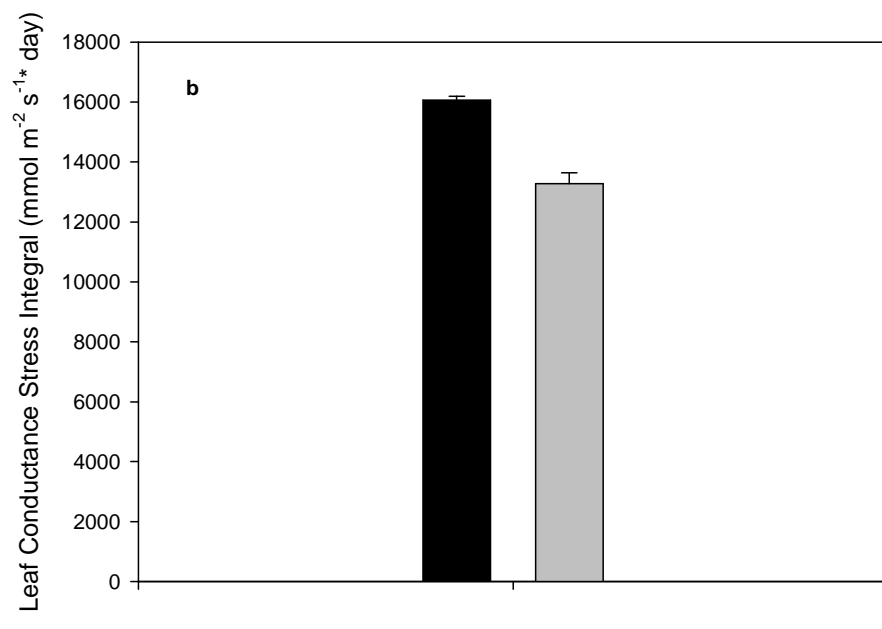
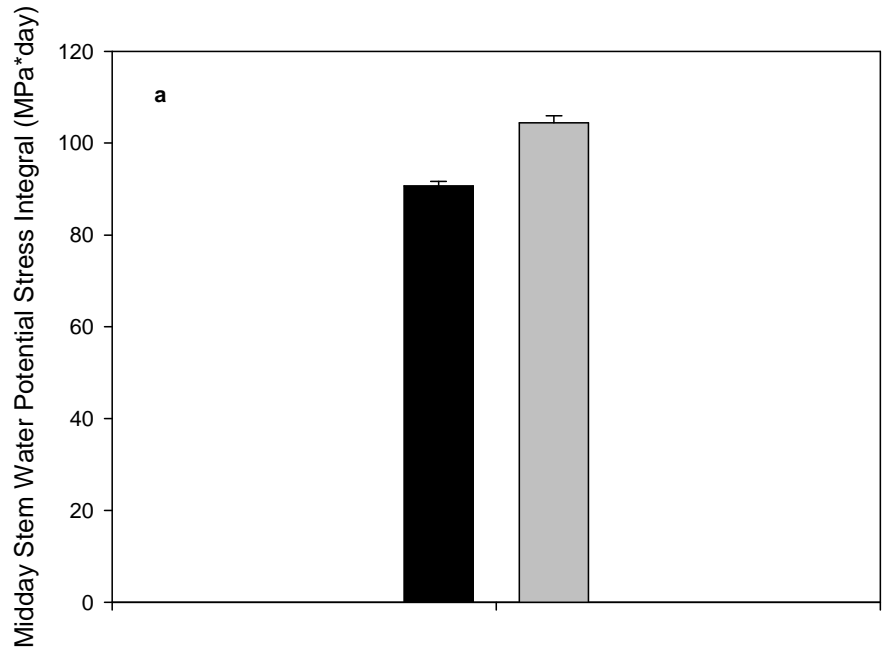


Table 1[Click here to download Tables: table1.docx](#)

Table 1. Fruit characteristics in the two surveys (49 days after the beginning of stress (DABD) and 63 DABD). Each value is the average of 10 data. Asterisk in the same column indicates significant differences ($p < 0.05$, LSD Test).

Treatment	49 DABD				63 DABD			
	Longitudinal Diameter (mm)	Tranversal Diameter (mm)	L/T ratio	Volumen (cm ³)	Longitudinal Diameter (mm)	Tranversal Diameter (mm)	L/T ratio	Volumen (cm ³)
Control	20.83	16.71	1.24	3.07	21.38	18.06	1.18	3.68
Estressed	21.82	16.91	1.29	3.30	23.42	18.42	1.27	4.18
LSD	0.18 *	0.11n.s.	0.007 *	0.066 *	0.208 *	0.100 *	0.009*	0.070*