

1 **Assessment of discretely measured indicators and maximum daily trunk**
2 **shrinkage for detecting water stress in pomegranate trees**

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23

24 **Abstract**

25 Measurements obtained by the continuous monitoring of trunk diameter
26 fluctuations were compared with discrete measurements of midday stem water
27 potential (Ψ_{stem}) and midday leaf conductance (g_l) in adult pomegranate trees
28 (*Punica granatum* (L.) cv. Mollar de Elche). Control plants (T0) were irrigated
29 daily above their crop water requirements in order to attain non-limiting soil
30 water conditions, while T1 plants were subjected to water stress by depriving
31 them of irrigation water for 34 days, after which time irrigation was restored and
32 plant recovery was studied for 6 days. The water relations in T0 pomegranate
33 plants confirmed that they had not suffered waterlogging. In contrast, T1 plants
34 showed a substantial degree of water stress, which developed slowly. Maximum
35 daily trunk shrinkage (MDS) was seen to be the most suitable plant-based
36 indicator for precise irrigation scheduling in adult pomegranate trees, because
37 its signal:noise ratio was higher than that for Ψ_{stem} and g_l . MDS increased in
38 response to water stress, but when the Ψ_{stem} fell below -1.67 MPa, the MDS
39 values decreased. Reference or baseline relationships for MDS measurements
40 can be obtained by pooling data across several seasons using crop reference
41 evapotranspiration (ET_o), mean daily air vapour pressure deficit, mean daily air
42 temperature and solar radiation. In this way, ET_o was seen to be the best
43 predictor of MDS. These findings open up the possibility of normalizing MDS
44 measurement at a given time respect to the expected value under non-limiting
45 water conditions, which can be calculated from the reference relationships.

46

47 *Key words:* Plant water relations; *Punica granatum*; trunk diameter fluctuations;
48 water stress

49 **1. Introduction**

50 Pomegranate (*Punica granatum* L.) is one of the oldest known edible fruits,
51 being among the seven kinds of fruit mentioned in the Bible (Blumenfeld et al.,
52 2000). However, despite being grown commercially in many regions of the
53 world, including countries of the Mediterranean Basin (Stover and Mercure,
54 2007; Holland et al., 2009), it has frequently been considered a minor crop. The
55 way at which it is regarded is beginning to change and there is a growing
56 interest in the consumption of its fruits for their organoleptic characteristics and
57 their perceived health benefits (Michel et al., 2005; Lansky and Newman, 2007).
58 Moreover, pomegranate is a very interesting fruit tree species because it has
59 drought tolerance characteristics common in xeromorphic plants, such as high
60 leaf relative apoplastic water content, and it is able to confront water stress by
61 developing complementary stress avoidance and stress tolerance mechanisms
62 (Rodríguez et al., 2012). For these reasons, this drought-hardy crop thrives well
63 in arid and semiarid areas, even under desert conditions (Sarkhosh et al., 2006;
64 Zamani et al., 2008).

65 Frequent situations of imbalance between water supply and demand
66 occur in Mediterranean agrosystems, which are facing increasing pressure to
67 reduce water use. Indeed, there is a constant need to improve water use
68 efficiency, and among the tools that growers can use to achieve this goal are
69 more precise irrigation scheduling procedures that will protect water resources
70 and their integrity for their future use (Naor and Cohen, 2003; Katerji et al.,
71 2008)

72 Bhantana and Lazarovitch (2010) measured the evapotranspiration (ET),
73 crop coefficients (K_c) and growth in two young pomegranate tree cultivars grown

74 in lysimeters to varying electrical conductivity of the irrigation water. Also,
75 Intrigliolo et al. (2011a) suggested tentative preliminary irrigation
76 recommendations for pomegranate trees. However, to our knowledge, no
77 specific studies have been conducted on irrigation water requirements in adult
78 pomegranate plants under field conditions. Moreover, to reach optimal growth,
79 yield and fruit quality in arid and semiarid conditions, pomegranate trees require
80 regular irrigation, particularly during the dry season (Holland et al., 2009;
81 Prasad et al. 2003; Shaliendra and Narendra, 2005; Sulochanamma et al.,
82 2005). Hence, studies on pomegranate water requirements and related criteria
83 for precise irrigation management practices are needed.

84 The use of plant-based water status indicators has become very popular
85 for planning precise irrigation, because plant water status is the best way for
86 predicting crop performance to a given irrigation scheduling regime. Since the
87 plant water status controls many physiological processes and crop productivity,
88 this information can be highly useful in irrigation scheduling (Fernández and
89 Cuevas, 2010; Ortuño et al., 2010). Measurements of trunk diameter
90 fluctuations (TDF) using LVDT (linear variable differential transducer) sensors
91 provide continuous and automated recording of maximum daily trunk shrinkage
92 (MDS), which seem suitable for the development of automated irrigation
93 scheduling in fruit trees (Conejero et al., 2007; Ortuño et al, 2009a; Moriana et
94 al., 2010).

95 Because plants are in the middle of the soil-plant-atmosphere continuum,
96 plant water status is the result of the soil water availability and the evaporative
97 demand. Therefore, absolute water stress indicator values recorded without
98 considering the evaporative demand might be meaningless. For this reason, it is

99 better to use the concept of signal intensity (SI) for irrigation scheduling,
100 normalizing the indicator absolute values with respect to values in non-limiting
101 soil water conditions (Naor and Cohen, 2003; Goldhamer and Fereres, 2001;
102 Ortuño et al., 2005, 2006). Water stress indicator SI is a dimensionless variable,
103 where values above unity indicate water stress levels, while SI values of unity
104 indicate the absence of irrigation-related stress (Goldhamer and Fereres, 2004).
105 One option for obtaining water stress indicator reference values could be to
106 previously define the effects of the evaporative demand on the plant water
107 status indicator and later to use this relationship or baseline as a reference to
108 correct the actual water stress indicator values obtained.

109 Intrigliolo et al. (2011b) suggested i) that differences in pomegranate
110 water status could be detected earlier for midday stem water potential (Ψ_{stem})
111 than for MDS, ii) also, these authors found a significant, but relatively low,
112 correlation between MDS and crop reference evapotranspiration (ET_o) (MDS
113 (μm) = 23.0 ET_o (mm) + 8.8. $r^2 = 0.44^{***}$), whereas the relationships between
114 MDS and air temperature and air vapour pressure deficit were even weaker,
115 and iii) as a final point, these authors indicated that the best fit between MDS
116 and Ψ_{stem} was obtained with a linear regression, which changed in concordance
117 with some changes in the fruit growth pattern or fruit removal. These behaviors
118 present some differences respect to those observed in other fruit trees. For
119 example, i) MDS has been frequently found as more sensitive than the other
120 indicators in detecting plant water stress (Ortuño et al., 2010), ii) some authors
121 have showed that it is possible to predict adequately MDS reference values in
122 crop trees when meteorological variables measured on a whole-day basis are
123 used (Moreno et al., 2006; Ortuño et al., 2009b; Conejero et al., 2011), and iii)

124 Ortuño et al. (2010) indicated that in several fruit tree species under drought
125 stress the decrease in Ψ_{stem} is associated with an increase in MDS, but this
126 pattern changes at values below a Ψ_{stem} threshold and any further reduction in
127 Ψ_{stem} is associated with a decrease in MDS values.

128 For these reasons, the objective of the present study was to compare the
129 sensitivity of MDS and discretely measured indicators of the plant water status
130 in adult pomegranate trees in response to a cycle of water deprivation and
131 recovery, establishing the relationship between MDS and the plant water status.
132 The feasibility of obtaining baselines for tree water status indicators in trees
133 under non-limiting water conditions and their inter-season constancy was also
134 investigated.

135

136 **2. Materials and Methods**

137 *2.1. Plant material, experimental conditions and treatments*

138 *Experiment 1 (2009)*

139 The experiment was carried out in 2009 on a farm located near the city of
140 Murcia (Spain) (37°57' N, 0°56'W). The plant material consisted of own rooted
141 10-year old pomegranate trees (*Punica granatum* L.) cv. Mollar de Elche, with
142 an average trunk diameter of about 15 cm. Tree spacing followed a 3 m × 6 m
143 pattern, with an average ground cover of about 59 %.

144 The soil of the orchard was a weakly saline (2.1 dS m⁻¹) Xeric
145 Torriorthent, with silt loam texture, high lime content (46 % calcium carbonate),
146 very low organic matter content (0.92 %), low cationic exchange capacity (7.93
147 meq 100 g⁻¹), and low available potassium and phosphorus levels. The irrigation
148 water had an electrical conductivity of between 1.7 and 2.2 dS m⁻¹ and the Cl⁻

149 concentration in the irrigation water ranged from 36 to 48 mg l⁻¹.

150 Control plants (treatment T0) were irrigated above crop water
151 requirements (115 % ETo), using six emitters (each delivering 4 l h⁻¹) per plant.
152 Irrigation in T1 plants was withheld for 34 days (from day of the year (DOY) 209
153 to 243, second half of rapid fruit growth period). The recovery of plants was
154 ensured by re-irrigation at the levels used in T0 for 6 days (from DOY 244 to
155 250). Total water amounts applied in experimental period were 261 and 38 mm
156 for T0 and T1 treatments, respectively.

157

158 *Experiment 2 (2010)*

159 The experiment was performed in 2010 on other farm also located near the city
160 of Murcia (Spain) (37°47'N, 1°25'W). The plant material was own rooted 10-year
161 old pomegranate trees (*Punica granatum* L.) cv. Mollar de Elche, with an
162 average trunk diameter of about 17 cm. Tree spacing followed a 3 m × 6 m
163 pattern, with an average ground cover of about 68 %.

164 The soil of the orchard was a Hyposalic Calciorthid moderately saline
165 (5.9 dS m⁻¹), with a silt loam texture, moderate lime content (20 % calcium
166 carbonate), very low organic matter content (1.1 %), low cationic exchange
167 capacity (9.32 meq 100 g⁻¹), low available potassium and high available
168 phosphorus levels. The irrigation water used had an electrical conductivity of
169 between 0.8 and 1.0 dS m⁻¹. The Cl⁻ concentration in the irrigation water ranged
170 from 62 to 70 mg l⁻¹ during the experimental period.

171 In 2010, from DOY 210 to 294 (from the beginning of the second half of
172 rapid fruit growth period to the beginning of leaf fall), only over irrigated plants
173 (T0) were considered. In order to guarantee non-limiting soil water conditions,

174 control plants (treatment T0) were irrigated above crop water requirements (107
175 % ETo), using three emitters (each delivering 4 l h⁻¹) per plant. Total water
176 amount applied during the experimental period to T0 plants was 414 mm.

177 During both experiments pest control and fertilization practices were
178 those usually used by local growers, and no weeds were allowed to develop
179 within the orchard. Irrigation was carried out daily and during the night using a
180 drip irrigation system with one lateral pipe per tree row.

181

182 *2.2. Measurements*

183 Micrometeorological data, namely air temperature, solar radiation, air relative
184 humidity, rainfall and wind speed 2 m above the soil surface, were collected
185 every 15 min by automatic weather stations located near the experimental sites.
186 Mean daily air vapour pressure deficit (VPD_m) and daily crop reference
187 evapotranspiration (ETo) were calculated according to Allen et al. (1998).

188 Midday (12 h solar time) stem water potential (Ψ_{stem}) was measured on
189 the south facing side and the middle third of the trees, in two fully developed
190 leaves per tree of each replicate, enclosing leaves in small black plastic bags
191 covered with aluminium foil for at least 2 h before measurements in the
192 pressure chamber (model 3005, Soil Moisture Equipment Co., Santa Barbara,
193 CA, USA).

194 Midday leaf conductance (g) in attached leaves was measured with a
195 steady-state porometer (LI-1600, LICOR Inc., Lincon, USA) on the abaxial
196 surface of the leaves and in a similar number and type of leaves as used for the
197 Ψ_{stem} measurements.

198 The micrometric trunk diameter fluctuations (TDF) were measured
199 throughout the experimental periods in four trees per treatment, using a set of
200 linear variable displacement transducers (LVDT) (model DF \pm 2.5 mm, accuracy
201 \pm 10 μ m, Solartron Metrology, Bognor Regis, UK) attached to the trunk, with a
202 special bracket made of invar, an alloy of Ni and Fe with a thermal expansion
203 coefficient close to zero (Katerji et al., 1994), and aluminium. Sensors were
204 placed on the north side and were covered with silver thermoprotected foil to
205 prevent heating and wetting of the device. Measurements were taken every 2 s
206 and the datalogger (model CR10X with AM25T multiplexer, Campbell Scientific,
207 Logan, UT) was programmed to report 15 min means. Maximum daily trunk
208 shrinkage (MDS) was calculated as the difference between the daily maximum
209 diameter (reached early in the morning) and the minimum diameter (usually
210 reached in the afternoon).

211 For comparing the sensitivity of the above mentioned plant-based
212 indicators for use as water stress indicators, it is important to take into account
213 that the absolute values of these indicators are influenced not only by the soil
214 water availability but also by the evaporative demand, and consequently it is
215 more suitable to compare their values relative to those of the control trees (Naor
216 and Cohen, 2003). For this, the signal intensity of both continuous and discrete
217 plant water status measurements were defined as the relative values ($T1/T0$ or
218 $T0/T1$), while variability or noise was defined as the coefficient of variation of the
219 mean. Thus, the signal:noise ratio integrates both the indicator strength and its
220 variability, and is important for assessing the usefulness of plant-based water
221 stress indicators for irrigation scheduling (Moreno et al., 2006; Goldhamer et al.,
222 2000; Ortuño et al., 2004).

223

224 *2.3. Statistical design and analysis*

225 The design of the experiments was completely randomized with four
226 replications, each replication consisting of three adjacent tree rows, each with
227 eleven (2009) or thirteen (2010) trees. Measurements were taken on the inner
228 tree of the central row of each replicate, which were very similar in appearance
229 (leaf area, trunk cross sectional area, height, ground shaded area, etc.), while
230 the other trees served as border trees. Data were analyzed using SPSS
231 software (SPSS Inc., 2002). Analysis of variance was performed and mean
232 values were compared by an $LSD_{0.05}$ test. Values for each replicate were
233 averaged before the mean and the standard error of each treatment were
234 calculated. Linear regression analysis was carried out to explore relationships
235 between variables, and linear regression differences were determined using
236 covariance analysis.

237

238 **3. Results**

239 During the 2009 and 2010 experimental periods, average daily maximum and
240 minimum air temperatures were 32.5 and 20.2 °C and 28.9 and 16.3 °C,
241 respectively (Fig. 1). VPD_m ranged from 0.98 to 2.84 kPa in the 2009
242 experimental period and from 0.67 to 2.96 kPa in the 2010 experimental period
243 (Fig. 1), and accumulated ETo was 226 and 387 mm in the 2009 and 2010
244 experimental periods, respectively (Fig. 1). There was no rainfall during the
245 2009 experimental period, but in the 2010 experimental period total rainfall was
246 51 mm, which took place mainly on DOY 214 (7 mm), 232 (20 mm) and 291 (6
247 mm) (Fig. 1).

248 Ψ_{stem} values in control (T0) plants were high and quite constant, ranging
249 between -0.73 and -0.98 MPa and between -0.76 and -0.92 MPa during the

250 2009 and 2010 experimental periods, respectively (Figs. 2A and 3A). During the
251 2009 water withholding period, Ψ_{stem} values in T1 plants gradually declined, the
252 decrease started to be significant from DOY 212 onwards and reaching
253 minimum values at the end of the stress period. When plants were rewatered,
254 Ψ_{stem} values gradually increased, reaching similar values to those of the T0
255 plants at the end of the measurement period (Fig. 2A).

256 The g_i in T0 plants were high and showed fluctuations, especially during
257 the 2009 experimental period (Figs. 2B and 3A). In T1 plants, water deficit
258 caused a gradual decrease in g_i , the reduction started to be significant after ten
259 days and reaching minimum values at the end of the stress period, before
260 recovering when irrigation was renovated (Fig. 2B).

261 MDS values showed substantial fluctuations during the 2009
262 experimental period both in T0 and T1 plants (Fig. 2C). Differences between T0
263 and T1 treatments were evident as early as four days after the imposition of
264 water stress, due to the MDS increase in T1 plants (Fig. 2C). When T1 plants
265 were rewatered, MDS values fell and were similar in both treatments during the
266 recovery period. MDS values in T0 plants during the 2010 experimental period
267 fluctuated sharply before DOY 280, although from this day onwards, when
268 evaporative demand decreased (Fig. 1B), MDS values were low and quite
269 constant (Fig. 3B).

270 Taking into consideration the Ψ_{stem} values obtained during the 2009
271 experimental period and the MDS values taken at the same times, a polynomial
272 relationship between both parameters ($\text{MDS (mm)} = 0.419 + 0.979\Psi_{\text{stem}} \text{ (MPa)}$
273 $+ 1.118\Psi_{\text{stem}}^2 \text{ (MPa)} + 0.331\Psi_{\text{stem}}^3 \text{ (MPa)}$, $r^2 = 0.685$, $\text{MSE} = 0.003$) was evident
274 in the range of water stress studied (Ψ_{stem} values from -0.72 to -1.98 MPa)

275 (Fig. 4). This relationship was characterized by two different phases. Above
276 Ψ_{stem} values of -1.67 MPa, MDS values increased sharply as Ψ_{stem} decreased,
277 and when Ψ_{stem} values were below this threshold value the relationship changed
278 and any further reduction in Ψ_{stem} was associated with a decrease in MDS. Also,
279 despite the wider scatter, linear regression ($\text{MDS (mm)} = 0.056 - 0.168\Psi_{\text{stem}}$
280 (MPa), $r^2 = 0.587$, $\text{MSE} = 0.004$) may be a good approach for modeling the
281 relationship between Ψ_{stem} and MDS in the water stress range studied (Fig. 4).

282 For precise irrigation scheduling based on changes in the plant water
283 status, it is necessary to use plant-based water stress indicators able to develop
284 an immediate, consistent and reliable response to water deficit. As a
285 consequence, to compare the sensitivity of the measured indicators we looked
286 at MDS as a continuously recorded plant-based indicator and at Ψ_{stem} and g_i as
287 discretely measured plant-based indicators. In response to water stress the SI
288 values in the three considered plant-based water stress indicators increased,
289 with the particular characteristic that, at the beginning of the stress period, the
290 MDS and Ψ_{stem} SI (T1/T0) increased earlier than g_i SI (T0/T1), while MDS SI
291 values during the water stress period showed more pronounced oscillations
292 than the other indicators (Fig. 5). Moreover, when irrigation was restored, the SI
293 values sharply decreased, minimum values of around unity occurring during the
294 last days of the experiment (Fig. 5).

295 In a complementary manner, we studied the signal intensity, noise, and
296 signal:noise ratio for MDS, Ψ_{stem} and g_i during increasing intervals of time from
297 the beginning to the end of the stress period (Table 1). The data indicated that
298 at the beginning of the water stress period MDS, Ψ_{stem} and g_i (DOY 209-217)
299 presented similar mean SI and mean noise values. However, as the interval of

300 time considered grew (DOY 209-238 and DOY 209-243), the Ψ_{stem} SI was
301 significantly higher than those for MDS and g_l , while Ψ_{stem} and g_l average noise
302 values showed a tendency to be similar and higher than those observed in MDS
303 (Table 1). For these reasons, the MDS signal:noise ratio was the highest ratio
304 for all the intervals of time considered, indicating that the MDS is more sensitive
305 than the other indicators for detecting the plant water stress in our experimental
306 conditions.

307 During the 2009 and 2010 experimental periods, the observations of
308 MDS in trees under non-limiting soil water conditions correlated significantly
309 with meteorological variables measured on a whole-day basis (ET_o , VPD_m , T_m
310 and R_s) (Table 2). These baselines in pomegranate trees suggested that the
311 best predictor for MDS reference values is the ET_o because the relation
312 between both variables was the tightest, whereas the relation between MDS
313 and T_m presented the widest scatter (Table 2). Moreover, the covariance
314 analysis of the MDS seasonal baselines using ET_o , VPD_m , T_m and R_s as
315 independent variables showed that differences in slopes and intercepts
316 between both experimental seasons were not statistically significant. This
317 suggests that it is possible to evaluate MDS by means of a first-order fit and by
318 pooling data across both experimental seasons (Fig. 6). The pooled data over
319 both experimental seasons confirmed a tighter correlation for the regression of
320 MDS versus ET_o than those of MDS versus VPD_m , R_s and T_m (Fig. 6).

321

322 **4. Discussion**

323 The fact that Ψ_{stem} and g_l values in T0 plants were high throughout both
324 experimental periods (Figs. 2A and 3A) suggesting that control pomegranate

325 plants, despite being under non-limiting soil water conditions, never became
326 waterlogged. In this sense, water relations under flooding conditions are
327 characterized by a similar behaviour to those observed under water stress due
328 to chemical signals from roots and an increase in the resistance to water flowing
329 through the plant (Ruiz-Sánchez et al., 1996; Ortuño et al., 2007).

330 Ψ_{stem} values in T1 plants at the end of the water withholding period (Fig.
331 2A) and the stomatal regulation observed (Fig. 2B) pointed to a relatively strong
332 water stress situation. However, the fact that Ψ_{stem} values decreased at a rate of
333 around 0.025 MPa d^{-1} indicates that the water stress developed quite slowly
334 (Hale and Orcutt, 1987).

335 It is well known that water stress triggers hormonal changes in leaves,
336 such as an increase in abscisic acid and/or decrease in cytokinins, both
337 mechanisms that delay stomatal aperture after rehydration (Mansfield, 1987;
338 Davies and Zhang, 1991; Ruiz-Sánchez et al., 1997). In contrast, the speedy
339 recovery of g_l values in T1 plants when irrigation was restarted could indicate
340 that, at the level of water stress reached at the end of the irrigation withholding
341 period, the stomatal regulation achieved was not mediated by hormonal
342 changes in the leaf (Torrecillas et al., 1995; Mellisho et al., 2012).

343 Considering that the three measured plant-based water stress indicators
344 have different dimensions, it is not possible to evaluate their sensitivity using
345 their absolute values, and it is more meaningful to compare their values relative
346 to those of the control trees (Fig. 5) (Naor and Cohen, 2003). For this, and
347 bearing in mind that the strength of an indicator signal intensity must be seen in
348 the context of its variability, their signal:noise ratio was compared at different
349 time intervals (Table 1). The finding suggested that MDS is the most suitable

350 indicator for pomegranate irrigation scheduling, because its signal:noise ratio
351 was higher than that for Ψ_{stem} and g_l for all the intervals of time considered. In
352 this sense, Ortuño et al. (2004, 2006) showed that continuously measured plant
353 water status indicators were more immediate and sensitive than discretely
354 measured indicators. Moreover, Remorini and Massai (2003) indicated that
355 trunk diameter fluctuations differed between irrigation treatments even in the
356 absence of differences in Ψ_{stem} , and Goldhamer et al. (1999) indicated that MDS
357 responded sooner than Ψ_{stem} to water stress.

358 The changes in stem diameter are closely related to changes in water
359 content in the whole plant (Simonneau et al., 1993). For this, several authors
360 have observed that MDS values are a good indicator of transpiration intensity
361 when the soil water content is not strongly depleted, and increases in MDS
362 have been associated with decreases in water potential (Huguet et al., 1992;
363 Herzog et al., 1995). In accordance with these ideas, our results indicated that
364 the best fit between MDS and Ψ_{stem} was obtained with a polynomial regression
365 model (Fig. 4), which is the behaviour more frequent in most species and it is
366 characterized by the existence of a Ψ_{stem} threshold value (-1.67 MPa) below
367 which any further reduction in Ψ_{stem} is associated with a decrease in MDS
368 values (Ortuño et al., 2010; Moriana et al., 2000). However, Intrigliolo et al.
369 (2011b), suggested that the best fit between MDS and Ψ_{stem} in pomegranate
370 trees was obtained with a linear regression, and that there was not a single
371 unique relationship between both variables valid for the whole season due to
372 changes in fruit growth pattern and fruit removal. In this sense, our data also
373 revealed the possibility of presenting the relationship between MDS and Ψ_{stem}
374 by means of a first-order fit but the correlation obtained worsened respect to

375 that obtained with the polynomial regression model (Fig. 4). In our opinion, to
376 clarify the model that defines the relationship between both variables, a higher
377 number of MDS data point corresponding to Ψ_{stem} values below -1.67 MPa
378 would have been necessary.

379 The significant relations between MDS and ET_o , VPD_m , T_m and R_s in
380 pomegranate trees growing under non-limiting soil water conditions (Table 2,
381 Fig. 6) indicated that MDS, as well as reflecting tree water status, was clearly
382 influenced by weather conditions (Fernández and Cuevas, 2010; Ortuño et al.,
383 2010, 2009b; Conejero et al., 2011; Moriana et al., 2011). Moreover, the
384 possibility of obtaining these individual baselines or reference equations
385 between MDS and environmental variables confirmed the possibility of
386 obtaining MDS reference values to normalize the actual absolute values of MDS
387 with respect to those in non-limiting soil water conditions.

388 Taking into consideration that MDS reflects the continuous trunk
389 diameter records on a diurnal basis, being an integrative indicator could explain
390 its direct relation with climatic variables measured on a whole-day basis (ET_o ,
391 VPD_m , T_m and R_s) (Table 2, Fig. 6). However, it is difficult to explain why the
392 behaviour of MDS under non-limiting soil water conditions can be adequately
393 predicted by changes in T_m because it is known that temperature is not an
394 accurate indicator of the evaporative demand of the atmosphere (Hatfield and
395 Fuchs, 1990). Nevertheless, earlier reports in almond (Ferreeres and Goldhamer,
396 2003), plum (Intrigliolo and Castel, 2006) olive (Moreno et al., 2006) and lemon
397 (Ortuño et al., 2009b) showed that air temperature is a good predictor of MDS.
398 In any case, it must be considered that T_m is a climatic variable that is easier
399 and less costly to measure than ET_o , VPD_m and R_s . The fact that Intrigliolo et

400 al. (2011b) indicated that relationships between MDS and air temperature and
401 air vapour pressure deficit were weaker than that between MDS and ETo
402 confirmed that in pomegranate trees the best predictor for MDS reference
403 values is the ETo.

404 In spite of the existence of some differences in the location of
405 experimental plots and tree size, the inter-seasonal constancy in the reference
406 equations to estimate MDS as a function of ETo, VPD_m, T_m and Rs (Fig. 6) was
407 in agreement with the results showed by other authors in lemon and peach
408 trees (Ortuño et al., 2009b; Conejero et al., 2011). These authors showed the
409 constancy of MDS reference equations in trees with very different crop load
410 levels.

411 Overall, the results indicated that MDS is a reliable plant-based water
412 stress indicator in adult pomegranate trees. In addition, the fact that LVDT
413 sensors used in the experiment did not have to be repositioned, together with
414 other operational advantages over discretely measured indicators, such as the
415 low labour costs involved and the possibility of connection to remotely operated
416 automata, confirm that MDS is a suitable plant-based indicator for precise
417 irrigation scheduling practices. Moreover, MDS reference equations can be
418 obtained by pooling data across several seasons for ETo, VPD_m, T_m and Rs,
419 meaning that it is possible to compare MDS measurements at a given time with
420 the expected value under non-limiting water conditions, which can be calculated
421 from the reference relationships.

422

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428

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Table 1

Maximum daily trunk shrinkage (MDS), midday stem water potential (Ψ_{stem}) and midday leaf conductance (g_l) mean signal intensity, mean noise, and signal/noise ratio at different intervals of the 2009 water stress period. For each interval, mean signal or mean noise values that do not have a common letter are significantly different according to the $\text{LSD}_{0.05}$ range test.

DOY		Mean signal	Mean noise	Signal/noise
209-217	MDS	1.24a	0.17a	7.31
	Ψ_{stem}	1.27a	0.21a	6.16
	g_l	1.04a	0.15a	6.93
209-224	MDS	1.31ab	0.17b	7.84
	Ψ_{stem}	1.46a	0.23a	6.27
	g_l	1.15b	0.15b	7.73
209-231	MDS	1.37ab	0.17b	7.95
	Ψ_{stem}	1.59a	0.22ab	7.13
	g_l	1.29b	0.24a	5.34
209-238	MDS	1.41b	0.17b	8.24
	Ψ_{stem}	1.72a	0.23a	7.39
	g_l	1.40b	0.23a	6.07
209-243	MDS	1.46b	0.18b	8.31
	Ψ_{stem}	1.81a	0.24a	7.63
	g_l	1.49b	0.26a	5.82

Table 2

Intercept (a), slope (b), coefficient of determination (r^2), number of data points (n) and mean square error (MSE) of best fit first-order linear equations ($y = ax + b$) between maximum daily trunk shrinkage (MDS, mm) and selected environmental variables for 2009 and 2010 seasons

Season	a	b	r^2	n	MSE
<i>MDS vs. ET_o (mm)</i>					
2009	-0,0841	0,0557	0,7519 ^{***}	49	0,0009
2010	-0,068	0,0519	0,7482 ^{***}	85	0,0017
<i>MDS vs VPD_m (kPa)</i>					
2009	0,0329	0,1001	0,598 ^{***}	49	0,0015
2010	-0,0249	0,137	0,6152 ^{***}	85	0,0025
<i>MDS vs T_m (°C)</i>					
2009	-0,2922	0,0192	0,4197 ^{***}	49	0,0021
2010	-0,2363	0,0181	0,5933 ^{***}	85	0,0027
<i>MDS vs R_s ($W m^{-2}$)</i>					
2009	-0,0615	0,001	0,665 ^{***}	49	0,0012
2010	-0,0739	0,001	0,6024 ^{***}	85	0,0025

ET_o daily crop reference evapotranspiration, T_m daily mean air temperature, VPD_m daily mean air vapour pressure deficit, R_s solar radiation

*** Significant at $P < 0.001$

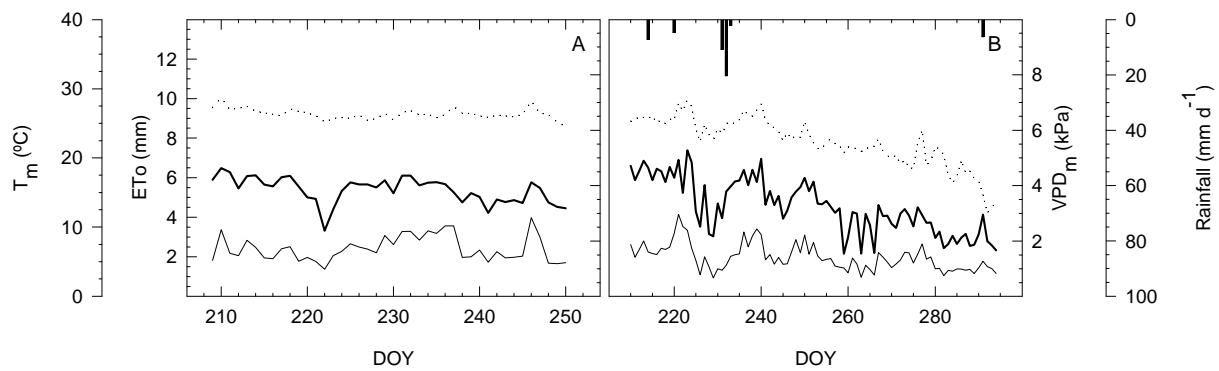


Fig. 1. Daily crop reference evapotranspiration (ET_o , solid thick line), daily mean air temperature (T_m , dotted line), mean daily air vapour pressure deficit (VPD_m) (solid thin line) and daily rainfall (vertical bars) during the 2009 (A) and 2010 (B) experimental periods.

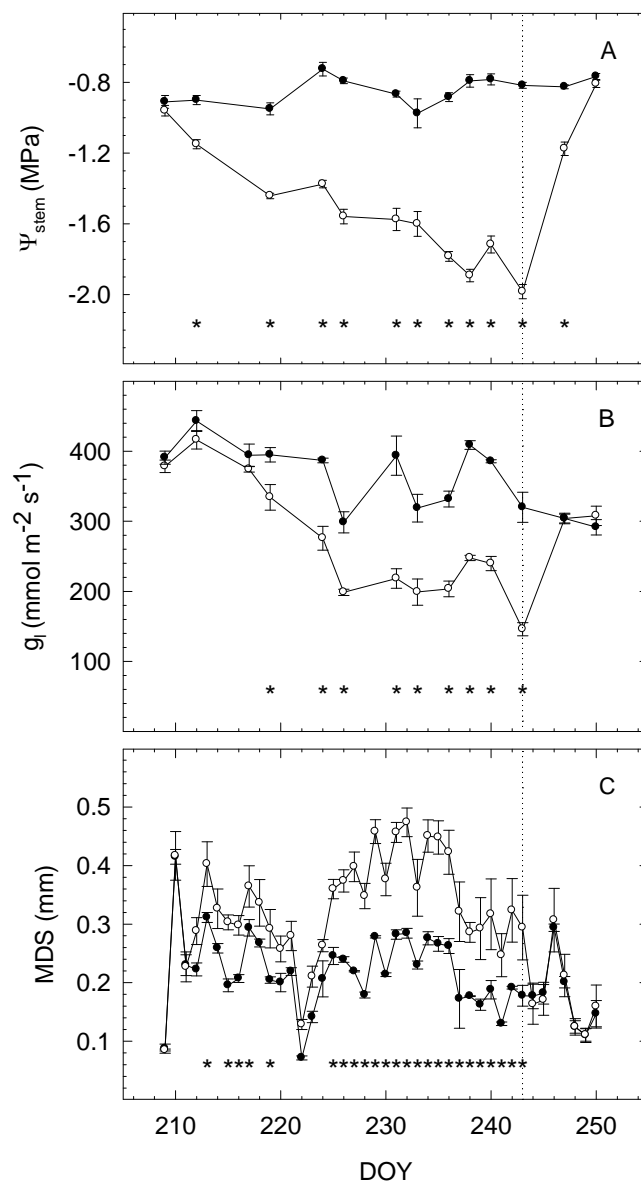


Fig. 2 Midday stem water potential (Ψ_{stem}) (A), midday leaf conductance (g_l) (B) and maximum daily trunk shrinkage (MDS) (C) in T0 (closed symbols) and T1 (open symbols) plants during the 2009 experimental period. Bars on data points are \pm S.E. of the mean (not shown when smaller than symbols). Vertical dotted line indicated the time at which irrigation was restored. Asterisks indicate statistically significant differences by least significant difference at 5% level ($\text{LSD}_{0.05}$) range test. Each point is the mean of four values

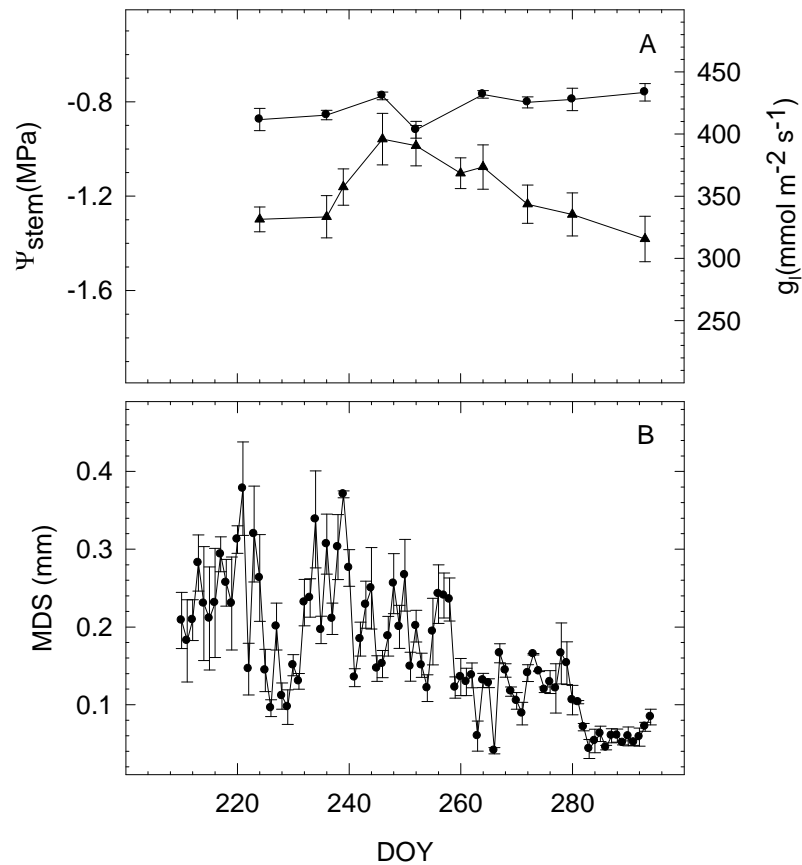


Fig. 3. Midday stem water potential (Ψ_{stem} , circles) (A), midday leaf conductance (g_l , triangles) (A) and maximum daily trunk shrinkage (MDS) (B) in T0 plants during the 2010 experimental period. Bars on data points are \pm S.E. of the mean (not shown when smaller than symbols). Each point is the mean of four values.

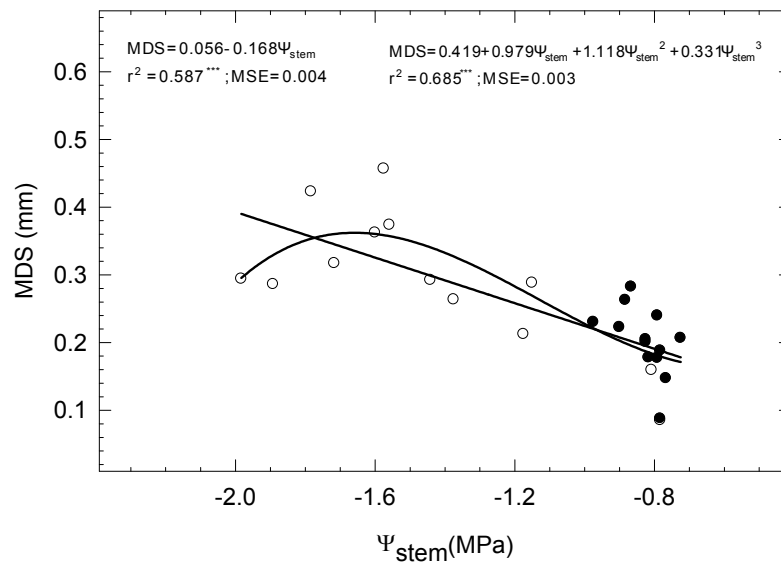


Fig. 4 Relationship between and maximum daily trunk shrinkage (MDS) and stem water potential (Ψ_{stem}) in T0 (closed symbols) and T1 (open symbols) plants during the 2009 water stress period. Each value is the mean of four measurements

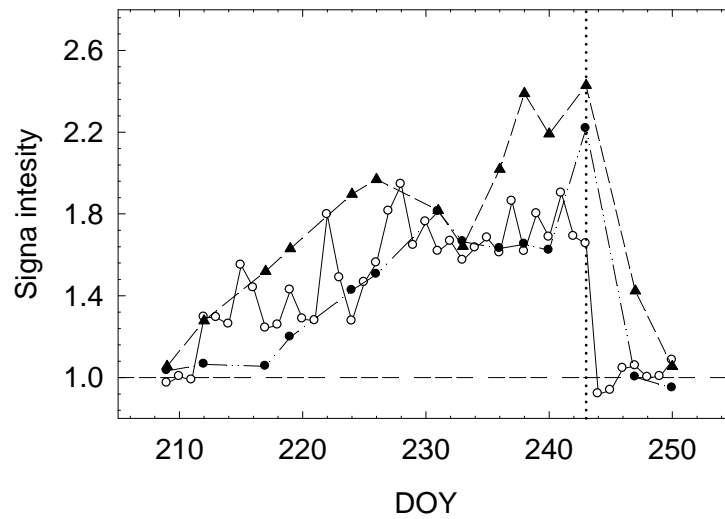


Fig. 5 Maximum daily trunk diameter shrinkage (MDS, open circles), midday stem water potential (Ψ_{stem} , triangles) and midday leaf conductance (g_l , closed circles) signal intensities during the 2009 experimental period. Each value is the mean of four measurements. Vertical dotted line indicated the time at which irrigation was restored

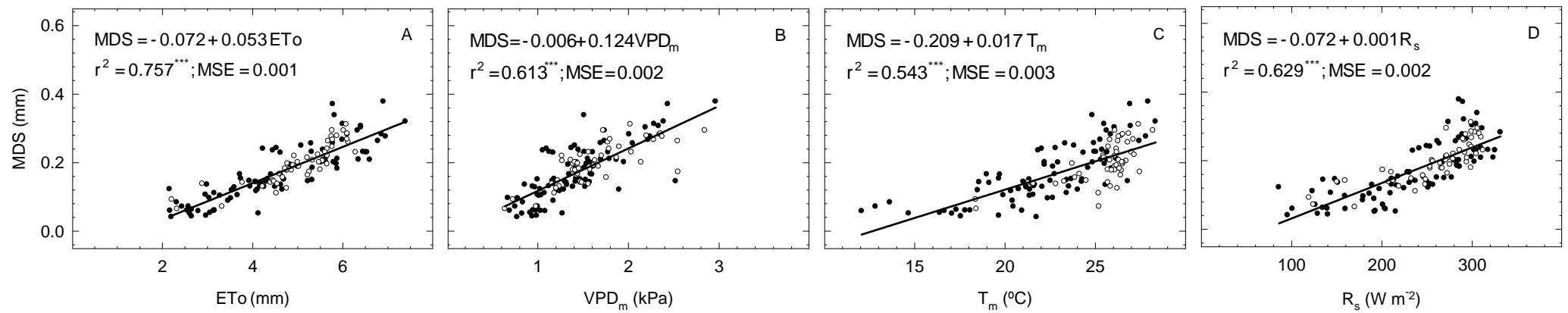


Fig. 6 Relationships for trees under non-limiting soil moisture conditions (T0) between maximum daily trunk shrinkage (MDS) and daily values of reference crop evapotranspiration (ETo) (A), mean daily air vapour pressure deficit (VPD_m) (B), mean daily air temperature (T_m) (C) and solar radiation (R_s) (D), using all data pooled (2009, open circles; 2010, closed circles). Each data point is the mean of four values