1 **Title**

2 Effect of irrigation on sap flux density variability and water use estimate in cherry

3 (*Prunus avium*) for timber production: azimuthal profile, radial profile and sapwood

4 estimation

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16

17 **Abstract**

18 Information on tree water use in plantations for high quality wood is scarce, thus studies

19 are needed to properly estimate the irrigation demand of these plantations. Plant water

20 use estimation with sap flow sensors has been used extensively. However, biases in tree

21 sap flow estimate can arise from variations on radial and azimuthal profiles of sap flux

22 density and also from the sapwood area considered for the up-scaling from sap flux

23 density to sap flux. This work aimed to 1) study the spatial variations of sap flux density

24 in cherry trees in a timber orientated plantation, 2) compare several methods to estimate

25 sapwood depth in cherry trees and 3) to evaluate the effect of drip irrigation on these

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26   factors. The results showed that most of the studied trees had decreasing radial sap flux

27   density profiles with depth as expected. However, the three irrigated trees of bigger

28   sizes still showed high sap flux densities in their inner tissues, at contrast with the rest

29   of the trees and especially with the non irrigated ones of similar size with values close

30   to 20 % of the sap flux density measured at 1 cm depth from cambium. On the other

31   hand, the different methods tested to estimate sapwood depth gave significantly

32   different results and only the two methods of visual identification in wood cores based

33   on color change and measurements of sap flux densities along the xylem radius may be

34   considered suitable for scaling purposes. Moreover, azimuthal variation pattern was

35   found to be random in all the studied trees, and the ranking between the aspects (north,

36   south, east and west) was not affected by either drip irrigation or sun exposition, and

37   thus measuring sap flux density in any particular aspect has been shown to be suitable to

38   estimate the overall tree sap flux. We conclude that more studies are necessary to

39   properly assess the radial profile of sap flux density, especially when considering the

40   high sap flux density in the inner tissues of the three bigger irrigated trees as compared

41   to the other trees, and also how this pattern seemed to indicate sapwood depths values

42   very contrasted to the ones estimated from color change in wood cores.

43

45

46   **1. Introduction and objectives**

47   Plantations of Angiosperm trees for high quality timber production (commonly named

48   hardwood) have increased in recent years in Europe as a consequence of two main

49   causes, a permanent demanding market that is not entirely satisfied by the own

50   production, and the EU regulations promoting their establishment due to their important

environmental role in CO2 capture (Cambria et al., 2012). The later is especially noticeable when considering that timber from the tree species normally used, such as walnut or cherry, take between 30 and 50 years to get its maximum market value (Cisneros, 2004).

The plantations for high quality timber production are normally developed in areas subject to depopulation, where the normal cultivars are not economically viable. Management of these plantations consisted of several operations such as pruning, thinning, soil tillage, fertilization and irrigation (Cambria and Pierangeli, 2012), and as in any other economical activity, the related cost should be carefully evaluated. Irrigation demand of these plantations is frequently estimated using the FAO procedures with crop factors of fruit plantations, because specific crop factors for timber plantations are still scarce. However, the orchard management of timber plantations is normally quite different than that of fruit plantations (leading for instance to different tree architecture and tree density), and this could lead to important biases in the evapotranspiration estimate.

Sap flow sensors based on heat pulse methods have been widely demonstrated as a valuable tool for measuring water use by trees (Burgess et al., 2001; Green et al., 2003). There are, however, several factors to take into account when scaling up to the whole tree, such as the spatial variations of sap flux density within the tree, i.e., the radial and the azimuthal variations (Chang et al., 2014; Kume et al., 2012; Nadezhdina et al., 2002), and a correct estimation of the sapwood depth (Čermák and Nadezhdina, 1998). Furthermore, orchard management techniques such as branch punning and localized irrigation could contribute to add variability (Cabibel and Isberie, 1997; Lu et al., 2000). Radial variation in sap flux density has been a topic widely studied in several tree species under different conditions (e.g., Nadezhdina et al., 2002; Gebauer et al., 2008;

Cohen et al., 2008). The general finding is a decreasing of the sap flux density from the outer to the inner sapwood, though this profile may show different shapes (Alvarado-Barrientos et al., 2013; Gebauer et al., 2008; Kubota et al., 2005) and it could vary with species, tree age and environmental conditions (Čermák and Nadezhdina, 1998). Azimuthal variation of sap flux density, for its part, is understudied as compared to radial variation (Kume et al., 2012), though it may be of higher magnitude (e.g., Shinohara et al., 2013; Lu et al., 2000). In the other hand, the observed results are more contrasting (e.g., Cabibel and Isberie, 1997; Kume et al., 2012; Tsuruta et al., 2010; Shinohara et al., 2013) and consequently general conclusions about tree circumference profile of sap flux density are difficult to be drawn.

Sapwood is the outer part of the xylem conducting sap, which contains living parenchyma cells (Čermák and Nadezhdina, 1998). It can be estimated by several methods, but they normally show contrasted results even for the same species (Cermark and Nadezhdina, 1998; Nadezhdina et al., 2002). As example, Poyatos et al. (2007) found that the sapwood area estimated from radial patterns of sap flux density was 1.5–2 times larger than sapwood area estimates made in the field based on visual inspection of wood cores.

For three years we have been monitoring water use by cherry trees in a timber orientated plantation in a Mediterranean area, under rainfed and irrigated conditions. To this end, sap flow sensors, based on heat pulse with two radial measurements within the sapwood were installed at the east aspect of the studied trees. Sapwood was determined by visual identification of sapwood and heartwood in tree cores.

The aim of this work was to evaluate the accuracy of the approach followed during these three years of experiment (2011-2013) to estimate tree water use with a deeper study in 2014 of the spatial variations of sap flux density (is representative enough

101 measuring sap flux density in two points and only in one aspect of trees?) and, on the

102 other hand, to compare several methods to estimate sapwood depth in cherry trees (was

103 sapwood depth well estimated?). Finally, the effect of drip irrigation on these aspects

104 was also evaluated.

105

106 **2. Materials and methods**

107 **2.1. Study site**

108 The study was carried out at the Torre Marimon site (Caldes de Montbui, Spain) in the

109 IRTA facilities (41º 36 ´47 ´´ N, 2º10´11 ´´E, 170 m.a.s.l). Climate is Mediterranean,

110 with mean annual (1999-2012) rainfall and potential evapotranspiration of 599.4 ± 33.4

111 and 846.8 ± 23.3 mm, respectively. Soils are basic and have two contrasting

112 characteristics, sandy-loam soils with about 60 % of gravels and loam soils with

113 negligible presence of gravels; these differences are due to an alluvium trend brought by

114 a close river did not affect to the same extent the study area.

115 Measurements were conducted during the growing season of 2014 in an 8 year-old

116 cherry tree (*Prunus avium* L.) plantation orientated to timber production with tree

117 spacing of 4x4 m (625 trees ha^{-1}). Trees are pruned every two years at the middle of the

118 growing season. Pruning follows the common practices, where the lower part of crowns

119 is removed, and it approximately accounted for one third of total aboveground biomass.

120 Meteorological conditions during the experiments were measured in an open area next

121 to the plantation. Mean daily values of potential evapotranspiration and maximum

122 temperature during the summer months (July and August) were 4.6±1.3 mm and 29±2.4

123 ºC respectively, while total rainfall accounted only for 45.4 mm.

124

2.2. Experiment 1: Radial variation in sap flux density and sapwood area estimation methods

Radial variation of sap flux density was studied by mean of two sensors based on the compensation heat pulse method (CHPM, Green et al., 2003) with 4 radial measurements at 0.5, 1.2, 2.1 and 3.2 cm from bark, and the bark of 2 mm depth was not previously removed to install the sensors. The CHPM sensors were programmed to measure each 30 minutes, and were charged with a battery of 80 mAh and 12 V connected to a CR-1000 logger (Campbell Scientific, USA). Each sensor consisted of three needles 40 mm in length and 1.8 mm in diameter. The needle placed in the centre is the heater that emits the heat pulse during 1 second. Then, the temperature increase is systematically measured during 8 minutes in the other two needles at 0.5 cm and 1 cm upstream and downstream respectively. Heat pulse velocity is estimated from the time taken to obtain the same temperature upstream and downstream.

The measurements were simultaneously conducted during seven days at 1.3 m height and at the east aspect of the trunk in two close trees with similar diameter but different irrigation treatment (drip irrigated versus non-irrigated trees). Seven days after the two CHPM sensors were moved to other two trees and this was repeated seven times, resulting in a total of 14 trees sampled and a sampling period of 49 days (Table 1). Irrigation treatment consisted of four emitters per tree (16 l h^{-1} tree^{-1}) located at 25 and 50 cm at north and south sides from trunk. Daily doses were estimated at the beginning of each week as the 60 % of the ET_0 for the previous week, and irrigation was not applied when ET_0 was lower than rainfall. Total irrigation per tree ranged from 8 to 125 l day^{-1} when applied.

Heat pulse velocity was corrected for effects of probe-induced wounding (Barret et al., 1995), following the numerical approximation proposed by Swanson and Whitfield

150 (1981) as a function of wound width. Since no wound width is available from literature

151 for the study specie, we adopted the results of Barret et al. (1995) confirmed by

152 Fernandez et al. (2006) in plum trees (*Prunus domestica* L.) of 1.8+2x0.3 mm. Sap flux

153 density was estimated from corrected heat pulse velocity following Barrett et al. (1995).

154 Once the seven days-period of CHPM measurements finalized in each couple of trees,

155 we proceeded to core the measured trees. Seven different methods were used to

156 determine the limit between sapwood and heartwood: (1) methyl orange or (2) lugol

157 staining, (3) visual differentiation based on colour change (VD_m), (4) dye injection

158 (DI_m), (5) radial variation of wood density (WD_m), (6) wood water content (WC_m) and

159 (7) radial profile of sap flux density.

160 Methyl orange and lugol methods were prior tested in several cores taken in neighbour

161 trees, as no colour change was observed in any of the samples these methods were not

162 further considered.

163 For the other methods we extracted one core from each tree with a Pressler increment

164 borer (Suunto Finland) at 35 cm below the sensor position. The cores collected were

165 immediately taken to laboratory and kept in a refrigerator. Visual identification of

166 sapwood and heartwood radiuses were measured (VD_m) and cores were sectioned into

167 small cylinder sections of 8-10 mm length, and volumetric fraction of water and basic

168 density of wood (WD_m and WC_m) were estimated following Nadezhdina et al. (2002).

169 The hole done by this first coring was then used to inject 0.1 % acid fuschin dye

170 (following Umebayashi et al., 2007) and 2-3 hours after we extracted a second core at

171 15 cm under the sensor position (at 20 cm distance from the first core) to estimate the

172 coloured part as sapwood (DI_m). Moreover, 4 extra trees were cored in the last week of

173 the experiment to determine sapwood through VD_m and DI_m, though the coring for DI_m

174   was taken at 5 cm from the first hole, instead of 20 cm. Sapwood depth was also

175   estimated from the radial profile of sap flux density (7) taking into account the point

176   where the sap flux density approached to zero by fitting linear regressions to data (e.g.,

177   Nadezhdina et al., 2002, Cohen et al., 2008).

178   Finally, sapwood depth was expressed as percent of xylem radius to compare between

179   the methods (SW_r). The t-student test for paired data was used for comparing the

180   methods.

181

182   **2.3. Experiment 2: Azimuthal variation in sap flux density**

183   Azimuthal variation in sap flux density was studied by mean of HRM sensors (Burgess

184   et al., 2001) with two radial measurements at 1.25 (outer measurement) and 2.75 (inner

185   measurement) cm from bark. The HRM sensors were programmed to measure every 60

186   minutes, and were charged with 12-W solar panels connected to their internal batteries

187   (ICT International, Australia). Each sensor consisted of three needles 35 mm in length

188   and 1.3 mm in diameter. The needle placed in the centre is the heater that emits the heat

189   pulse during 1 or 2 seconds, and the temperature increase is then systematically

190   measured during 100 seconds in the other two needles at 0.5 cm upstream and

191   downstream from the heater. Each couple of measures (inner and outer) is used to

192   estimate the heat pulse velocity by considering a thermal diffusivity average value of

193   0.002 cm^2 s^{-1} estimated from the cores taken in the first experiment for sapwood

194   determinations, following Burgess et al. (2001).

195   The measurements were continuously carried out from May to August 2014 in 6 trees

196   within 2 different DBH classes (Table 2). Four sensors were inserted in each tree

197   approximately 1.3 m above the ground at north, east, south and west aspects (following

198 Shinohara et al., 2013 and Lu et al., 2000). The sensors were placed at slightly different

199 heights to avoid interferences among the readings. All trees were drip irrigated from

200 May to June following the same irrigation scheme than in experiment 1. However, in

201 order to study whether the ranking between the aspects were maintained in each tree due

202 to induced soil water stress on azimuthal variation, drip irrigation was stopped from

203 July on (Figure 1).

204 The heat pulse velocity was corrected for the effects induced by probe misalignment

205 and wounding. Probe misalignment correction was done by comparing the baselines

206 corresponding to zero sap flow during the first leafless week of measurement to the

207 baselines from all the measurements. Wounding correction was applied in the same way

208 than in the radial experiment (1.3+2x0.3 mm) but following the mathematical

209 approximation of Burgess et al. (2001).

210 Sap flux density was estimated following the same methodology than that in the radial

211 experiment (Barrett et al., 1995). Finally, tree sap flux was calculated from the sap flux

212 density estimated for the inner and outer measurements assuming the sapwood area

213 divided into two concentric bands delimited by the mid-point between them (Bleby et

214 al., 2004). The calculations were made for the entire tree from each of the four aspects,

215 and also for the average sap flux density.

216

217 3. Results

218 3.1. Radial variation of sap flux density

219 As expected, mean sap flux density was higher in all the irrigated trees (Figure 2) except

220 for one couple of trees where the radial profile was very similar between the irrigated

221 tree and the non irrigated one (trees 5 and 6, data not shown) (similar to the radial

222   profile from tree 9). The differences in sap flux density were not constant along the

223   radial profile, and differed among trees. The highest mean difference among irrigated

224   and non irrigated trees was found in the most inner measurement at 3 cm from

225   cambium.

226   The results of expressing sap flux density as relative to the expected maximum (ratio

227   over the measurement at 1 cm from cambium) are presented in Figure 3. The radial

228   profile pattern was very similar for all the non irrigated trees, with maximum sap flux

229   densities from cambium to 1 cm depth (from 0.8 to 1.1 cm h^{-1} at the outer measurement)

230   and a decrease from this distance on (except for one tree with a value of 0.45 in the

231   outer measurement) until an average reduction of about 24 % at a distance of 3 cm from

232   cambium, regardless the difference on sapwood depth between the trees (from 2.7 to 3.6

233   cm). In contrast, radial profile of the irrigated trees was less consistent in the outer part

234   (average normalized values from 0.5 to 1), and also sap flux densities were lower. On

235   the other hand, in the other point measurements, the radial profile in the non-irrigated

236   trees (trees 1, 3, 5, 7, 9, 11 and 13) was very similar to those of the irrigated trees with

237   smaller size (tress 2, 4, 6 and 8) but quite different from those of the biggest irrigated

238   trees (trees 10, 12 and 14), still presenting high sap flux density in their inner xylem

239   tissues (Figure 3). In this sense, it is important to remark that the sapwood depth range

240   was very similar between the non-irrigated and the irrigated trees, from 2.9 to 3.6 and

241   from 2.8 to 4.3 cm respectively (see Table 1).

242   The unexpected, high difference found in the outermost measurements between the

243   irrigated and the non irrigated trees (mean normalized sap flux density of 0.5 versus 0.9)

244   made us reconsider the quality of the raw data measured with the CHPM sensors. In

245   Figure 4 we show two examples of heat pulse velocity from the four radial

246   measurements taken in two trees during one day: the inconsistencies in the

247 measurements were especially important in the outermost thermocouples (0.5 cm from

248 cambium) as indicated by high fluctuations in the heat pulse velocity values, while in

249 the other measurements no sudden fluctuations were observed during the day. These

250 fluctuations appeared in all the tested trees, so we decided to discard the outermost

251 measurements for the rest of the analysis. However, we decided to keep these data in

252 Figures 2 and 3 to show the effect of the presence of bark and cambium in the outermost

253 measurements in cherry trees.

254

255 **3.2. Comparison of methods to determine sapwood**

256 Basic density of wood and volumetric water content were not significantly different

257 between the irrigated and the non irrigated trees, ranging from 388.5 to 513.9 kg m^{-3}

258 and from 171.9 to 302.7 kg m^{-3} respectively. Radial variation of basic density of wood

259 and volumetric water content was different depending on tree, but a systematic decrease

260 with depth was found for volumetric water content in the irrigated trees (Figure 5).

261 However, this pattern was not consistent enough to distinguish between sapwood and

262 heartwood, and consequently this method was considered not useful to determine the

263 limit between these two tissues in cherry trees.

264 The results for the rest of methods are presented in Table 3. For the VD_m, three regions

265 were clearly observed in all cores, with the darkest part close to tree pith, identified as

266 heartwood, and turning lighter when moving outer. In the case of radial variation of sap

267 flux density, linear regressions (R^2 values higher than 0.9) were fitted to all data

268 excluding the outermost readings (as explained in Figure 4), except for trees 10, 12 and

269 14 where the inner sap flux density remained higher than 60 % of the maximum

270 expected, as indicated in Figure 3. On the other hand, with the DI_m we found a

271   discontinuous pattern for all the trees, with coloured bands (the expected conducting

272   tissue) and non coloured ones alternating along the xylem radius. In order to estimate

273   the sapwood depth, we assumed that the limit between sapwood and heartwood was

274   located in the most inner part of the last coloured band found.

275   Paired t-student tests used to compare the methods in Table 3 showed that SW_r was

276   significantly different between the methods, and estimation from DI_m resulted in the

277   lowest values. This type of results was also found when including the four

278   complementary measurements carried out during the last week of the experiment in 4

279   extra trees sampled at a closer distance between the cores (5 instead of 15 cm).

280

281   **3.3. The effect of irrigation on heartwood formation**

282   The influence of irrigation on heartwood formation was assessed by comparing the

283   relationships between tree diameter and heartwood diameter for irrigated and non

284   irrigated trees, obtained with the VD_m method (Figure 6). Heartwood diameter was very

285   similar in both treatments for diameter lower than 10 cm (non significant differences at

286   p-level < 0.001). However, for bigger trees, irrigated individuals had lower heartwood

287   diameters than those of the non irrigated ones, as indicated by a lower value of the a

288   parameter in the quadratic functions fitted to all the data (0.14 versus 0.53).

289

290   **3.4. Azimuthal variation of sap flux density**

291   Diurnal courses of sap flux densities followed a bell-shaped pattern in all the studied

292   aspects during both periods, with and without irrigation. The coefficient of variation

293   (CV) of the 24 hourly sap flux densities from the 4 aspects of each tree (from the mean

294   of the measurements at two sapwood depths), was higher in the period without

295   irrigation and ranged from 11 to 75 and from 27 to 81 % per tree respectively.

296   The azimuthal variation of sap flux density for each tree, i.e. the ranking between the

297   aspects for each tree, was expressed as the relative differences over the mean as follow:

298   RD = ([(SF$_i$ −SF$_{mean}$)/SF$_{mean}$]/N) · 100, where SF$_i$ was the daily average from one

299   particular aspect, SF$_{mean}$ the daily average from the 4 aspects measured, and N the

300   number of days studied.

301   During the irrigation period, RD was different depending on the sample tree. For

302   instance, tree 16 showed the lowest sap flux density at the north side (-85±2 %), while it

303   was the highest at this aspect for tree 18 (50±7 %). In contrast, tree 19 showed very

304   similar values in all the studied aspects (around 0 %) (Figure 7, left).

305   The soil water deficit induced by stopping irrigation produced a mean general decrease

306   of 92 % in tree sap flux. The variability between the aspects was higher than in the

307   irrigation period, with a mean increase of the CV values of 23±21 and 35±32 % for the

308   outer and inner measurements, respectively. In contrast, the ranking between the aspects

309   was quite constant for each tree between the two periods (except for tree 15), as

310   indicated by very similar average and standard deviation values for the RD between the

311   irrigation and the without irrigation periods for each tree (Figure 7).

312

313   Cumulative tree sap flux calculations were made for each of the four aspects

314   considering sap flux density in each of them as representative of all the sapwood area.

315   As a way to determine which aspect was most representative of the overall cumulative

316   tree sap flux, these calculations were expressed as a percentage of the mean of the four

317   aspects. Average± standard deviation values were 88.3±46.9, 106.4±34.4, 108.7±42.7

318   and 96.6±38.1 % for north, south, east and west aspect respectively.

319

320   **4. Discussion**

321   In this work we evaluated the effects of irrigation on radial and azimuthal variation of

322   sap flux density in 8 years old timber-orientated cherry tree plantation in a

323   Mediterranean area of Spain. Moreover, several methods for estimating the conducting

324   sapwood area have been tested in order to evaluate its likely effect on the scaling up

325   from sap flux density to tree sap flux.

326   **4.1. Radial variation of sap flux density**

327   Several studies have reported a decrease of sap flux density as a result of declines in soil

328   water content, naturally induced through a contrasted dry season or by comparing

329   different irrigation practices (Lu et al., 2000; Philipps et al., 1996). In this study we

330   simultaneously measured drip irrigated and non-irrigated ones, during the dry season

331   (from July to September). We found that irrigated trees showed higher sap flux densities

332   than the non-irrigated ones, but differences were not constant along the xylem radius,

333   with mean differences of 2.5, 2.6 and 5.4 times higher for distances of 1.2, 2.1 and 3.2

334   cm from bark, respectively. These differences were consequence of a different decrease

335   of sap flux density with sapwood depth related to tree size, with similar patterns for the

336   non irrigated trees and the smaller irrigated ones (7 trees and 4 trees respectively) and a

337   contrasted pattern for the bigger irrigated ones (3 trees) (Figure 3).

338   The decreasing of sap flux density with sapwood depth is frequently found in literature

339   (e.g., Ford et al., 2004; Cohen et al., 2008) and functions such as the Weibull or the

340   Gaussian ones are frequently used for describing the flow shape (Gebauer et al., 2008;

341   Kubota et al., 2005). In this sense, Nadezhdina et al. (2002) found a peaked distribution

342   on the radial sap flux density pattern (with the maximum at 80 % of total radius) of a

343   tree species very similar to the one studied here, i.e., *Prunus serotina*, but 19 years old.

344   In our study, the outer thermocouples, placed at 0.5 cm from cambium, were discarded

345   because of their inconsistent behaviors (Figure 4), and thus we could not conclude

346   whether the radial sap flux pattern of *P. avium* was characterized by a monotonous

347   decrease of sap flux with depth or by a peaked distribution, as the two most common

348   (Cohen et al., 2012). However, we believed that showing the effect of not removing the

349   non homogenous tissue (bark and cambium) has on the quality of the readings was

350   important due to this fact is not usually mentioned in the related literature, especially for

351   species with small bark thickness. This inconsistent behavior was the result of bark and

352   cambium interferences in the measurements (Steve Green, personal communication);

353   first, heat pulse emitted by the heater is likely affected by this sharp change on water

354   conductivity and then heat pulse is not propagated correctly (Steve Green, personal

355   communication), and second, the measurement radius of thermocouples should

356   probably be higher than 3 mm as in other heat pulse methods (taking into account that

357   our non conducting tissue was approximately of 2 mm depth) and thus readings would

358   be accordingly affected. Moreover, the high and persistent difference found between the

359   irrigated and the non irrigated trees seems to indicate that despite the difference on

360   depth of non conducting tissue was about 0.3 mm, this small difference could lead to

361   important effects of the abovementioned explaining aspects.

362   In addition, when only considering data from of all our non irrigated trees together with

363   the 4 smaller irrigated ones, the radial profile showed similar decreases on the

364   normalized data and consequently a consistent difference of about 2.5 times along the

365   radial profile in the absolute data. However, the other 3 irrigated trees (the biggest trees

366   in the experiment) showed lower radial variations and less pronounced decreases with

367   depth, and even high sap flux densities in the inner tissues (tree 14 in Figure 2). This

368   result is in contrast with the results observed in maritime pines of different sizes, with

369   steeper declines in the trees with the biggest sizes (Delzon et al., 2004). These trees had

370   a small difference of about 1 cm in the sapwood depth as compared to the non irrigated

371   ones with bigger size (see next section), thus tree size was not the only responsible of

372   these differences (Čermák and Nadezhdina, 1998) but also the irrigation applied during

373   4 growing seasons (during the experiment presented here and the 3 previous years). It is

374   widely accepted that leaves are connected to new xylem and though xylem can be viable

375   to a large depth in the xylem, sap movement will not necessary occur due to the

376   increasing resistance to lateral flow with xylem depth (Cohen et al., 2012). We found

377   significant differences on leaf area index between irrigated and not irrigated trees of

378   bigger size (data not shown), and also high differences in sap flux density (Figure 2).

379   This supports that, though xylem is viable for both type of trees, irrigation seems to

380   induce changes in the xylem which is actively conducting water in order to maintain the

381   ratio of cross sectional area of actively conducting xylem to leaf area constant (Cohen et

382   al., 2012).

383   **4.2. Sapwood determination and heartwood formation**

384   Our results have shown that several methods, which have been used for delimiting

385   sapwood depth in other species (methyl orange, lugol, and radial variation of xylem

386   density and xylem water content), were not suitable for delimiting the sapwood depth in

387   *Prunus avium* and thus these methods are not recommended for scaling purposes for

388   this species. In this sense, Čermák and Nadezhdina (1998) found that the radial

389   variation of xylem water content was useful to delimit the sapwood depth for some

390   species but not for others. In contrast, the rest of the methods showed clear

391 differentiation between sapwood and heartwood tissues but significantly different

392 values for the sapwood depths. Visual identification carried out in ring cores is related

393 with the different chemical processes taking place in the two tissues, and our results are

394 in agreement with those from Nadezhdina et al. (2002), who found three different

395 colored parts in ring cores of *Prunus serotina* and a sapwood depth very similar to ours

396 (75 % of the xylem radius versus 80 %). However, while these authors found a clear

397 relationship between these three different colored parts and the sap flux density

398 measured along the xylem radius, normally considered as a robust proxy for sapwood

399 depth estimation (Cohen et al., 2008; Poyatos et al., 2007), this was not the case in the

400 present study. Though we found a significant difference between these two methods (13

401 % in average), this bias may be considered as negligible for scaling purposes (from

402 point measurement to plantation transpiration), because of the low value of sap flux

403 density at deeper depths (and consequently the sap flux at these depths) in the trees

404 where the estimation of sapwood depth was accomplished with the sap flow

405 measurements (11 out of 14 trees). In contrast, the high sap flux densities at the inner

406 depths of the other 3 bigger irrigated trees did not match with the sapwood depth values

407 found in the other two suitable methods, and how it was discussed before it remains an

408 open question for further studies, especially when considering that this could introduce

409 important errors when scaling from measurement to tree transpiration.

410 In addition, a particular result was found for the dye injection method, with a systematic

411 lower value (average of 25 %) of the xylem radius in all the tested trees, as compared

412 with those from either the radial profile of sap flux density or the visual identification.

413 This may be related with the discontinuous pattern of the water conducting areas

414 showed by the dye injection method. This pattern is more related to ring porous species

415 with water conducting regions and non conducting regions within the sapwood (Tsuruta

416   et al. 2010), and thus was unexpected due to the semi-diffuse porous wood of *Prunus*

417   *avium* (García-Esteban et al., 2003). In this sense, the results of Boumghar (2012) when

418   using dye to differentiate between sapwood and heartwood showed that all the sapwood

419   was coloured in the studied ring porous species so this discontinuous pattern was not

420   found. We thus hypothesize that an artifact could be introduced with the dye injection

421   method and thus complementary anatomical studies are needed in this species.

422   On the other hand, heartwood formation was affected by irrigation for tree diameters

423   bigger than 10 cm (Figure 6), pointing out again the hypothesis that the inner tissues of

424   the bigger trees in our experiment, subjected to cumulate water stress periods underwent

425   anatomical changes as the result of faster growth and bigger diameter. This result seems

426   to be an open research question to be resolved for the wood industry, because the

427   presence of heartwood in high quality woods is nowadays increasing their market

428   values (Vilanova, personal communication).

429

430   **4.3. Azimuthal variation of sap flux density**

431   Sap flux density was measured in four different aspects of six trees (24 sensors) during

432   two periods, the first characterized by irrigation and the second one by no irrigation and

433   probably a certain degree of water stress given by summer conditions (high potential

434   evapotranspiration, low rain).

435   The decrease in tree sap flux from one period to the other was as expected for the

436   species, with a general reduction of 92 % in tree sap flux compared to a reduction of 85

437   % when comparing between irrigated trees and non irrigated trees observed during one

438   experiment carried out in summer under Mediterranean climate (Cabibel and Isbérie,

439   1997). This indicates the high sensibility of this species to dry periods characterized by

440 high soil water deficit and water evaporative demand, as other authors have already

441 pointed out (e.g. Juhász et al., 2013; Cabibel and Isbérie, 1997). On the other hand, the

442 sap flux density variability within each tree (CV of the 4 aspects) was higher during the

443 water deficit period, with a mean increase of about 30 % respect to the irrigation period.

444 In contrast, the ranking between the aspects for each tree was quite constant between the

445 periods, pointing to a relevant weight in the azimuthal pattern of the individual

446 structural conditions in each tree.

447 Azimuthal variability in sap flux density can be larger than the radial variability (e.g.,

448 Shinohara et al., 2013). However, this variability has been found less predictable,

449 because it can be explained by differences on tree exposure to sun (Granier, 1987), soil

450 water content around the tree (Cabibel and Isbérie, 1997), anatomical xylem structure

451 (Tateishi et al., 2008) or crown architecture (Lu et al., 2000). In this sense, we expected

452 the azimuthal variability to be highly controlled by the soil water content around trees

453 with a virtually negligible effect of ET_0, as suggested by the results of Cabibel and

454 Isbérie (1997) in a drip irrigation experiment (with 2 emitters of 4 L h^{-1}, 1 m east and 1

455 m west from trunk) in cherry trees. However, we found no connection between the soil

456 water content around the tree (emitters placed north and south sides from trunk, 0.8 m^2

457 each wet bulb) and the ranking in the sap flux density of the different aspects during the

458 irrigation period. We thus postulate that most of the primary roots were affected by drip

459 irrigation, and consequently no clear connections or preferential fluxes between roots

460 and leafs from the same aspect could appear. In addition, the way the trees were pruned

461 led to not systematic differences on tree architecture and hence on azimuthal variability

462 of anatomical xylem structure, which could also affect the azimuthal pattern of sap flox

463 density in the studied trees. Therefore, when we compared the overall tree sap flux

464 estimated considering the sap flux density from each aspect and the one considering the

465 mean sap flux density, all of them performed similarly for all the studied trees, with

466 average tree sap flux from each aspect not being significantly different than tree sap flux

467 estimated from the average sap flux density.

468 **Conclusions**

469 As presented in this work, the maximum sap flux density in our cherry trees of 8 years

470 old is located at 1 cm from cambium or closer, because the values in inner tissues were

471 of lower magnitude in all the studied trees and consequently decreasing profiles with

472 depth were observed. Our sensors also measured sap flux density at 0.5 mm from

473 cambium, but the readings were finally discarded due to interferences promoted by the

474 presence of bark and cambium on the readings. However, we did not find any advice

475 regarding this fact in either the CHPM manual or articles using this method, so we

476 decided to show the effect that this thin non conducting tissue had on the readings. In

477 contrast, in the HRM method, as the one we used in the azimuthal experiment and in the

478 previous measurements from 2011 to 2013, the outermost measurements (at 1.25 cm

479 from cambium) are not affected by bark+cambum depth lower than 5 mm, which is

480 properly described in the corresponding manual. Therefore, further studies are necessary

481 to assess the sap flux density close to the cambium in order to a better assessment of the

482 radial profile shape in cherry trees for timber production.

483 The different methods to estimate sapwood gave contrasted results and a number of

484 them are considered as not suitable. The differences found between visual identification

485 and radial profile methods can be considered negligible for scaling purposes due to

486 lower values of sap flux density at these depths.

487 The effect of irrigation on spatial variations of sap flux density affected to different

488 extents. All the irrigated trees gave higher sap flux densities along the xylem radius and

489 the radial profiles of normalized values were similar between irrigated and non irrigated

490 trees. However, more measurements are needed in the inner depths of irrigated trees of

491 bigger sizes as shown in our results. On the other hand, the azimuthal variation was

492 found to be unpredictable and we consequently conclude that any aspect can be selected

493 to measure sap flow in young cherry trees drip irrigated and pruned to timber

494 production.

501

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594 living tree by the dye injection method. Tree Physiology, 27: 993–999.

595

596 **Figures Captions**

597   **Figure 1**. Mean sap flux density (1 data every hour) of two sampled trees in the

598   azimuthal experiment (see Table 2 for details).
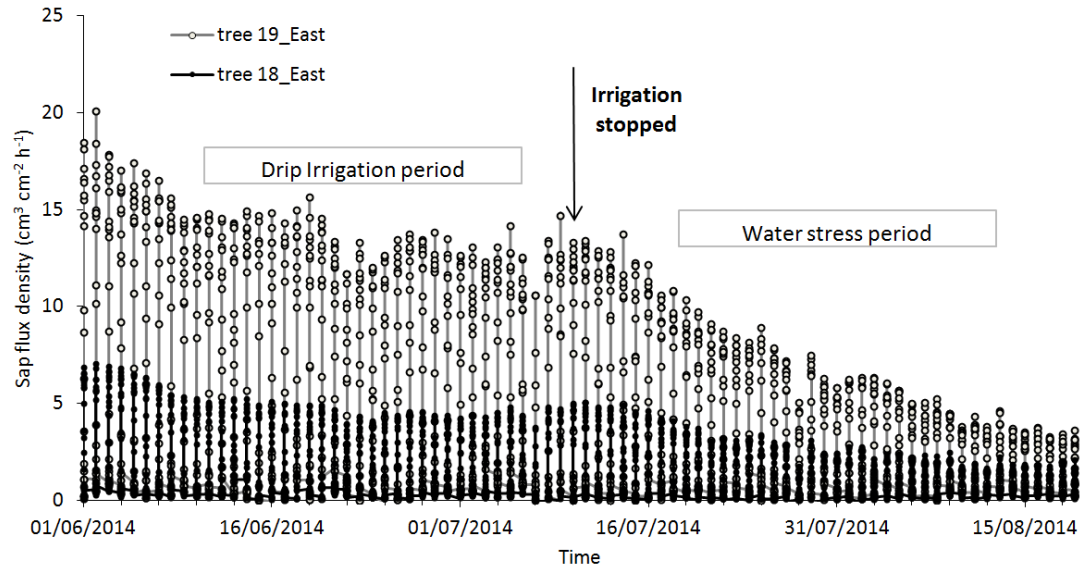
599

600   **Figure 2**. Examples of radial patterns of sap flux density in 4 irrigated trees (I) and 4

601   non- irrigated trees (NI). Data shown per tree is the average of all measurements during

602   7 seven days and standard deviations are not shown to improve the understanding. Tree

603   characteristics are shown in Table 1. Note that all the studied trees were not shown

604   because they behaved in a similar manner that the ones presented

605
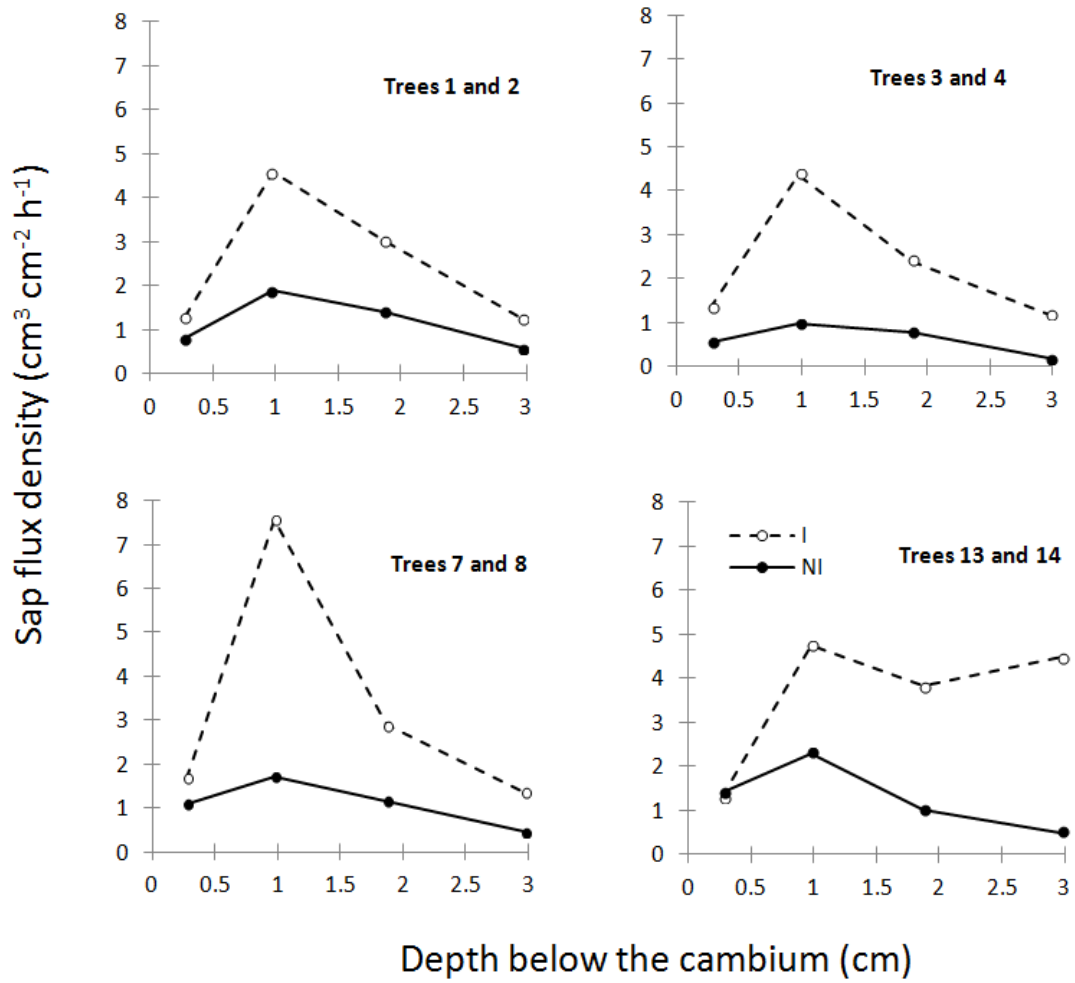
Figure 3. Normalized sap flux density (over the measurement at 1.2 cm from bark) in
the irrigated (I) and the non-irrigated trees (NI). Tree characteristics are shown in Table
1.

609
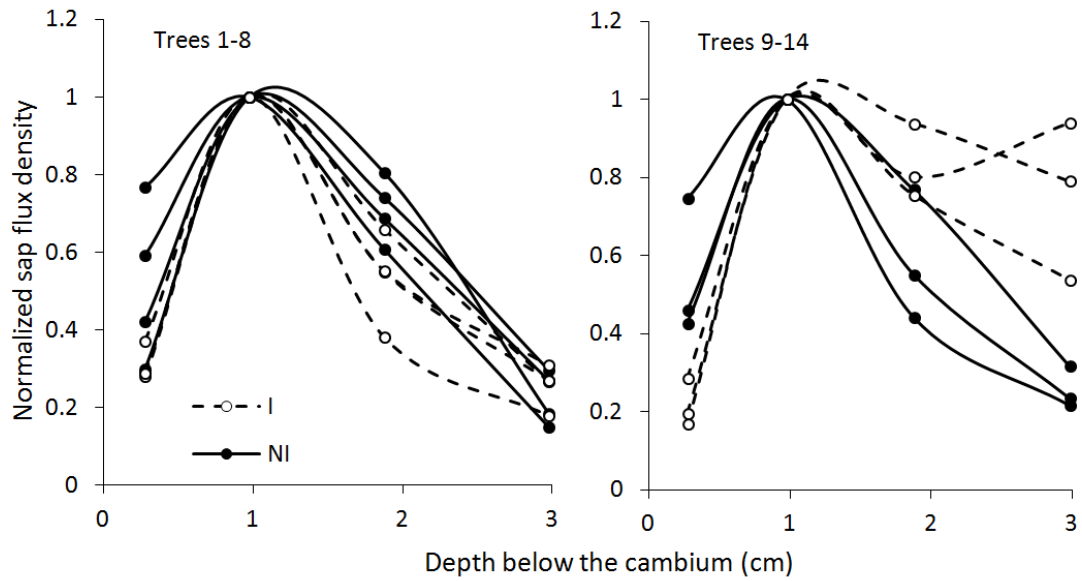
**Figure 4**. Raw data (heat pulse velocity) from two CHPM sap flow sensors during 1

day of measurements in two trees (one measurement each 30 minutes) at four depths

from cambium.

613
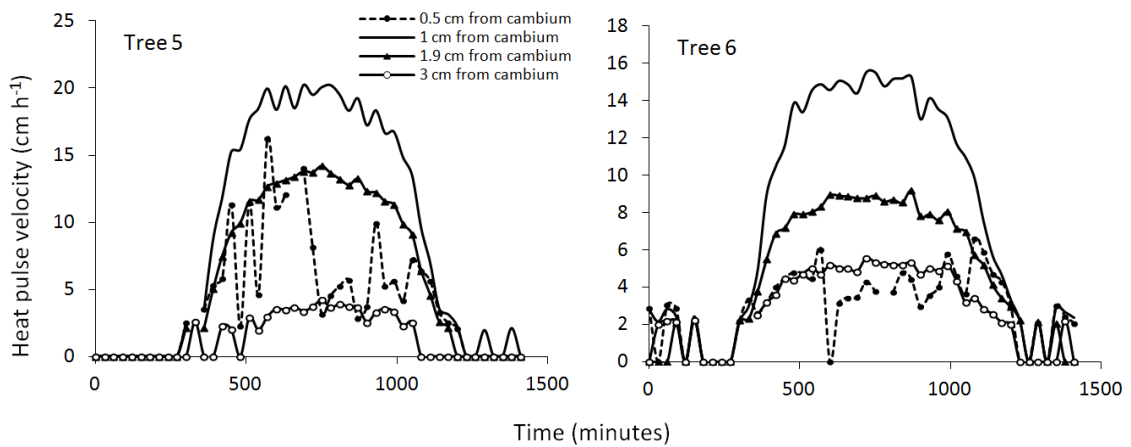
Figure 5. Radial profiles of volumetric water content and basic density (kg m^{-3}) in the

irrigated (open symbols) and the non-irrigated trees (closed symbols). Only R^2 values

from significant linear regressions were presented.

617
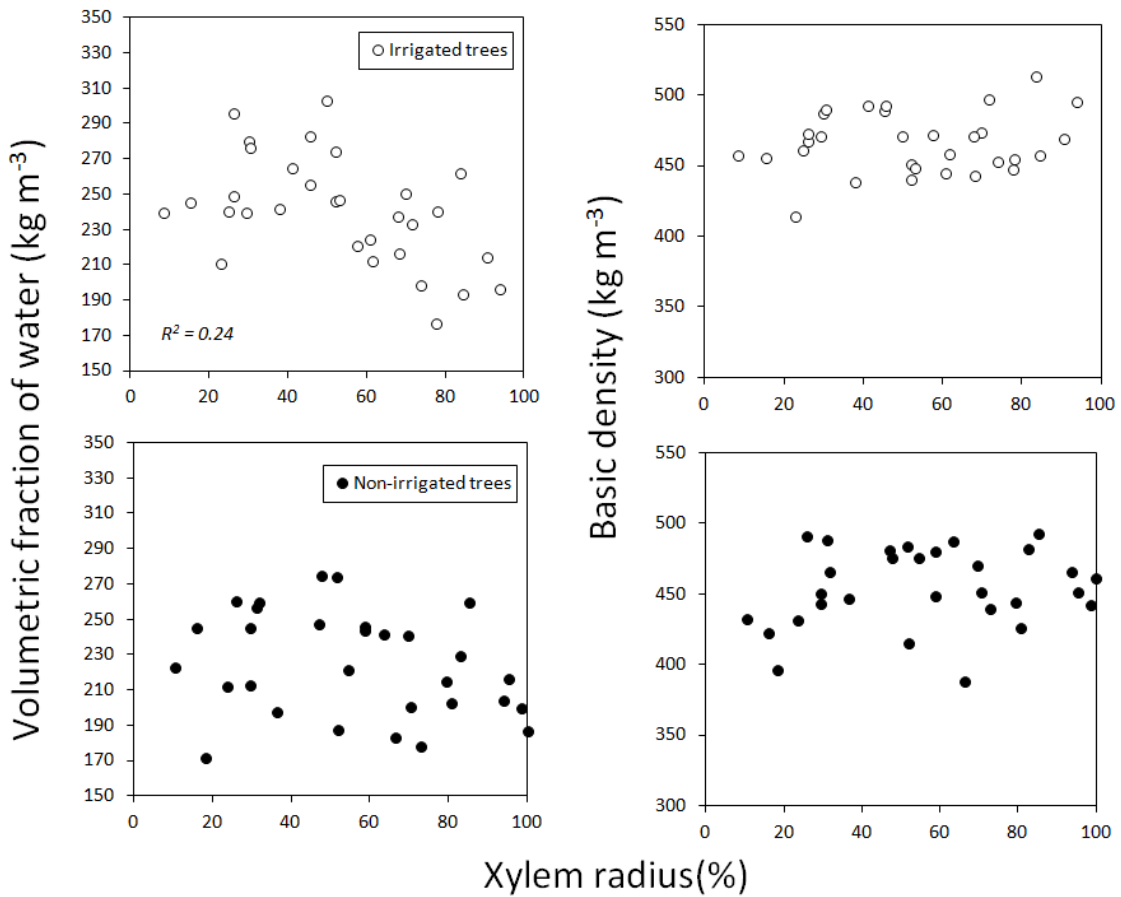
Figure 6. Relationship between trunk diameter (cm) and heartwood diameter (cm) for
the irrigated and the non-irrigated trees. Quadratic functions ($y=a\ x^2+b\ x$) were fitted
and forced through the origin.
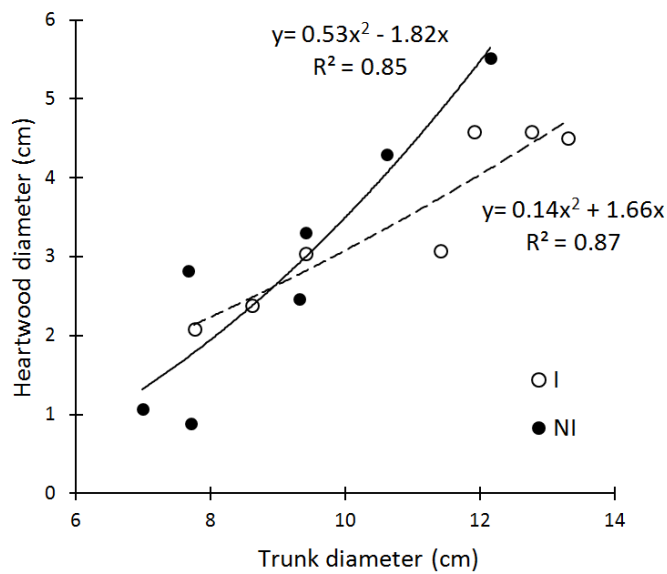
621

622 **Figure 7**. Relative differences over the mean (average and standard deviation values,

623 %) for each study tree and for the both studied periods, the irrigation and non irrigation

624 ones.
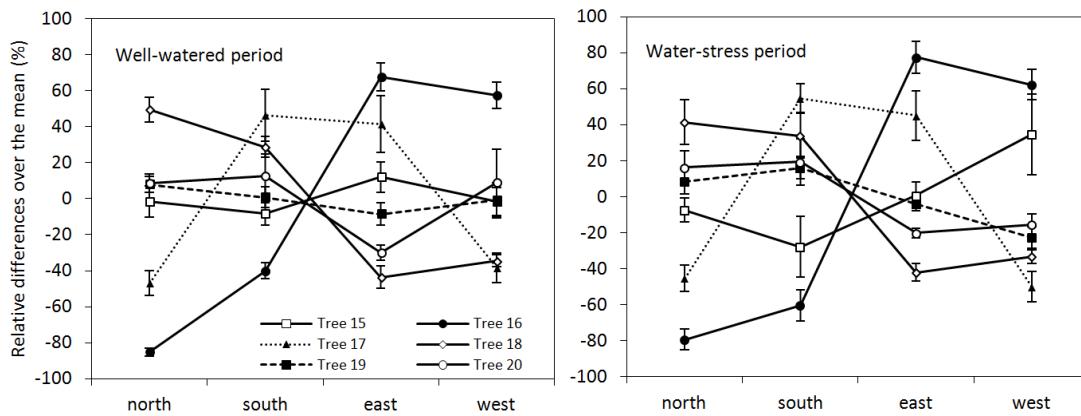
625

626 **Table 1**. Diameter at breast height (DBH) and sapwood depth, estimated from colour

627 change in wood cores (VD_m, see text for details), of the studied trees in the radial

628 experiment. Drip irrigated (I) and non-irrigated trees (NI).

Irrigation	Tree nº	DBH (cm)	Sapwood depth (cm)	Measurement period (DOY)
NI	1	7.0	2.9	
I	2	7.7	2.8	198-204
NI	3	7.7	2.7	
I	4	8.6	3.3	205-211
NI	5	7.7	3.4	
I	6	9.4	3.5	191-197
NI	7	9.3	3.0	
I	8	11.4	4.7	184-190
NI	9	9.4	3.2	
I	10	11.9	3.8	212-218
NI	11	10.6	3.4	
I	12	13.3	4.4	219-225
NI	13	12.1	3.6	
I	14	12.8	4.3	226-232

629

630 **Table 2**. Diameter at breast height (DBH) and sapwood depth, estimated from the

631 relationship with diameter obtained with the VD_m (see text for details), of the studied

632 trees in the azimuthal experiment. I: Irrigation period, NI: water stress period (see

633 Figure 1 for details).

Tree nº	DBH (cm)	Sapwood depth (cm)	Measurement period (DOY)
15	8.1	3.5	96-190 (I),191-234 (NI)
16	8.7	3.7	96-190 (I),191-234 (NI)
17	9.0	3.8	96-190 (I),191-234 (NI)
18	10.1	4.2	96-190 (I),191-234 (NI)
19	10.3	4.3	96-190 (I),191-234 (NI)
20	11.2	4.6	96-190 (I),191-234 (NI)

634

635 **Table 3**. Sapwood depth relative to xylem radius determined from: visual

636 differentiation based on colour change (VD_m), dye injection (DI_m) and radial profile of

637 sap flux density (SF_r). Note that for trees 11, 12 and 14 linear regressions were not fitted

638 to the sap flow data. Different letters indicate significant mean differences in the paired

639 t-student tests used to compare among methods. SD is standard deviation.

Tree	VD_m	DI_m	SF_r
1	0.7	0.3	1.0
2	0.7	0.5	1.2
3	0.7	0.6	0.9
4	0.8	0.4	0.8
5	0.6	0.6	0.8
6	0.7	0.6	0.9
7	0.7	0.6	0.6
8	0.8	0.5	0.8
9	0.7	0.4	0.8
10	0.9	0.5	0.7
11	0.7	0.5	-
12	0.7	0.5	-
13	0.6	0.5	0.7
14	0.6	0.5	-
Mean	*0.7a*	*0.5b*	*0.8c*
SD	*0.1*	*0.1*	*0.2*

640

641