

1 (Review paper)

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3 **Plant-based sensing to monitor water stress: applicability to commercial orchards**

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54 **Abstract**

55

56         Despite their potential for water stress monitoring, sap flow (SF), trunk diameter  
57 variation (TDV) and leaf turgor pressure (LTP) related measurements are rarely used in  
58 commercial orchards. The reasons for this lack of popularity are analysed here, as well  
59 as possible solutions for the identified limitations. I worked with data collected from  
60 different olive orchards as well as with findings from the literature reported for other  
61 fruit tree species. SF sensors are difficult to install but easy to maintain. TDV sensors  
62 are easier to install, but required greater maintenance. Both methods are highly  
63 demanding in terms of data processing, especially sap flow. The usefulness of SF  
64 records for monitoring water stress is curtailed on recovery periods, due to the delayed  
65 recovery of stomatal conductance. TDV records, on the other hand, depend on plant  
66 water status, but also on plant age, phenological stage and crop load, among other  
67 factors. For correct data interpretation, therefore, a deep understanding of the response  
68 of the monitored variable to plant and environmental conditions is required. For LTP  
69 related measurements we used ZIM probes. They showed to be easy to install and use,  
70 and robust enough to withstand field conditions for long irrigation seasons. Severe  
71 water stress, however, limited their performance. New approaches are being developed  
72 to increase the potential of the tested methods for being used in commercial orchards.  
73 These includes combining the plant-based methods with remote imagery, deriving more  
74 user-friendly water stress indices from the collected records and hiring the services of  
75 specialized companies which provides the user with easy-to-interpret summaries of the  
76 collected information. With the help of new tools and applications, and the hiring of  
77 specialized companies if required, the assessed plant-based methods can be reliable and

78 profitable tools for monitoring water stress and scheduling irrigation in commercial  
79 orchards.

80

## 81 **1. Introduction**

82

83         The need for a sustainable water use in agriculture has impelled the scientific  
84 community to develop new methods for monitoring water stress. Special interest has  
85 been paid to plant-based methods, since plant measurements have the advantage of  
86 integrating the soil and atmospheric water status, as well as the response of the plant to  
87 the surrounding conditions (Jones, 2004). Conventional plant-based methods to monitor  
88 water stress, such as those based on the use of Scholander-type chambers, are  
89 destructive and time and labour consuming. In the last decades, however, new methods  
90 have been developed for non-destructive, automatic and continuous measurements,  
91 easily implemented with data transmission systems for the user to have nearly real time  
92 access to the collected data from a remote computer, smart phone or similar. Most of  
93 these methods and related systems are highly sensitive and capable of working under  
94 field conditions for long periods of time. These characteristics confer them a great  
95 potential both for monitoring water stress and scheduling irrigation in commercial  
96 orchards (Fernández and Cuevas, 2010).

97         The new plant-based methods are based on a wide range of variables.  
98 Measurements of stem electrical conductivity with TDR probes inserted in the trunk  
99 (Nadler and Tyree, 2008; Nadler et al., 2008) and of electric potential differences  
100 between plant tissues (Gurovich and Hermosilla, 2009; Oyarce and Gurovich, 2011) can  
101 be used to monitor plant water status. The temperature of a fraction (Akkuzu et al.,  
102 2010; Çamoğlu 2013) or the whole canopy (Ben-Gal et al., 2010, Agam et al., 2013) has

103 also proven to be a useful indicator of water stress for a variety of fruit trees, and  
104 airborne thermal images are being used to assess the heterogeneity in water status in  
105 commercial orchards (Zarco-Tejada et al., 2009; Gonzalez-Dugo et al., 2013). The most  
106 widely studied plant-based methods, however, are those based on measurements or  
107 estimations of sap flow (SF), trunk diameter variation (TDV) and leaf turgor pressure  
108 (LTP). Sap flow methods have a potential for in situ determinations of plant water  
109 consumption and transpiration dynamics ([www.wgsapflow.com](http://www.wgsapflow.com)). Comparisons between  
110 several stress-related indices derived from SF records and other water stress indicators  
111 have been made (Escalona et al., 2002; Ortuño et al., 2006; Intrigliolo and Castel,  
112 2006), and the potential of SF-related measurements to schedule irrigation has been  
113 explored (Fernández et al., 2001, 2008a,b). The usefulness of TDV records both for  
114 monitoring water stress and scheduling irrigation has been evaluated for a great number  
115 of species (Fernández and Cuevas, 2010; Ortuño et al., 2010), and comparative studies  
116 between TDV and other water stress indicators have also been made (Ehrenberger et al.,  
117 2012a; Cuevas et al., 2013). More recently, the leaf patch clamp pressure probe or ZIM-  
118 probe (Zimmerman et al., 2008) is being used to monitor water stress from estimations  
119 of relative changes in LTP (Fernández et al., 2011a; Bramley et al., 2013).

120 Several irrigation protocols based on the three methods mentioned above have  
121 been suggested and some of them have been successfully tested (Fernández et al.,  
122 2008a; Ortuño et al., 2010; Zimmermann et al., 2013). However, and although the  
123 methods are being widely used in research, their use with commercial purposes, i.e. to  
124 improve irrigation in commercial orchards, is much lower. Some believe that the new  
125 plant-based methods based on automatic and continuous recording are superior to  
126 conventional water stress indicators, and that they will become popular when prices of  
127 the sensors and related systems decrease. Others, however, find these methods too

128 complicated to install and maintain, and their records too difficult to interpret, to be  
129 used in commercial orchards. No detailed studies, however, have been published on the  
130 reasons for these methods not being adopted by farmers and orchardists. I hypothesized  
131 that methods based on SF, TDV and LPT records are useful both for monitoring water  
132 stress and scheduling irrigation in commercial orchards, provided they are used in  
133 combination with new technologies and approaches that make them inexpensive and  
134 user-friendly. When used alone, or by non-trained users, they are expensive and errors  
135 can easily arise from difficulties on installation and maintenance, as well as on the  
136 analysis and interpretation of the data.

137 To test our hypothesis, in this work I review the potential of SF, TDV and LTP  
138 related records to monitor water stress in commercial orchards. I first identify main  
139 limitations on the performance of each method imposed by the plant response to  
140 environmental water conditions. I then address difficulties to the requirements of each  
141 method for installation, maintenance, and data processing and interpretation. Eventually  
142 I explore solutions that are being developed to overcome such limitations.

143

## 144 **2. Experimental details and data collection**

145

146 I have reviewed main findings on the use of SF, TDV and LPT related  
147 measurements reported by other authors for a variety of species and conditions. In  
148 addition, most of the figures and experimental approaches commented below are  
149 derived from the work on the topic made by my research group. We worked at three  
150 different olive (*Olea europaea* L.) orchards, all within a radius of 30 km from Seville,  
151 southwest Spain. La Hampa orchard had ‘Manzanilla de Sevilla’ trees (from now on  
152 ‘Manzanilla’) planted at 7 m × 5 m in 1969 (Cuevas et al., 2010). La Nava orchard had

153 'Arbequina' trees planted at 7 m × 6 m in 1998 (Fernández et al., 2011b). In both cases  
154 trees had a round canopy and a single trunk with 2-4 main branches from 0.7 to 1.2 m  
155 above ground. The Sanabria orchard had 'Arbequina' trees planted at 4 m × 1.5 m in  
156 2007 (Fernández et al., 2013). In this case the trees had a monoconic canopy, with a  
157 single trunk and main branches from 0.6-0.7 m above ground. Both at La Hampa and La  
158 Nava the soil had a useful depth of 1.5-2 m, and a moderate-to-high water retention  
159 capacity. At the Sanabria orchard the soil was sandy, with a low soil water retention  
160 capacity and a maximum depth of 0.6 m. Climate in the area is Mediterranean, with a  
161 wet, mild season from October to April and a dry, hot season from May to September.  
162 Average potential evapotranspiration ( $ET_0$ ) and rainfall ( $R$ ) are ca. 1250 mm and ca. 500  
163 mm, respectively. The three orchards were irrigated during the dry seasons, normally  
164 from May to early September (La Hampa) or late October (La Nava and Sanabria). Drip  
165 irrigation was applied in all cases, with one lateral per tree row and 4-5 drippers per  
166 tree. A variety of irrigation strategies were tested in the orchards, as detailed in the  
167 mentioned publications. Basically, we applied several deficit irrigation (DI) strategies,  
168 including low frequency irrigation (LFI), sustained deficit irrigation (SDI) and regulated  
169 deficit irrigation (RDI). We also had fully irrigated trees, with daily water supplies to  
170 replace the crop evapotranspiration ( $ET_c$ ) minus the effective rainfall.

171         Details on water stress measurements at the three orchards are also given in the  
172 literature. Basically, SF measurements were made with the Green's heat-pulse velocity  
173 system (Green et al., 2003; Fernández et al., 2006), TDV records were collected with  
174 linear variable displacement transducers (Fernández et al., 2011c) and Plantsens radial  
175 dendrometers (Fernández et al., 2011b), and LTP related measurements were made with  
176 ZIM-probes (Fernández et al., 2011a). Scholander-type pressure chambers were used to  
177 assess plant water status, and gas exchange measurements were made with portable gas

178 analysers. Both frequency domain and time domain sensors were used to monitor soil  
179 water status. The collected values of volumetric soil water content ( $\theta_v$ ) were used to  
180 calculate the relative extractable water (REW), i.e. a value between 0 and 1 for which  
181 the actual water content was expressed relative to the difference between the minimum  
182 soil water content measured during the experiments (0) and the soil water content at  
183 field capacity (1).

184

### 185 **3. Limiting factors to the use of plant-based indicators in commercial orchards**

186

187 As reported in the Introduction, the limited acceptance of SF, TDV and LTP  
188 related measurements to improve water use in commercial orchards could be related to  
189 sensors and related equipment being expensive, and to the high time and training  
190 requirements for installing and maintaining the systems, and for processing and  
191 interpreting the collected data. Sometimes, however, the limitation is not in the method,  
192 but in a poor understanding of its fundamentals and applicability. In this section I warn  
193 potential users on the need for understanding the impact of main morphological and  
194 physiological features of the instrumented species on the collected data, and on the  
195 importance of choosing the right index for obtaining reliable information. Limitations  
196 related to installation and maintenance of the systems are also discussed. In Section 4 I  
197 address different approaches that can be used to minimize those drawbacks.

198

#### 199 *3.1. Characteristics of the instrumented plant*

200

201 In addition to the soil water status and atmospheric conditions, the species, age,  
202 nutritional status, phenological stage, crop load and other of characteristics of the



203 instrumented plant affect the performance of plant-based water stress indicators. There  
204 is abundant literature on the topic, for SF (Fernández et al., 2008a), TDV (Fernández  
205 and Cuevas, 2010; Ortuño et al., 2010) and LTP (Ehrenberger et al., 2012b; Bramley et  
206 al., 2013). The same can be said for conventional water stress indicators such as those  
207 based on plant water status and gas exchange measurements (Jones, 2004; Naor, 2006;  
208 Moriana et al., 2012; Naor et al., 2013).

209         Concerning the species, it has been reported that TDV records are more  
210 informative of the plant water status in peach, for example, than in olive or grapevine  
211 (Fernández and Cuevas, 2010). The species also conditions the method for SF  
212 measurements. Thus, while heat-balance methods can be suitable for trunks of reduced  
213 diameter with thin barks, invasive heat-pulse methods are required for measurements in  
214 large diameter trunks with thick, insulating barks (Vandegehuchte and Steppe, 2013).  
215 ZIM-probes may be difficult to use if species with rough leaves, since a close contact of  
216 the probe with the leaf surface is required for reliable measurements (Zimmerman et al.,  
217 2008). Plant age and size also influence TDV records, since phloem thickness and trunk  
218 growth rates, among other factors, change with both variables (Fernández and Cuevas,  
219 2010). Both SF and LTP records, which are less related to trunk growth, are less  
220 affected by plant age and size. The performance of a water stress indicator can also be  
221 markedly affected by phenology. This has been reported when analysing trunk growth  
222 rates in olive (Pérez-López et al., 2008; Moriana et al., 2013) and grapevine (Intrigliolo  
223 and Castel, 2007). It is known that TDV records depend not only on water status but  
224 also on carbon status (Flore and Layne, 1997; Intrigliolo and Castel, 2004) so it is not  
225 surprising that the performance of TDV-derived indices depends on crop load and fruit  
226 development. From an experiment in olive, both the transpiration and tree's capacity to  
227 take up water from the soil increased with crop load (Naor et al., 2013). However, the

228 relationships between the stem water potential ( $\Psi_{\text{stem}}$ ) and stomatal conductance ( $g_s$ )  
229 were different for trees with different crop loads. These, and other examples mentioned  
230 in the literature, advise for a careful consideration of the plant characteristics when  
231 using plant-based measurements for water stress monitoring.

232

### 233 3.2. *Plant response to water stress conditions*

234

235 The performance of any plant-based water stress indicator depends on  
236 morphological and physiological adaptations of the plant to water conditions. For a  
237 correct data interpretation, the user must be aware of the impact of the mechanisms  
238 governing those adaptations on the collected records. Below I give examples on the  
239 extent at which both adaptive features of plants and changes in the environment may  
240 affect the performance of water stress indicators.

241

#### 242 3.2.1 *Osmotic adjustment*

243

244 The potential of combining SF and TDV records to monitor water stress was  
245 assessed by Fernández et al. (2011c) at La Hampa orchard. They work with fully  
246 irrigated (FI) trees and trees under deficit irrigation (60DI). The latter received  
247 decreasing irrigation amounts until day of year (DOY) 199. From that day on, a  
248 recovery irrigation strategy was applied. In total, the FI trees received 107% of  $ET_c$  and  
249 the 60DI trees 61% of  $ET_c$ . Figure 1 shows the seasonal courses of two water stress  
250 related indices,  $D_{\text{MXTD}}$  and  $D_{Ep}$ , derived from TDV and SF measurements in  
251 representative trees of both treatments. Values of  $D_{\text{MXTD}}$  were calculated as the daily  
252 difference between the maximum trunk diameter (MXTD) recorded in the 60DI trees

253 and the MXTD recorded in the FI trees. The  $D_{E_p}$  values were calculated as the daily  
254 difference between the plant transpiration ( $E_p$ ) derived from SF records in the 60DI  
255 trees and the  $E_p$  value derived from the SF records in the FI trees. In the figure the time  
256 courses of  $D_{MXTD}$  and  $D_{E_p}$  are plotted against REW values in the root zone of the trees  
257 (Fig. 1A). The figure shows that the  $D_{MXTD}$  index responded to soil water depletion in  
258 the DI60 treatment from DOY 189, when  $REW \approx 0.5$ . The  $D_{E_p}$  index, however, showed  
259 a similar trend all along the experimental period, with no response to soil water  
260 depletion. The authors reported that the great capacity of the olive tree to take up water  
261 from drying soils was behind the lack of response of  $D_{E_p}$  to the decreasing soil water  
262 content, and that that feature certainly curtailed the usefulness of the  $D_{E_p}$  index for  
263 assessing crop water needs in commercial orchards. They also stated that the  
264 simultaneous recording of  $D_{E_p}$  and  $D_{MXTD}$  could overcome such limitation. This,  
265 however, could be acceptable for research purposes, but having two different methods  
266 for monitoring water stress will be difficult to afford in most commercial orchards.

267 The described results show that the high capability of the olive tree to take up  
268 water from drying soils curtails the earliness of  $D_{E_p}$ , i.e. delays its response to the onset  
269 of water stress. One of the physiological mechanisms responsible for that behaviour is  
270 osmotic adjustment. The plant transpiration is given by the equation  $E_p = (\Psi_s - \Psi_l) / R_p$   
271 [Eq. 1], where  $\Psi_s$  is the ‘effective’ soil water potential at the root surface,  $\Psi_l$  is the  
272 ‘effective’ leaf water potential for the whole canopy and  $R_p$  is the plant hydraulic  
273 resistance (Jones, 1983). A minimum  $\Psi_s - \Psi_l$  gradient ( $\Delta \Psi$ ) is required for water to  
274 travel from roots to leaves and the plant to transpire. The  $\Psi_s$  value is given by the soil  
275 water and solute status, but the  $\Psi_l$  value can be adjusted, to some extent, by the plant.  
276 Thus,  $\Psi_l$  depends on the turgor potential ( $\Psi_p$ ) and on the osmotic potential ( $\Psi_\pi$ ), such  
277 that  $\Psi_l = \Psi_p - \Psi_\pi$ . The olive tree, as many other species of arid and semi-arid areas, is

278 able to adjust  $\Psi_{\pi}$  under soil drying conditions, which leads to high values of  $\Delta\Psi$   
279 (Fernández, 2014). This physiological feature typical of the species contributes to  
280 explain the low water stress levels reported for olive trees growing in drying soils,  
281 especially in the case of old trees with large root zones (Fernández et al., 1997; Cuevas  
282 et al., 2010). These results are in agreement with the  $\Psi_s$  value for permanent wilting  
283 point in olive being much lower than -1.5 MPa, the accepted permanent wilting point  
284 for most species. Actually, measurable transpiration and photosynthesis activities have  
285 been detected in olive plants at  $\Psi_s < -5$  MPa (Moriana et al., 2003; Perez-Martin et al.,  
286 2009).

287         The user of water stress indices calculated from SF measurements, such as  $D_{Ep}$ ,  
288 must bear in mind that the earliness of the index could be markedly curtailed when used  
289 in plants with an outstanding capacity to take up water from drying soils, such as olive.  
290 This applies mainly to plants growing in soils of medium to high soil water retention  
291 capacity. In cases of trees with small root zones growing in soils with low water-holding  
292 capacities, this limitation could be negligible. In an experiment at the Sanabria orchard  
293  $D_{Ep}$  and  $D_{MXTD}$  values were derived from measurements in 4-year-old ‘Arbequina’  
294 trees. Those had very small root zones, of ca. 0.12 m<sup>3</sup>, and the soil had a water retention  
295 capacity of 66 mm only. Under these conditions  $D_{Ep}$  responded slightly earlier than  
296  $D_{MXTD}$  to the onset of water stress (Cuevas et al., 2013).

297

298                     Figure 1 about here

299

300

301

302

### 303 3.2.2. Root-to-shoot signalling

304

305 The capability of a plant to take up water from the soil depends not only on  
306 water availability, but also on water distribution. Figure 2 shows data from an  
307 experiment at La Hampa orchard, when trees were 39 years old (Torres-Ruiz, 2012).  
308 Three water treatments were considered: rain-fed, with negligible rainfall events during  
309 the period shown in the figure; drip irrigation, with trees drip irrigated daily from May  
310 14 to October 2 with enough water to replace  $ET_c$ ; pond irrigation, with trees irrigated  
311 with a grid of pipes covering a surface of  $8\text{ m} \times 6\text{ m}$ , with a dripper every  $0.4\text{ m} \times 0.4$   
312 m. The tree was in the middle of the grid and irrigation supplies were enough to keep  
313 non-limiting soil water conditions in the whole root zone, all throughout the irrigation  
314 season. In the Drip trees part of the roots remained under soil drying conditions during  
315 the irrigation season, since a fraction of the root zone only was wetted by the localized  
316 irrigation. In a parallel experiment we had 2-year-old ‘Manzanilla’ trees growing in  
317 pots. Each tree was planted in the middle of two 50 L pots, with about half of the root  
318 system within each pot. This allowed for reproducing the water treatments applied in  
319 the field. Data shown in Fig. 2 refers to the diurnal courses of  $\Psi_l$  and  $g_s$  measured at the  
320 end of the dry season, both in field (Fig. 2A,B) and potted (Fig. 2C,D) trees. While no  
321 differences in  $\Psi_l$  were found between Drip and Pond trees, either for field and potted  
322 conditions, significant differences in  $g_s$  were recorded. This has been defined for olive  
323 as a near-isohydric behaviour (Cuevas et al., 2010). Leaf transpiration ( $E_l$ ) depends on  
324  $g_s$ , such that  $E_l = g_s D_{l-a} / 100P$  [Eq. 2], where  $D_{l-a}$  is the leaf-to-air vapour pressure  
325 deficit and  $P$  the atmospheric pressure. Figure 2, therefore, illustrates the capacity of  
326 olive to limit transpiration through stomatal closure, a water-saving strategy widely  
327 observed in plants well adapted to drought. Basically, the plant tries to keep hydraulic

328 functioning with minimum water consumption, by an effective control of transpiration  
329 through stomatal closure (Tognetti et al., 2009; Boughalleb and Hajlaoui, 2011). Thus,  
330 and although the responses of the stomata both to plant water status and environmental  
331 conditions are not well understood (Buckley, 2005), it seems that “stomatal guard cells  
332 respond by negative feedback to  $\Psi_p$ , which is related to  $\Psi_l$  and that to  $\Psi_x$  (xylem water  
333 potential). Thus, the plant can operate near the embolism threshold, generating the  
334 required  $\Delta\Psi$  for transpiration at the same time that the risk for excessive  $R_p$  is  
335 minimized” (Fernández, 2014).

336 For plants of several species growing in soils with a highly variable water  
337 distribution, it has been reported that stomatal closure is induced by chemical signals  
338 generated in roots remaining in drying soil (Wilkinson and Davies, 2002; Chaves et al.,  
339 2010). In addition, the overall hydraulic conductance around the roots is expected to be  
340 lower in plants with a fraction of their root zone remaining dry, e.g. the Drip trees in  
341 Fig. 2, than in plants with the whole root zone wetted by irrigation, e.g. the Pond trees.  
342 Hydraulic signals with a capacity to induce stomatal closure can also be generated  
343 (Tardieu and Davies, 1993). Other findings suggest that signals emanating from roots in  
344 contact with drying soil are likely to integrate both chemical and hydraulic signals, and  
345 that soil-drying-induced ABA signals arising in the shoot (upon receipt of signals from  
346 the root) are more important for the regulation of stomatal aperture than ABA generated  
347 in the roots (Wilkinson and Hartung, 2009). Whatever the mechanism involved, Fig. 2  
348 suggests that, for plants showing an isohydric or near-isohydric behaviour, transpiration  
349 rates are not always closely related to plant water status. Caution has to be taken,  
350 therefore, when using SF related measurements to monitor water stress in those types of  
351 plants. They can show a wide range of  $g_s$  values for similar water stress levels, which

352 may leads to poor agreement between SF and leaf or stem water potential values. This is  
353 explained in detail in the next section.

354 Figure 2 also shows that  $\Psi_1$  and  $\Psi_{\text{stem}}$  may not always reflect properly the effect  
355 of stressing conditions on  $g_s$ . For olive,  $g_s$  is closely related to the net CO<sub>2</sub> assimilation  
356 ( $A$ ) (Moriana et al., 2002; Naor et al., 2013), except for maximum values of  $g_s$  obtained  
357 under non-limiting conditions (Fernández et al., 2008c). It can be assumed, therefore,  
358 that  $\Psi_1$  and  $\Psi_{\text{stem}}$ , although being trusty indicators of plant water status, they may not  
359 always provide reliable information on the impact of water scarcity on crop  
360 performance. This leads to be cautious when using  $\Psi_{\text{stem}}$  vs.  $g_s$  and  $\Psi_{\text{stem}}$  vs. yield  
361 relationships already published for olive and other species with an isohydric or near-  
362 isohydric behaviour (Naor et al., 2004, 2013).

363

364 Figure 2 about here

365

366 *3.2.3. Recovery from water stress*

367

368 Understanding how the plant recovers from water stress is crucial for a rational  
369 water management, especially when certain DI strategies are used. This is the case of  
370 RDI, which implies drastic and sudden changes in water supplies and, therefore, periods  
371 of increasing water stress followed by re-watering periods. Olive roots are able to take  
372 up water from the soil immediately this is available, even if the tree has been under soil  
373 drying conditions for a long period (Fernández et al., 2001). Thus,  $\Psi_1$  recovers within a  
374 period of hours to a few days, depending both on the severity of the suffered water  
375 stress and on the amount of water supplied for re-watering. Both  $g_s$  and  $A$ , however,  
376 take longer to recover (Fernández et al., 1997; Perez-Martin et al., 2014). It has been

377 suggested that ABA accumulated in roots during drought is delivered to the leaves  
378 during rehydration, thus contributing to the slow recovery of gas exchange (Lovisolo et  
379 al., 2008). Also reported is a possible effect of hydraulic signals generated in the leaf on  
380 the down-regulation of stomatal conductance (Torres-Ruiz et al., 2014). Whatever the  
381 reason, the slow  $g_s$  recovery after re-watering of the olive tree induces reduced  
382 transpiration (Eq. 2).

383 Figure 3 illustrate this phenomenon. Data refers to measurements made at the  
384 Sanabria orchard, from a few days before resuming daily irrigation for the 60RDI trees  
385 at the end of August to a few days after. In July and August, a period of low sensitivity  
386 to water stress, the 60RDI trees were irrigated two days per week only, with a total  
387 supply on that period of 20-30% of the crop water needs. In 2010 it took nearly one  
388 month for  $g_s$  to recover, and in 2012 the  $g_s$  values of the 60RDI trees remained below  
389 those of the FI trees. Only in 2011  $g_s$  recovered a few days after resuming daily  
390 irrigation. In agreement with the  $g_s$  values, in 2010  $D_{Ep}$  did not reach values close to one  
391 until the full recovery of  $g_s$  (Fig. 3D). In 2011  $D_{Ep}$  values increased quickly, showing a  
392 full recovery of  $E_p$  in the 60RDI trees at some two weeks after the beginning of the re-  
393 watering phase (Fig. 3E). The dynamics of  $D_{Ep}$  in 2012 suggest that the  $E_p$  of the 60RDI  
394 trees did not recover (Fig. 3F). Fig. 3 shows, in fact, that the slow recovery of  $g_s$  in olive  
395 after re-watering may curtail the earliness and sensitivity of water stress indices derived  
396 from SF measurements, such as  $D_{Ep}$ .

397

398 Figure 3 about here

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401



#### 402 3.2.4. *Weather conditions*

403

404 Water stress indicators based on plant-based measurements are influenced by  
405 weather conditions (Jones, 2004; Naor, 2006). The influence is sometimes beyond the  
406 expected effect from certain meteorological conditions, such as the vapour pressure  
407 deficit and the incoming radiation, being main driving variables for the plant water  
408 consumption. On stormy days, for instance, both high air humidity and especially rain  
409 affects the swelling and shrinking of cortical tissues, to the point that TDV records  
410 become useless (Fernández and Cuevas, 2010). Concerning SF records, Figure 4  
411 illustrates how a marked and sudden decrease in atmospheric demand curtails de  
412 performance of  $D_{Ep}$ . The figure refers to an experiment at the Sanabria orchard (Cuevas  
413 et al., 2013). It shows the seasonal courses of atmospheric demand, soil water status and  
414 the  $D_{Ep}$  and  $D_{MXTD}$  indices for the irrigation season of 2010. High decreases of  $ET_o$  were  
415 recorded on the three days marked in the figure with vertical lines (Fig. 4A). The  $D_{Ep}$   
416 values increased from DOY 224 to DOY 229, when  $ET_o$  decreased. From DOY 230 to  
417 235 they decreased as  $ET_o$  increased (Fig. 4C). This was despite of non-significant  
418 changes in soil water content for any of the two considered irrigation treatments (Fig.  
419 4B). The  $D_{MXTD}$  values, however, were not affected by the changing atmospheric  
420 conditions (Fig. 4D). As suggested by Cuevas et al. (2013), a sudden decrease in  $ET_o$   
421 can have a stronger influence in the  $E_p$  of the FI trees than in that of the 60RDI trees,  
422 leading to peak  $D_{Ep}$  values non related to soil water conditions. This occurs when the  $E_p$   
423 of the 60RDI trees is already markedly reduced, as compared to that of the FI trees, by  
424 the lack of water in the soil. Based on these results, the authors stated that “an increase  
425 in  $D_{Ep}$  does not necessarily mean an improvement of the soil water status” and that “the  
426 user must take into account main peculiarities of the response of sap flow rates to

427 environmental conditions, for correctly interpreting the information provided by the  $D_{Ep}$   
428 index". This last statement applies, actually, to any water stress indicator relying on  
429 plant-based measurements.

430

431 Figure 4 about here

432

### 433 3.3. *Installation, maintenance and data processing*

434

435 Installation and maintenance of most plant-based water stress indicators require  
436 specific training. This applies also to both the processing of the collected records to  
437 derive an appropriate index for the orchard conditions and to the interpretation of the  
438 information provided by the index. Sap flow methods are particularly demanding in  
439 terms of installation. Most of the SF methods suitable for fruit trees are invasive, such  
440 that sensors must be installed within the trunk of the trees. For some methods this  
441 implies careful drilling and sensor insertion (Fernández et al., 2006). TDV methods are  
442 less demanding in terms of installation, since attaching holders and dendrometres to the  
443 trunk is relatively easy. The maintenance of TDV sensors, however, is more demanding  
444 than that of SF sensors. Dendrometres may be affected by weed growing, animals, and  
445 traffic in the orchard. In addition, dendrometres could have to be readjusted several  
446 times along the irrigation season, due to trunk growth (Fernández and Cuevas, 2010).  
447 Of the three methods considered here, the ZIM-probe is the less demanding in terms of  
448 installation and maintenance. The probes are easily attached to leaves, and can stand  
449 orchard conditions for many months (Fernández et al., 2011a).

450 In terms of data processing, SF and TDV records are collected with the help of  
451 dataloggers which, depending on the models, are more or less user-friendly. Sap flow

452 methods are based on time and temperature records provided by the system, which have  
453 to be analysed first to derive values of variables such as sap flux density ( $J_p$ ) and sap  
454 flux ( $Q$ ), and then to calculate different water stress indices (Fernández et al., 2001,  
455 2008a). Data processing is also required to calculate most of the water stress indices that  
456 can be obtained from TDV records (Fernández and Cuevas, 2010). ZIM-probes are  
457 connected to radio transmitters that send the collected information to a datalogger  
458 nearby. This automatically sends the collected data to a server belonging to the maker,  
459 which provides the user with a link to visualize, through the Internet, the  $P_p$  records in  
460 tables and figures. As for the other methods, different water stress indices can be  
461 obtained from the ZIM records, some of them requiring complex calculations  
462 (Fernández et al., 2011a; Bramley et al., 2013). In summary, the use of SF, TDV and  
463 LTP methods require specific training for analysing the collected information, which  
464 limits the suitability of these plant-based methods to monitor water stress in commercial  
465 orchards.

466         An additional complication relies on choosing the most appropriate index for the  
467 orchard conditions. For SF measurements I have already shown that  $D_{Ep}$  could be  
468 adequate for trees with small root zones growing in soils with low water retention  
469 capacity, but it lacks earliness when used in mature trees with large root systems,  
470 especially if belonging to species with effective control of transpiration through  
471 stomatal closure. In these cases, the effect of stressing conditions on stomatal closure,  
472 and therefore on water consumption, could be better monitored by the ratio between  $J_p$   
473 values derived from measurements deep into the xylem and those derived from  
474 measurements closer to the cambium (Fernández et al., 2008a). When using TDV  
475 records, it is widely accepted that the maximum daily shrinkage (MDS) is an  
476 appropriate water stress index for many species (Fernández and Cuevas, 2010). In olive,

477 however, the trunk growth rate could be most appropriate than MDS (Cuevas et al.,  
478 2010; Moriana et al., 2010). In addition, it is know that, for several woody species  
479 including olive, the MDS vs  $\Psi_{\text{stem}}$  relationship has a parabolic form, with decreasing  
480 slope from ca.  $\Psi_{\text{stem}} < -1.5$  MPa (Ortuño et al., 2010). Under severe water stress  
481 conditions, the information given by the  $P_p$  records taken with the ZIM probe is limited  
482 by the increase of air in the spongy mesophyll of the leaf, which attenuates the pressure  
483 transfer through the leaf tissues (Fernández et al., 2011a; Ehrenberger et al., 2012b).  
484 Caution should also be taken when using a water stress indicator such as  $\Psi_{\text{stem}}$  in plants  
485 showing isohydric behaviour, as detailed above. These examples advice for a careful  
486 choice of the water stress index.

487

#### 488 **4. Improving the applicability of plant-based indicators to commercial orchards**

489

490 What it is said above suggests that considerable training and expertise are  
491 required for using SF, TDV and LTP related measurements as water stress indicators. If  
492 this is added to sensors and related systems being not cheap, we could think of these  
493 methods as not suitable for monitoring water stress and scheduling irrigation in  
494 commercial orchards. New approaches, however, are being developed to decrease the  
495 impact of those limitations. These approaches, detailed below, make the systems less  
496 costly and easier to use, increasing their potential for use in commercial orchards.

497

##### 498 *4.1. Making the system inexpensive*

499

500 The suitability of any water stress indicator for its use in commercial orchards  
501 depends mainly on the tree-to-tree variability, signal strength, sensitivity, reliability and

502 robustness (Fernández and Cuevas, 2010). Other requirements for a water stress  
503 indicator to be used with commercial purposes are mentioned in Table 1. The tree-to-  
504 tree variability is quantified by the coefficient of variation (CV). High CV values mean  
505 that a greater number of trees must be instrumented to reduce the uncertainty or noise of  
506 the collected information. The variability must be considered relative to the signal  
507 strength, or signal intensity (SI). With the SI approach, the value of the index derived  
508 from measurements in the plants which water stress we want to monitor (actual value) is  
509 related to the value of the index derived from similar plants growing under non-limiting  
510 soil water conditions (reference value) (Goldhamer and Fereres, 2001). Thus, if the  
511 chosen index is MDS,  $SI = \text{actual MDS} / \text{reference MDS}$ . The use of reference plants  
512 accounts for the fact that absolute values of any plant-based water stress indicator mean  
513 little if not considered relative to similar measurements made in plants growing in soils  
514 with non-limiting available water. The sensitivity, or signal:noise ratio, relates CV and  
515 SI, such that  $\text{sensitivity} = SI / CV$ .

516         The SI approach has been widely used in research experiments in which water  
517 stress indices were derived from plant-based measurements (Fernández and Cuevas,  
518 2010; Ortuño et al., 2010). In most commercial orchards, however, the variability in  
519 soil, plant and microclimate conditions is high. Therefore, the achievement of  
520 acceptably low CV values, as required by the SI approach, implies either to instrument a  
521 large number of trees or to choose the trees to be instrumented from an area, within the  
522 orchard, with highly uniform water-stress characteristics. None of these two options is  
523 satisfactory, the first because it is expensive and the second because it provides biased  
524 information. The use of remote imagery may provide, however, an acceptable  
525 alternative to choose the trees to sample. Recent developments on remote imagery  
526 techniques, involving miniaturized cameras installed in GPS controlled, unmanned

527 aerial vehicles, allow for detailed, inexpensive infrared images of the orchard. These  
528 images can be used to divide the orchard into a small number of areas with contrasting  
529 water-stress characteristics (Gonzalez-Dugo et al., 2013; Jimenez-Bello et al., 2013).  
530 The trees to be instrumented can then be chosen within each area, such that a reduced  
531 number of trees may provide reliable information for the whole orchard. This approach  
532 allows for precise irrigation within the orchard, since the irrigation sectors can be  
533 reallocated to match the water requirements of each area. The combined use of plant-  
534 based measurements and remote imagery is, in fact, recommended for lowering the cost  
535 and increasing precision in plant-based water stress monitoring in commercial orchards  
536 (Fernández and Cuevas, 2010). Still, this approach will not avoid a certain tree-to-tree  
537 variability, since this also depends on the location of the sensor in the plant and other  
538 tree-dependent factors.

539

540 Table 1 about here

541

#### 542 *4.2. User-friendly data recording, processing and interpretation*

543

544 As mentioned in Section 3.3, calculating a water stress related index from SF,  
545 TDV or LTP records may be complex, which certainly limits the use of these methods  
546 in commercial orchards. But this is not always the case. The  $J_p$  ratio mentioned in  
547 Section 3.3 is based on changes in SF radial profiles, and does not require calculating  
548 absolute values of  $Q$  (Fernández et al., 2008a). This simplifies data processing when SF  
549 is the chosen method for water stress monitoring. If we use TDV, the collected records  
550 can provide useful information on the level of water stress in the sampled trees, without  
551 any data processing. This can be seen, for instance, in the work by Fernández and

552 Cuevas (2011) with ‘Arbequina’ trees under two irrigation treatments, LFI and SDI.  
553 They showed that the time course of the TDV records collected from the LFI trees  
554 allowed to identify low, moderate or severe water stress levels in the sampled trees (Fig.  
555 5).

556

557 Figure 5 about here

558

559 Similarly to the case of SF and TDV records, calculating reliable water stress  
560 indices from leaf patch output pressure ( $P_p$ ) values recorded with the ZIM-probe does  
561 not always implies complex calculations. It has been proved, for a variety of species,  
562 that the shape of the daily  $P_p$  curve indicates the level of water stress suffered by the  
563 instrumented plant. An example for olive is given in Figs. 6 and 7. Figure 6 shows three  
564 different shapes of the  $P_p$  curve, and the corresponding levels of water stress as deduced  
565 from concomitant measurements of  $\Psi_{\text{stem}}$  with a Scholander-type pressure chamber.  
566 Measurements were made in 2010 for assessing the potential of  $P_p$  records for  
567 monitoring water stress at the Sanabria orchard (Fernández et al., 2011a). The three  
568 levels of water stress shown in the figure were identified as States 1, 2 and 3, after  
569 Ehrenberger et al. (2012b). In 2011, the occurrence of States 1, 2 and 3 at the orchard  
570 was compared with the seasonal course of  $\Psi_{\text{stem}}$ . Measurements were made in trees with  
571 different levels of water stress, belonging to the FI, 60RDI and 30RDI treatments.  
572 Results showed that this approach, which can be automatically displayed in nearly real  
573 time via the Internet, was effective for identifying low (State 1), moderate (State 2) and  
574 severe (State 3) levels of water stress in the orchard (Fig. 7). This is a powerful tool for  
575 growers with little training to adjust both the timing and quantity of irrigation.

576 Additional algorithms to fine tune the water stress indices from  $P_p$  records and to  
577 facilitate data processing are being developed.

578

579                   Figures 6 and 7 about here

580

581           For certain orchard conditions, however, the most suitable index could not be  
582 one of the easily-derived indices mentioned above. In this case the orchardist still has  
583 the possibility of hiring the services of a specialized company which, in addition to  
584 installing the sensors and related equipment, collects and processes the data, and  
585 provides easy-to-interpret summaries through the Internet. The data analysis system of  
586 some of these companies includes weather forecast models which allows for an early  
587 adjustment of water supplies under changing weather conditions. In most cases the  
588 orchardist has to combine the information provided by these companies with in situ  
589 observations on phenological stage, crop load, shoot growth, and other crop  
590 characteristics related to water management. New data capture tools, such as digital  
591 pens, allow for an easy data collection and arrangement in spread data sheets, such that  
592 the additional information taken by the orchardist can be easily combined with that  
593 provided by the company. All these new tools and approaches increase the suitability of  
594 the new plant-based water stress indicators for commercial orchards.

595

## 596 **5. Conclusions**

597

598           The three plant-based methods evaluated in this work have a great potential for  
599 monitoring water stress and scheduling irrigation in commercial orchards. They allow  
600 for non-destructive, automatic and continuous data recording and are easily



601 implemented with data transmission systems for a nearly real time access to the  
602 collected records from a remote computer. These are great advantages for improving  
603 water use in modern agriculture. But the methods have also drawbacks that curtail their  
604 use by farmers and orchardists. Some are difficult to use and maintain, and the sensors  
605 and related systems are expensive. In addition, the standard user often lacks the required  
606 knowledge on the impact both of the species' main ecophysiological features and  
607 environmental conditions on the monitored variable required for a proper interpretation  
608 of the collected records. These limitations, however, can be successfully overcome with  
609 the help of new approaches that are being developed to facilitate the use of these  
610 methods in commercial orchards. First, the cost of implementing these methods can be  
611 reduced to acceptable limits by using remote imagery, which reduces the number of  
612 plants to be instrumented. It also allows for precision irrigation even in large orchards  
613 with high water-stress variability. Second, some of the water stress indices that can be  
614 derived from any of the three tested methods are easy to obtain and use, and new  
615 applications are being developed by manufacturers to decrease the complexity of data  
616 processing and interpretation. Finally, there are specialized companies that install and  
617 maintain the required equipment, and collect and interpret the data. These companies  
618 provide the user with online access to easy-to-interpret summaries both of the collected  
619 information and derived indices, at an affordable cost. Some of these companies have  
620 implemented weather forecast models that improve irrigation management under  
621 weather changing conditions. If combined with these new approaches, the plant-based  
622 methods evaluated here become useful tools for monitoring water stress and scheduling  
623 irrigation in commercial orchards.

624

625

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637

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842

843 **Fig. 1.** Time courses of (A) the relative extractable water (REW,  $\text{avg} \pm \text{SE}$ ,  $n = 7$ )  
844 derived from soil water measurements at La Hampa orchard, in the root zones of 38-  
845 year-old 'Manzanilla' olive trees under two water treatments. The fully irrigated (FI)  
846 trees were irrigated daily, with an average water supply for the whole irrigation season  
847 of 107% of the crop water needs ( $\text{ET}_c$ ). The deficit irrigated (60DI) trees received  
848 decreasing irrigation amounts until day of year (DOY) 199. From that day on, a  
849 recovery irrigation strategy was applied. The 60DI trees received a total of 61% of  $\text{ET}_c$ .  
850 Also shown are the values of two water stress indices,  $D_{\text{MXTD}}$  and  $D_{E_p}$ . The first (B) was  
851 calculated as the daily difference between the maximum trunk diameter (MXTD) of the  
852 60DI trees and that of the FI trees. The second (C) was calculated as the difference  
853 between the daily transpiration ( $E_p$ ) of the 60DI trees that of the FI trees. Both for  
854  $D_{\text{MXTD}}$  and  $D_{E_p}$ , the mentioned differences were calculated with the average of three  
855 trees per treatment, after considering the values recorded at the beginning of the  
856 irrigation season as zero. The  $D_{\text{MXTD}}$  index showed greater earliness and sensitivity than  
857  $D_{E_p}$  (After Fernández et al., 2011c).

858

859 **Fig. 2.** Diurnal courses of leaf water potential ( $\Psi_l$ ) and stomatal conductance ( $g_s$ ) in  
860 sunlit leaves of (A, B) 39-year-old 'Manzanilla' trees in the field and (C, D) 2-year-old  
861 'Manzanilla' trees in pots ( $\text{avg} \pm \text{SE}$ ,  $n = 6$ ). Measurements were made at La Hampa  
862 orchard, at the end of the dry season of 2007. Measurements were made in rain-fed trees  
863 and in trees under two irrigation treatments. LI trees received enough water to replace  
864 the crop water needs, but they were under localized irrigation such that part of the root  
865 system remained under soil drying conditions during the irrigation season. In the Pond  
866 trees, the whole rhizosphere was kept under non-limiting soil water conditions all  
867 throughout the irrigation season. Different letters indicate significant differences

868 between the two irrigated treatments at  $p < 0.05$ . No letter means no effect. GMT =  
869 Greenwich mean time. The data show a near-isohydric behaviour of the olive trees.

870

871 **Fig. 3.** Courses of (A, B, C) maximum stomatal conductance ( $g_{s-max}$ ,  $avg \pm SE$ ,  $n = 8$ )  
872 measured in ‘Arbequina’ olive trees of the Sanabria orchard. Trees were under a full  
873 irrigation (FI) treatment supplying 100% of the crop water needs ( $ET_c$ ) and a regulated  
874 deficit irrigation treatment supplying 60% of  $ET_c$  (60RDI). The shown periods  
875 correspond to a few days before and after the recovery irrigation applied to the 60RDI  
876 trees from the end of August, in three experimental years. In 2010 the trees were 4 year  
877 old. The arrows show the beginning of the re-watering periods. Different letters indicate  
878 significant differences between treatments at  $p < 0.05$ . No letter means no effect. Also  
879 shown are (D, E, F) the  $D_{Ep}$  values (see Fig. 1 for explanation) on the represented  
880 periods. DOY = day of year. The earliness of the  $D_{Ep}$  index was limited by the slow  
881 recovery of stomatal conductance typical in olive.

882

883 **Fig. 4.** Seasonal courses of (A) the FAO56 Penman-Monteith potential  
884 evapotranspiration ( $ET_o$ ) calculated with records from a weather station close to the  
885 orchard; (B) the relative extractable water (REW,  $avg \pm SE$ ,  $n = 8$ ) calculated from soil  
886 water measurements in the root zone of FI and 60RDI ‘Arbequina’ olive trees at the  
887 Sanabria orchard (see Fig. 3 for explanation on treatments). Different letters indicate  
888 significant differences between treatments at  $p < 0.05$ . No letter means no effect; (C) the  
889  $D_{Ep}$  and (D) the  $D_{MXTD}$  values (see Fig. 1 for explanation). Vertical lines within graphs  
890 indicate three days on which marked decreases in  $ET_o$  were recorded. The  $D_{Ep}$  index  
891 lacked reliability on these days (After Cuevas et al., 2013).

892

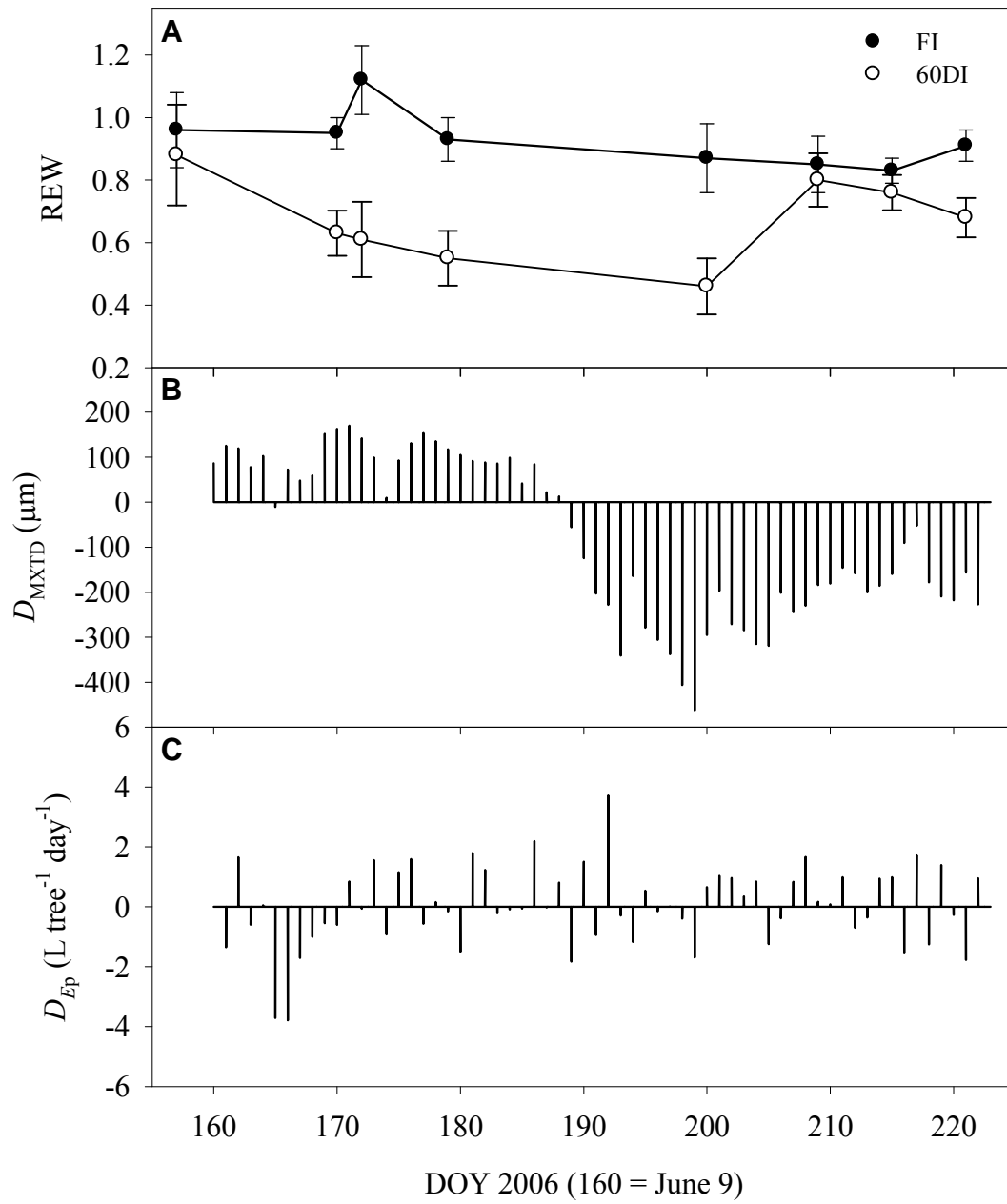
893 **Fig. 5.** Time courses of (A) the trunk diameter variation (TDV) recorded in 12-year-old  
894 ‘Arbequina’ olive trees at La Nava orchard, under sustained deficit irrigation (SDI) and  
895 low frequency irrigation (LFI). Also shown are (B) the time courses of the average  
896 relative extractable water (REW) calculated from soil water measurements in the root  
897 zone of four trees per treatment. Dashed lined limits the periods of recovery from water  
898 stress (R), moderate stress (MS) and severe stress (SE) as deduced from the TDV values  
899 recorded in the LFI trees. DOY = day of year. (After Fernández and Cuevas, 2011)

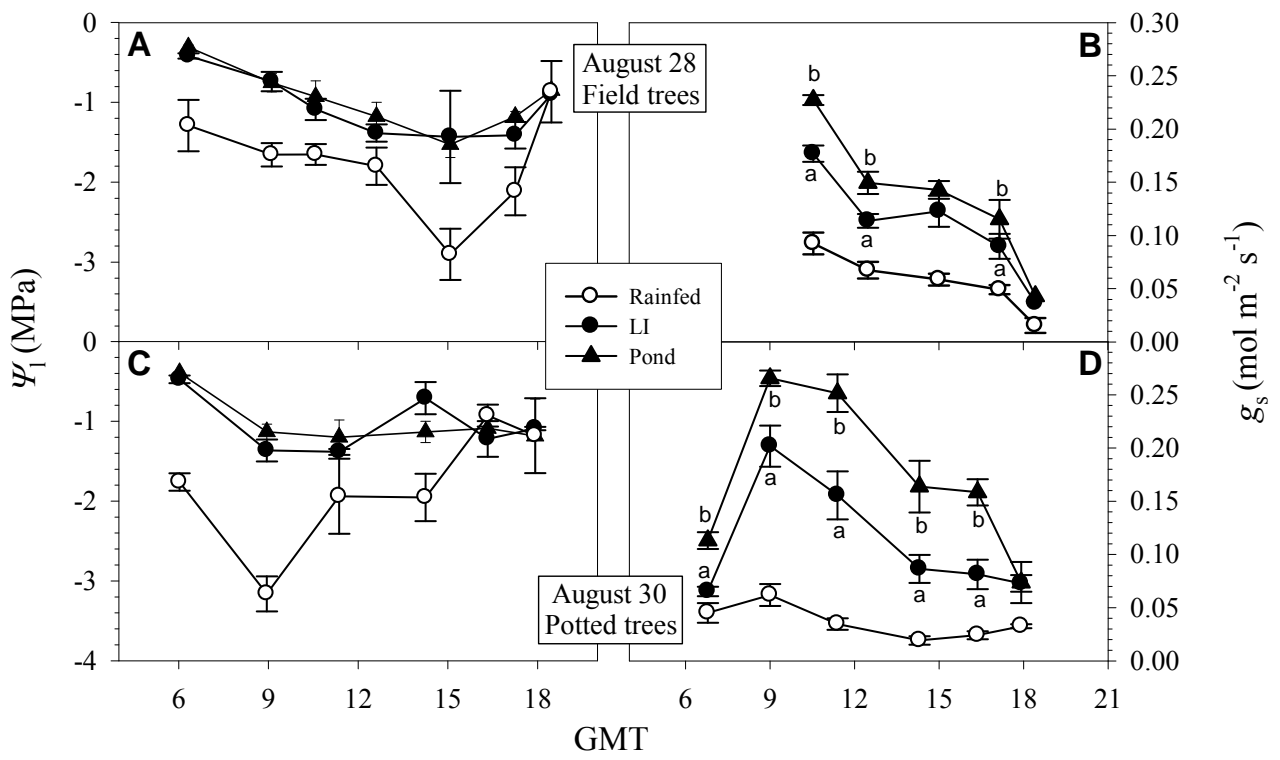
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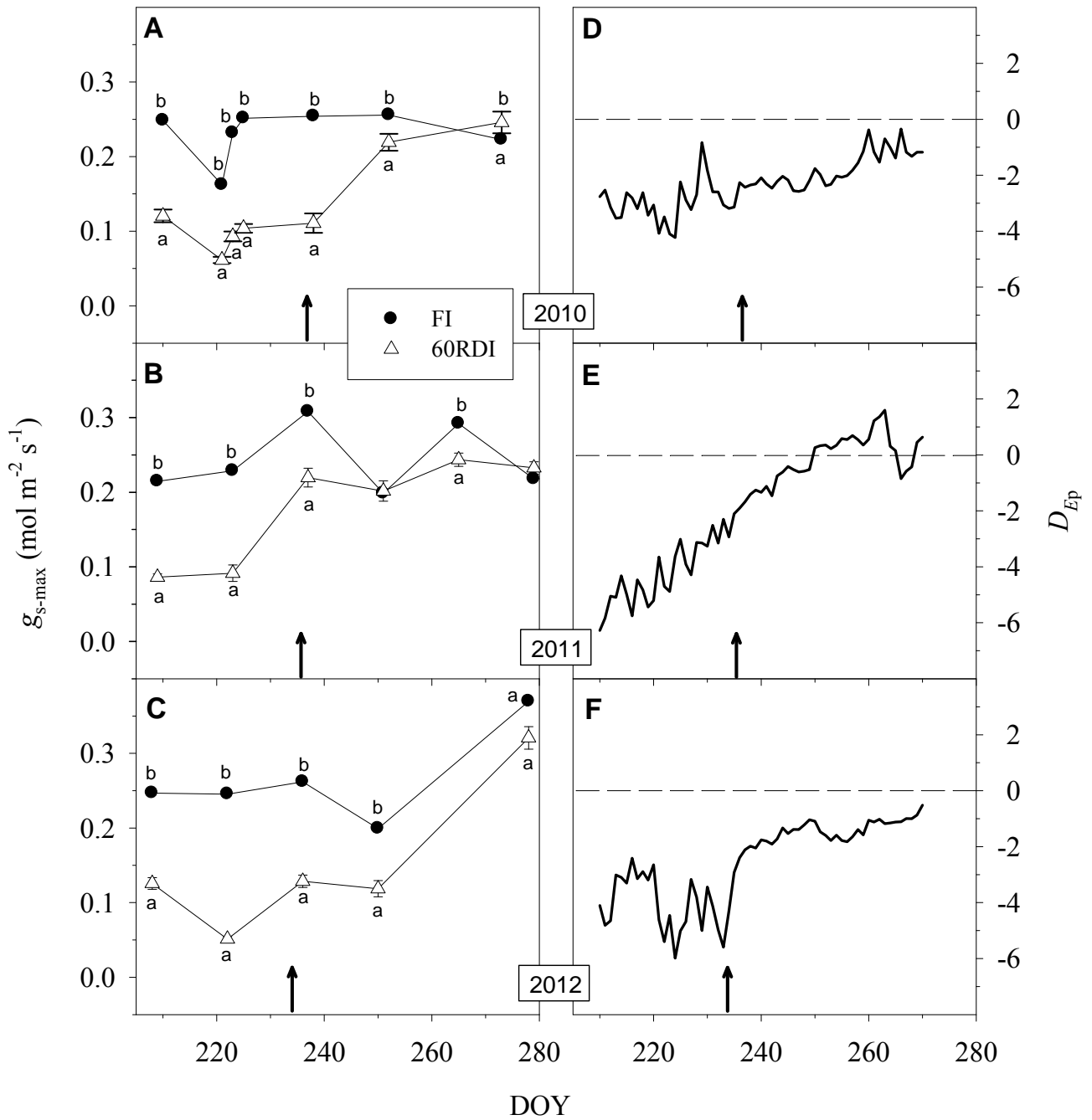
901 **Fig. 6.** Daily leaf patch clamp pressure ( $P_p$ ) curves recorded in 2010 by ZIM probes in  
902 ‘Arbequina’ olive trees of the Sanabria orchard under different levels of water stress.  
903 The shown curve shapes are typical of three levels of water stress identified in the figure  
904 by values both of leaf turgor pressure ( $P_c$ ) and midday stem water potential ( $\Psi_{\text{stem}}$ )  
905 (After Fernández et al., 2011a and Ehrenberger et al., 2012b).

906

907 **Fig. 7.** Seasonal courses of midday stem water potential ( $\Psi_{\text{stem}}$ , avg  $\pm$  SE,  $n = 8$ )  
908 measured in 2011 in FI, 60RDI and 30RDI trees of the Sanabria orchard (see Fig. 1 for  
909 details on treatments). Different letters indicate significant differences between  
910 treatments at  $p < 0.05$ . No letter means no effect (top graph). Horizontal colour bars  
911 below the graph represent the occurrence, along the season, of  $P_p$  curves showing State  
912 1 (low water stress), State 2 (moderate stress) or State 3 (severe stress) (see Fig. 6 for  
913 details).  $P_p$  data were collected with three ZIM probes per treatment, one per tree,  
914 except in the 30RDI treatment, where two trees only were instrumented. For each  
915 irrigation treatment, periods between dashed lines indicates  $\Psi_{\text{stem}}$  values below -1.4  
916 MPa, a threshold for water stress in olive trees with high crop load (Moriani et al.,  
917 | 2012).

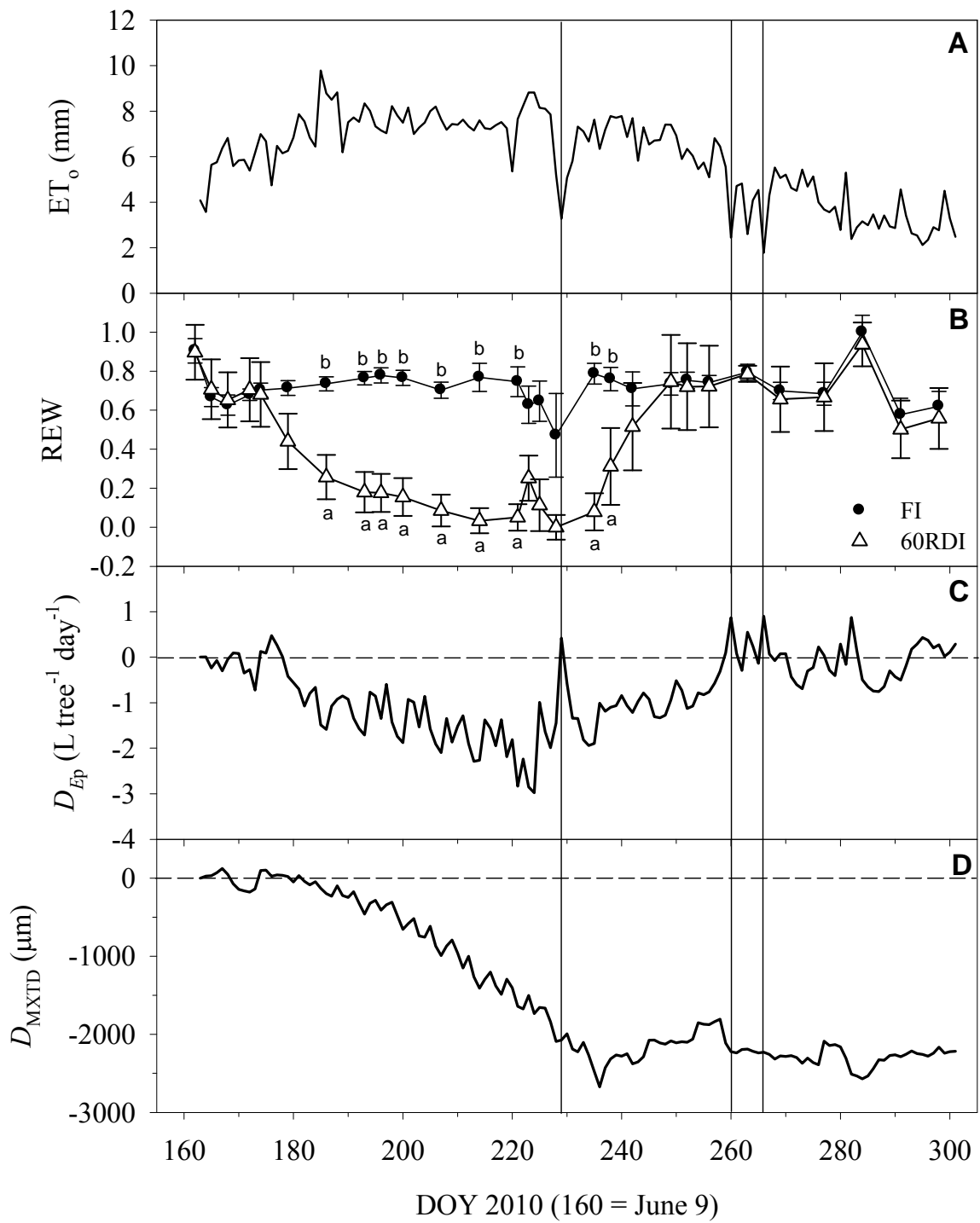


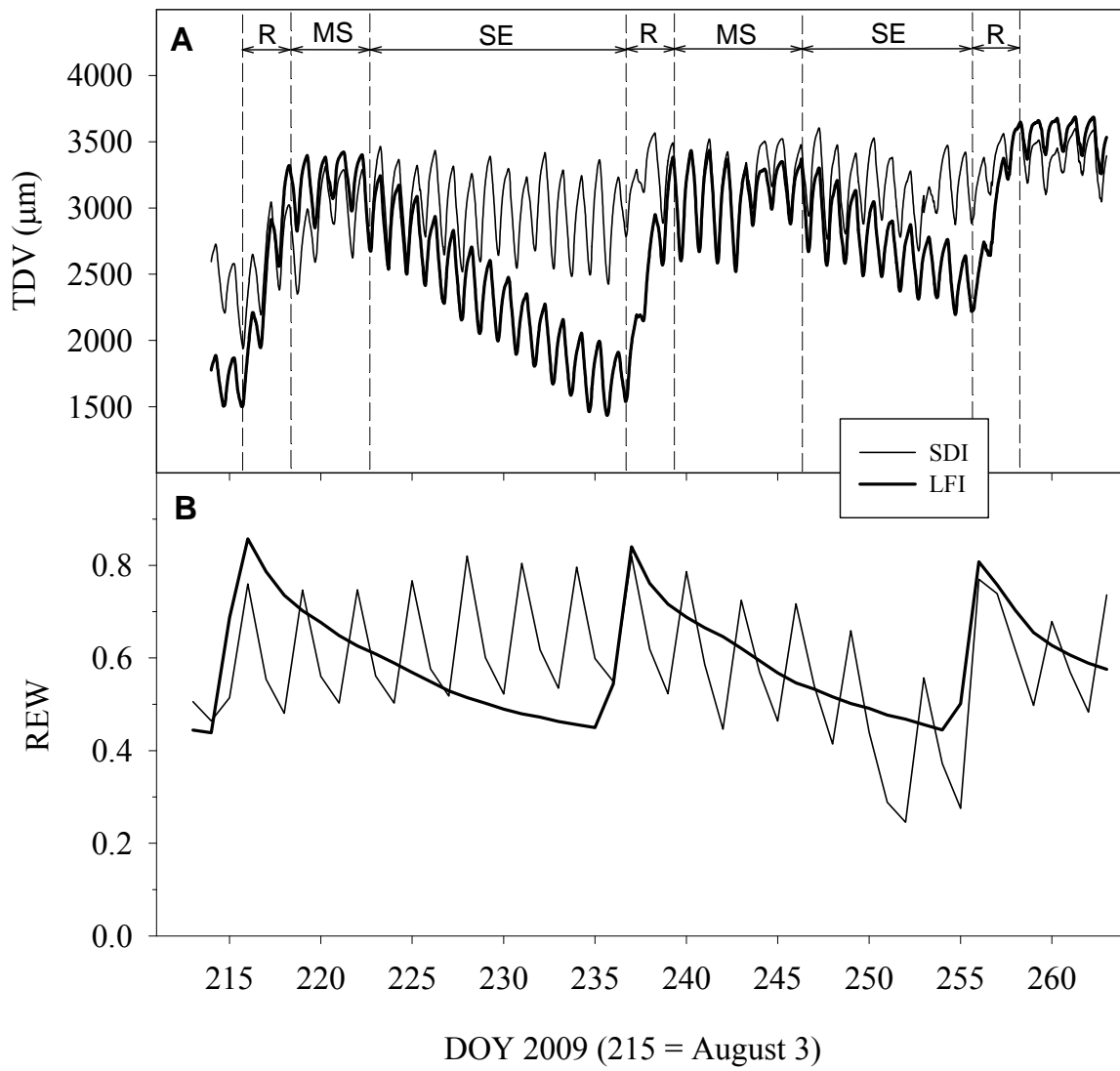




(For 2010 and 2011, DOY 220 = August 8; for 2012, DOY 220 = August 7)







922 |

