Title

Effect of irrigation on sap flux density variability and water use estimate in cherry (*Prunus avium*) for timber production: azimuthal profile, radial profile and sapwood estimation

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Abstract

Information on tree water use in plantations for high quality wood is scarce, thus studies are needed to properly estimate the irrigation demand of these plantations. Plant water use estimation with sap flow sensors has been used extensively. However, biases in tree sap flow estimate can arise from variations on radial and azimuthal profiles of sap flux density and also from the sapwood area considered for the up-scaling from sap flux density to sap flux. This work aimed to 1) study the spatial variations of sap flux density in cherry trees in a timber orientated plantation, 2) compare several methods to estimate sapwood depth in cherry trees and 3) to evaluate the effect of drip irrigation on these factors. The results showed that most of the studied trees had decreasing radial sap flux density profiles with depth as expected. However, the three irrigated trees of bigger sizes still showed high sap flux densities in their inner tissues, at contrast with the rest of the trees and especially with the non irrigated ones of similar size with values close to 20 % of the sap flux density measured at 1 cm depth from cambium. On the other hand, the different methods tested to estimate sapwood depth gave significantly different results and only the two methods of visual identification in wood cores based on color change and measurements of sap flux densities along the xylem radius may be considered suitable for scaling purposes. Moreover, azimuthal variation pattern was found to be random in all the studied trees, and the ranking between the aspects (north, south, east and west) was not affected by either drip irrigation or sun exposition, and thus measuring sap flux density in any particular aspect has been shown to be suitable to estimate the overall tree sap flux. We conclude that more studies are necessary to properly assess the radial profile of sap flux density, especially when considering the high sap flux density in the inner tissues of the three bigger irrigated trees as compared to the other trees, and also how this pattern seemed to indicate sapwood depths values very contrasted to the ones estimated from color change in wood cores.

Keywords: wood, heartwood, cherry tree, heat pulse, tree circumference

1. Introduction and objectives

Plantations of Angiosperm trees for high quality timber production (commonly named hardwood) have increased in recent years in Europe as a consequence of two main causes, a permanent demanding market that is not entirely satisfied by the own production, and the EU regulations promoting their establishment due to their important environmental role in CO_2 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 measuring sap flux density in two points and only in one aspect of trees?) and, on the other hand, to compare several methods to estimate sapwood depth in cherry trees (was sapwood depth well estimated?). Finally, the effect of drip irrigation on these aspects was also evaluated.

2. Materials and methods

2.1. Study site

The study was carried out at the Torre Marimon site (Caldes de Montbui, Spain) in the IRTA facilities (41º 36 ´47 ´´ N, 2º10´11 ´´E, 170 m.a.s.l). Climate is Mediterranean, with mean annual (1999-2012) rainfall and potential evapotranspiration of 599.4 ± 33.4 and 846.8 ± 23.3 mm, respectively. Soils are basic and have two contrasting characteristics, sandy-loam soils with about 60 % of gravels and loam soils with negligible presence of gravels; these differences are due to an alluvium trend brought by a close river did not affect to the same extent the study area.

Measurements were conducted during the growing season of 2014 in an 8 year-old cherry tree (*Prunus avium* L.) plantation orientated to timber production with tree spacing of 4x4 m (625 trees ha^{-1}). Trees are pruned every two years at the middle of the growing season. Pruning follows the common practices, where the lower part of crowns is removed, and it approximately accounted for one third of total aboveground biomass.

Meteorological conditions during the experiments were measured in an open area next to the plantation. Mean daily values of potential evapotranspiration and maximum temperature during the summer months (July and August) were 4.6±1.3 mm and 29±2.4 ºC respectively, while total rainfall accounted only for 45.4 mm.

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

(1981) as a function of wound width. Since no wound width is available from literature for the study specie, we adopted the results of Barret et al. (1995) confirmed by Fernandez et al. (2006) in plum trees (*Prunus domestica* L.) of 1.8+2x0.3 mm. Sap flux density was estimated from corrected heat pulse velocity following Barrett et al. (1995).

Once the seven days-period of CHPM measurements finalized in each couple of trees, we proceeded to core the measured trees. Seven different methods were used to determine the limit between sapwood and heartwood: (1) methyl orange or (2) lugol staining, (3) visual differentiation based on colour change (VD_m), (4) dye injection (DI_m), (5) radial variation of wood density (WD_m), (6) wood water content (WC_m) and (7) radial profile of sap flux density.

Methyl orange and lugol methods were prior tested in several cores taken in neighbour trees, as no colour change was observed in any of the samples these methods were not further considered.

For the other methods we extracted one core from each tree with a Pressler increment borer (Suunto Finland) at 35 cm below the sensor position. The cores collected were immediately taken to laboratory and kept in a refrigerator. Visual identification of sapwood and heartwood radiuses were measured (VD_m) and cores were sectioned into small cylinder sections of 8-10 mm length, and volumetric fraction of water and basic density of wood (WD_m and WC_m) were estimated following Nadezhdina et al. (2002). The hole done by this first coring was then used to inject 0.1 % acid fuschin dye (following Umebayashi et al., 2007) and 2-3 hours after we extracted a second core at 15 cm under the sensor position (at 20 cm distance from the first core) to estimate the coloured part as sapwood (DI_m). Moreover, 4 extra trees were cored in the last week of the experiment to determine sapwood through VD_m and DI_m, though the coring for DI_m

was taken at 5 cm from the first hole, instead of 20 cm. Sapwood depth was also estimated from the radial profile of sap flux density (7) taking into account the point where the sap flux density approached to zero by fitting linear regressions to data (e.g., Nadezhdina et al., 2002, Cohen et al., 2008).

Finally, sapwood depth was expressed as percent of xylem radius to compare between the methods (SW_r). The t-student test for paired data was used for comparing the methods.

2.3. Experiment 2: Azimuthal variation in sap flux density

Azimuthal variation in sap flux density was studied by mean of HRM sensors (Burgess et al., 2001) with two radial measurements at 1.25 (outer measurement) and 2.75 (inner measurement) cm from bark. The HRM sensors were programmed to measure every 60 minutes, and were charged with 12-W solar panels connected to their internal batteries (ICT International, Australia). Each sensor consisted of three needles 35 mm in length and 1.3 mm in diameter. The needle placed in the centre is the heater that emits the heat pulse during 1 or 2 seconds, and the temperature increase is then systematically measured during 100 seconds in the other two needles at 0.5 cm upstream and downstream from the heater. Each couple of measures (inner and outer) is used to estimate the heat pulse velocity by considering a thermal diffusivity average value of 0.002 cm^2 s^{-1} estimated from the cores taken in the first experiment for sapwood determinations, following Burgess et al. (2001).

The measurements were continuously carried out from May to August 2014 in 6 trees within 2 different DBH classes (Table 2). Four sensors were inserted in each tree approximately 1.3 m above the ground at north, east, south and west aspects (following

Shinohara et al., 2013 and Lu et al., 2000). The sensors were placed at slightly different heights to avoid interferences among the readings. All trees were drip irrigated from May to June following the same irrigation scheme than in experiment 1. However, in order to study whether the ranking between the aspects were maintained in each tree due to induced soil water stress on azimuthal variation, drip irrigation was stopped from July on (Figure 1).

The heat pulse velocity was corrected for the effects induced by probe misalignment and wounding. Probe misalignment correction was done by comparing the baselines corresponding to zero sap flow during the first leafless week of measurement to the baselines from all the measurements. Wounding correction was applied in the same way than in the radial experiment (1.3+2x0.3 mm) but following the mathematical approximation of Burgess et al. (2001).

Sap flux density was estimated following the same methodology than that in the radial experiment (Barrett et al., 1995). Finally, tree sap flux was calculated from the sap flux density estimated for the inner and outer measurements assuming the sapwood area divided into two concentric bands delimited by the mid-point between them (Bleby et al., 2004). The calculations were made for the entire tree from each of the four aspects, and also for the average sap flux density.

3. Results

3.1. Radial variation of sap flux density

As expected, mean sap flux density was higher in all the irrigated trees (Figure 2) except for one couple of trees where the radial profile was very similar between the irrigated tree and the non irrigated one (trees 5 and 6, data not shown) (similar to the radial profile from tree 9). The differences in sap flux density were not constant along the radial profile, and differed among trees. The highest mean difference among irrigated and non irrigated trees was found in the most inner measurement at 3 cm from cambium.

The results of expressing sap flux density as relative to the expected maximum (ratio over the measurement at 1 cm from cambium) are presented in Figure 3. The radial profile pattern was very similar for all the non irrigated trees, with maximum sap flux densities from cambium to 1 cm depth (from 0.8 to 1.1 cm h^{-1} at the outer measurement) and a decrease from this distance on (except for one tree with a value of 0.45 in the outer measurement) until an average reduction of about 24 % at a distance of 3 cm from cambium, regardless the difference on sapwood depth between the trees (from 2.7 to 3.6 cm). In contrast, radial profile of the irrigated trees was less consistent in the outer part (average normalized values from 0.5 to 1), and also sap flux densities were lower. On the other hand, in the other point measurements, the radial profile in the non-irrigated trees (trees 1, 3, 5, 7, 9, 11 and 13) was very similar to those of the irrigated trees with smaller size (tress 2, 4, 6 and 8) but quite different from those of the biggest irrigated trees (trees 10, 12 and 14), still presenting high sap flux density in their inner xylem tissues (Figure 3). In this sense, it is important to remark that the sapwood depth range was very similar between the non-irrigated and the irrigated trees, from 2.9 to 3.6 and from 2.8 to 4.3 cm respectively (see Table 1).

The unexpected, high difference found in the outermost measurements between the irrigated and the non irrigated trees (mean normalized sap flux density of 0.5 versus 0.9) made us reconsider the quality of the raw data measured with the CHPM sensors. In Figure 4 we show two examples of heat pulse velocity from the four radial measurements taken in two trees during one day: the inconsistencies in the measurements were especially important in the outermost thermocouples (0.5 cm from cambium) as indicated by high fluctuations in the heat pulse velocity values, while in the other measurements no sudden fluctuations were observed during the day. These fluctuations appeared in all the tested trees, so we decided to discard the outermost measurements for the rest of the analysis. However, we decided to keep these data in Figures 2 and 3 to show the effect of the presence of bark and cambium in the outermost measurements in cherry trees.

3.2. Comparison of methods to determine sapwood

Basic density of wood and volumetric water content were not significantly different between the irrigated and the non irrigated trees, ranging from 388.5 to 513.9 kg m^{-3} and from 171.9 to 302.7 kg m^{-3} respectively. Radial variation of basic density of wood and volumetric water content was different depending on tree, but a systematic decrease with depth was found for volumetric water content in the irrigated trees (Figure 5). However, this pattern was not consistent enough to distinguish between sapwood and heartwood, and consequently this method was considered not useful to determine the limit between these two tissues in cherry trees.

The results for the rest of methods are presented in Table 3. For the VD_m, three regions were clearly observed in all cores, with the darkest part close to tree pith, identified as heartwood, and turning lighter when moving outer. In the case of radial variation of sap flux density, linear regressions (R^2 values higher than 0.9) were fitted to all data excluding the outermost readings (as explained in Figure 4), except for trees 10, 12 and 14 where the inner sap flux density remained higher than 60 % of the maximum expected, as indicated in Figure 3. On the other hand, with the DI_m we found a discontinuous pattern for all the trees, with coloured bands (the expected conducting tissue) and non coloured ones alternating along the xylem radius. In order to estimate the sapwood depth, we assumed that the limit between sapwood and heartwood was located in the most inner part of the last coloured band found.

Paired t-student tests used to compare the methods in Table 3 showed that SW_r was significantly different between the methods, and estimation from DI_m resulted in the lowest values. This type of results was also found when including the four complementary measurements carried out during the last week of the experiment in 4 extra trees sampled at a closer distance between the cores (5 instead of 15 cm).

3.3. The effect of irrigation on heartwood formation

The influence of irrigation on heartwood formation was assessed by comparing the relationships between tree diameter and heartwood diameter for irrigated and non irrigated trees, obtained with the VD_m method (Figure 6). Heartwood diameter was very similar in both treatments for diameter lower than 10 cm (non significant differences at p-level < 0.001). However, for bigger trees, irrigated individuals had lower heartwood diameters than those of the non irrigated ones, as indicated by a lower value of the a parameter in the quadratic functions fitted to all the data (0.14 versus 0.53).

3.4. Azimuthal variation of sap flux density

Diurnal courses of sap flux densities followed a bell-shaped pattern in all the studied aspects during both periods, with and without irrigation. The coefficient of variation (CV) of the 24 hourly sap flux densities from the 4 aspects of each tree (from the mean of the measurements at two sapwood depths), was higher in the period without irrigation and ranged from 11 to 75 and from 27 to 81 % per tree respectively.

The azimuthal variation of sap flux density for each tree, i.e. the ranking between the aspects for each tree, was expressed as the relative differences over the mean as follow: $RD = ([(SF_i - SF_{mean})/SF_{mean}]/N) \cdot 100$, where SF_i was the daily average from one particular aspect, SF_{mean} the daily average from the 4 aspects measured, and N the number of days studied.

During the irrigation period, RD was different depending on the sample tree. For instance, tree 16 showed the lowest sap flux density at the north side (-85±2 %), while it was the highest at this aspect for tree 18 (50±7 %). In contrast, tree 19 showed very similar values in all the studied aspects (around 0 %) (Figure 7, left).

The soil water deficit induced by stopping irrigation produced a mean general decrease of 92 % in tree sap flux. The variability between the aspects was higher than in the irrigation period, with a mean increase of the CV values of 23±21 and 35±32 % for the outer and inner measurements, respectively. In contrast, the ranking between the aspects was quite constant for each tree between the two periods (except for tree 15), as indicated by very similar average and standard deviation values for the RD between the irrigation and the without irrigation periods for each tree (Figure 7).

Cumulative tree sap flux calculations were made for each of the four aspects considering sap flux density in each of them as representative of all the sapwood area. As a way to determine which aspect was most representative of the overall cumulative tree sap flux, these calculations were expressed as a percentage of the mean of the four aspects. Average± standard deviation values were 88.3±46.9, 106.4±34.4, 108.7±42.7 and 96.6±38.1 % for north, south, east and west aspect respectively.

4. Discussion

In this work we evaluated the effects of irrigation on radial and azimuthal variation of sap flux density in 8 years old timber-orientated cherry tree plantation in a Mediterranean area of Spain. Moreover, several methods for estimating the conducting sapwood area have been tested in order to evaluate its likely effect on the scaling up from sap flux density to tree sap flux.

4.1. Radial variation of sap flux density

Several studies have reported a decrease of sap flux density as a result of declines in soil water content, naturally induced through a contrasted dry season or by comparing different irrigation practices (Lu et al., 2000; Philipps et al., 1996). In this study we simultaneously measured drip irrigated and non-irrigated ones, during the dry season (from July to September). We found that irrigated trees showed higher sap flux densities than the non-irrigated ones, but differences were not constant along the xylem radius, with mean differences of 2.5, 2.6 and 5.4 times higher for distances of 1.2, 2.1 and 3.2 cm from bark, respectively. These differences were consequence of a different decrease of sap flux density with sapwood depth related to tree size, with similar patterns for the non irrigated trees and the smaller irrigated ones (7 trees and 4 trees respectively) and a contrasted pattern for the bigger irrigated ones (3 trees) (Figure 3).

The decreasing of sap flux density with sapwood depth is frequently found in literature (e.g., Ford et al., 2004; Cohen et al., 2008) and functions such as the Weibull or the Gaussian ones are frequently used for describing the flow shape (Gebauer et al., 2008;

Kubota et al., 2005). In this sense, Nadezhdina et al. (2002) found a peaked distribution on the radial sap flux density pattern (with the maximum at 80 % of total radius) of a tree species very similar to the one studied here, i.e., *Prunus serotina*, but 19 years old. In our study, the outer thermocouples, placed at 0.5 cm from cambium, were discarded because of their inconsistent behaviors (Figure 4), and thus we could not conclude whether the radial sap flux pattern of *P. avium* was characterized by a monotonous decrease of sap flux with depth or by a peaked distribution, as the two most common (Cohen et al., 2012). However, we believed that showing the effect of not removing the non homogenous tissue (bark and cambium) has on the quality of the readings was important due to this fact is not usually mentioned in the related literature, especially for species with small bark thickness. This inconsistent behavior was the result of bark and cambium interferences in the measurements (Steve Green, personal communication); first, heat pulse emitted by the heater is likely affected by this sharp change on water conductivity and then heat pulse is not propagated correctly (Steve Green, personal communication), and second, the measurement radius of thermocouples should probably be higher than 3 mm as in other heat pulse methods (taking into account that our non conducting tissue was approximately of 2 mm depth) and thus readings would be accordingly affected. Moreover, the high and persistent difference found between the irrigated and the non irrigated trees seems to indicate that despite the difference on depth of non conducting tissue was about 0.3 mm, this small difference could lead to important effects of the abovementioned explaining aspects.

In addition, when only considering data from of all our non irrigated trees together with the 4 smaller irrigated ones, the radial profile showed similar decreases on the normalized data and consequently a consistent difference of about 2.5 times along the radial profile in the absolute data. However, the other 3 irrigated trees (the biggest trees in the experiment) showed lower radial variations and less pronounced decreases with depth, and even high sap flux densities in the inner tissues (tree 14 in Figure 2). This result is in contrast with the results observed in maritime pines of different sizes, with steeper declines in the trees with the biggest sizes (Delzon et al., 2004). These trees had a small difference of about 1 cm in the sapwood depth as compared to the non irrigated ones with bigger size (see next section), thus tree size was not the only responsible of these differences (Čermák and Nadezhdina, 1998) but also the irrigation applied during 4 growing seasons (during the experiment presented here and the 3 previous years). It is widely accepted that leaves are connected to new xylem and though xylem can be viable to a large depth in the xylem, sap movement will not necessary occur due to the increasing resistance to lateral flow with xylem depth (Cohen et al., 2012). We found significant differences on leaf area index between irrigated and not irrigated trees of bigger size (data not shown), and also high differences in sap flux density (Figure 2). This supports that, though xylem is viable for both type of trees, irrigation seems to induce changes in the xylem which is actively conducting water in order to maintain the ratio of cross sectional area of actively conducting xylem to leaf area constant (Cohen et al., 2012).

4.2. Sapwood determination and heartwood formation

Our results have shown that several methods, which have been used for delimiting sapwood depth in other species (methyl orange, lugol, and radial variation of xylem density and xylem water content), were not suitable for delimiting the sapwood depth in *Prunus avium* and thus these methods are not recommended for scaling purposes for this species. In this sense, Čermák and Nadezhdina (1998) found that the radial variation of xylem water content was useful to delimit the sapwood depth for some species but not for others. In contrast, the rest of the methods showed clear differentiation between sapwood and heartwood tissues but significantly different values for the sapwood depths. Visual identification carried out in ring cores is related with the different chemical processes taking place in the two tissues, and our results are in agreement with those from Nadezhdina et al. (2002), who found three different colored parts in ring cores of *Prunus serotina* and a sapwood depth very similar to ours (75 % of the xylem radius versus 80 %). However, while these authors found a clear relationship between these three different colored parts and the sap flux density measured along the xylem radius, normally considered as a robust proxy for sapwood depth estimation (Cohen et al., 2008; Poyatos et al., 2007), this was not the case in the present study. Though we found a significant difference between these two methods (13 % in average), this bias may be considered as negligible for scaling purposes (from point measurement to plantation transpiration), because of the low value of sap flux density at deeper depths (and consequently the sap flux at these depths) in the trees where the estimation of sapwood depth was accomplished with the sap flow measurements (11 out of 14 trees). In contrast, the high sap flux densities at the inner depths of the other 3 bigger irrigated trees did not match with the sapwood depth values found in the other two suitable methods, and how it was discussed before it remains an open question for further studies, especially when considering that this could introduce important errors when scaling from measurement to tree transpiration.

In addition, a particular result was found for the dye injection method, with a systematic lower value (average of 25 %) of the xylem radius in all the tested trees, as compared with those from either the radial profile of sap flux density or the visual identification. This may be related with the discontinuous pattern of the water conducting areas showed by the dye injection method. This pattern is more related to ring porous species with water conducting regions and non conducting regions within the sapwood (Tsuruta et al. 2010), and thus was unexpected due to the semi-diffuse porous wood of *Prunus avium* (García-Esteban et al., 2003). In this sense, the results of Boumghar (2012) when using dye to differentiate between sapwood and heartwood showed that all the sapwood was coloured in the studied ring porous species so this discontinuous pattern was not found. We thus hypothesize that an artifact could be introduced with the dye injection method and thus complementary anatomical studies are needed in this species.

On the other hand, heartwood formation was affected by irrigation for tree diameters bigger than 10 cm (Figure 6), pointing out again the hypothesis that the inner tissues of the bigger trees in our experiment, subjected to cumulate water stress periods underwent anatomical changes as the result of faster growth and bigger diameter. This result seems to be an open research question to be resolved for the wood industry, because the presence of heartwood in high quality woods is nowadays increasing their market values (Vilanova, personal communication).

4.3. Azimuthal variation of sap flux density

Sap flux density was measured in four different aspects of six trees (24 sensors) during two periods, the first characterized by irrigation and the second one by no irrigation and probably a certain degree of water stress given by summer conditions (high potential evapotranspiration, low rain).

The decrease in tree sap flux from one period to the other was as expected for the species, with a general reduction of 92 % in tree sap flux compared to a reduction of 85 % when comparing between irrigated trees and non irrigated trees observed during one experiment carried out in summer under Mediterranean climate (Cabibel and Isbérie, 1997). This indicates the high sensibility of this species to dry periods characterized by high soil water deficit and water evaporative demand, as other authors have already pointed out (e.g. Juhász et al., 2013; Cabibel and Isbérie, 1997). On the other hand, the sap flux density variability within each tree (CV of the 4 aspects) was higher during the water deficit period, with a mean increase of about 30 % respect to the irrigation period. In contrast, the ranking between the aspects for each tree was quite constant between the periods, pointing to a relevant weight in the azimuthal pattern of the individual structural conditions in each tree.

Azimuthal variability in sap flux density can be larger than the radial variability (e.g., Shinohara et al., 2013). However, this variability has been found less predictable, because it can be explained by differences on tree exposure to sun (Granier, 1987), soil water content around the tree (Cabibel and Isbérie, 1997), anatomical xylem structure (Tateishi et al., 2008) or crown architecture (Lu et al., 2000). In this sense, we expected the azimuthal variability to be highly controlled by the soil water content around trees with a virtually negligible effect of ET_0, as suggested by the results of Cabibel and Isbérie (1997) in a drip irrigation experiment (with 2 emitters of 4 L h^{-1}, 1 m east and 1 m west from trunk) in cherry trees. However, we found no connection between the soil water content around the tree (emitters placed north and south sides from trunk, 0.8 m^2 each wet bulb) and the ranking in the sap flux density of the different aspects during the irrigation period. We thus postulate that most of the primary roots were affected by drip irrigation, and consequently no clear connections or preferential fluxes between roots and leafs from the same aspect could appear. In addition, the way the trees were pruned led to not systematic differences on tree architecture and hence on azimuthal variability of anatomical xylem structure, which could also affect the azimuthal pattern of sap flox density in the studied trees. Therefore, when we compared the overall tree sap flux estimated considering the sap flux density from each aspect and the one considering the mean sap flux density, all of them performed similarly for all the studied trees, with average tree sap flux from each aspect not being significantly different than tree sap flux estimated from the average sap flux density.

Conclusions

As presented in this work, the maximum sap flux density in our cherry trees of 8 years old is located at 1 cm from cambium or closer, because the values in inner tissues were of lower magnitude in all the studied trees and consequently decreasing profiles with depth were observed. Our sensors also measured sap flux density at 0.5 mm from cambium, but the readings were finally discarded due to interferences promoted by the presence of bark and cambium on the readings. However, we did not find any advice regarding this fact in either the CHPM manual or articles using this method, so we decided to show the effect that this thin non conducting tissue had on the readings. In contrast, in the HRM method, as the one we used in the azimuthal experiment and in the previous measurements from 2011 to 2013, the outermost measurements (at 1.25 cm from cambium) are not affected by bark+cambum depth lower than 5 mm, which is properly described in the corresponding manual. Therefore, further studies are necessary to assess the sap flux density close to the cambium in order to a better assessment of the radial profile shape in cherry trees for timber production.

The different methods to estimate sapwood gave contrasted results and a number of them are considered as not suitable. The differences found between visual identification and radial profile methods can be considered negligible for scaling purposes due to lower values of sap flux density at these depths.

The effect of irrigation on spatial variations of sap flux density affected to different extents. All the irrigated trees gave higher sap flux densities along the xylem radius and the radial profiles of normalized values were similar between irrigated and non irrigated trees. However, more measurements are needed in the inner depths of irrigated trees of bigger sizes as shown in our results. On the other hand, the azimuthal variation was found to be unpredictable and we consequently conclude that any aspect can be selected to measure sap flow in young cherry trees drip irrigated and pruned to timber production.

Acknowledgments

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Figures Captions

Figure 1. Mean sap flux density (1 data every hour) of two sampled trees in the azimuthal experiment (see Table 2 for details).

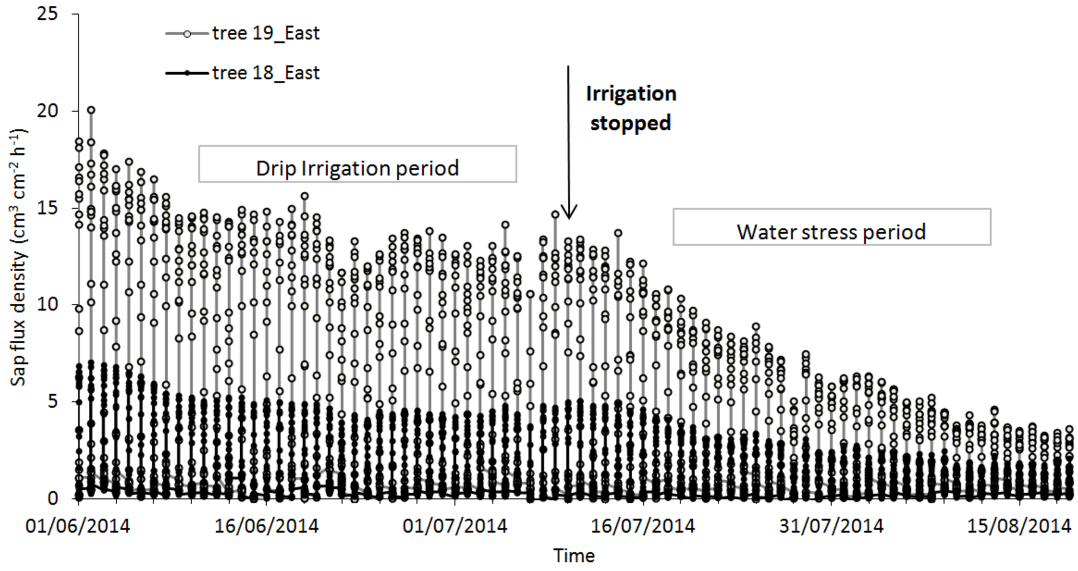

Figure 2. Examples of radial patterns of sap flux density in 4 irrigated trees (I) and 4 non- irrigated trees (NI). Data shown per tree is the average of all measurements during 7 seven days and standard deviations are not shown to improve the understanding. Tree characteristics are shown in Table 1. Note that all the studied trees were not shown because they behaved in a similar manner that the ones presented

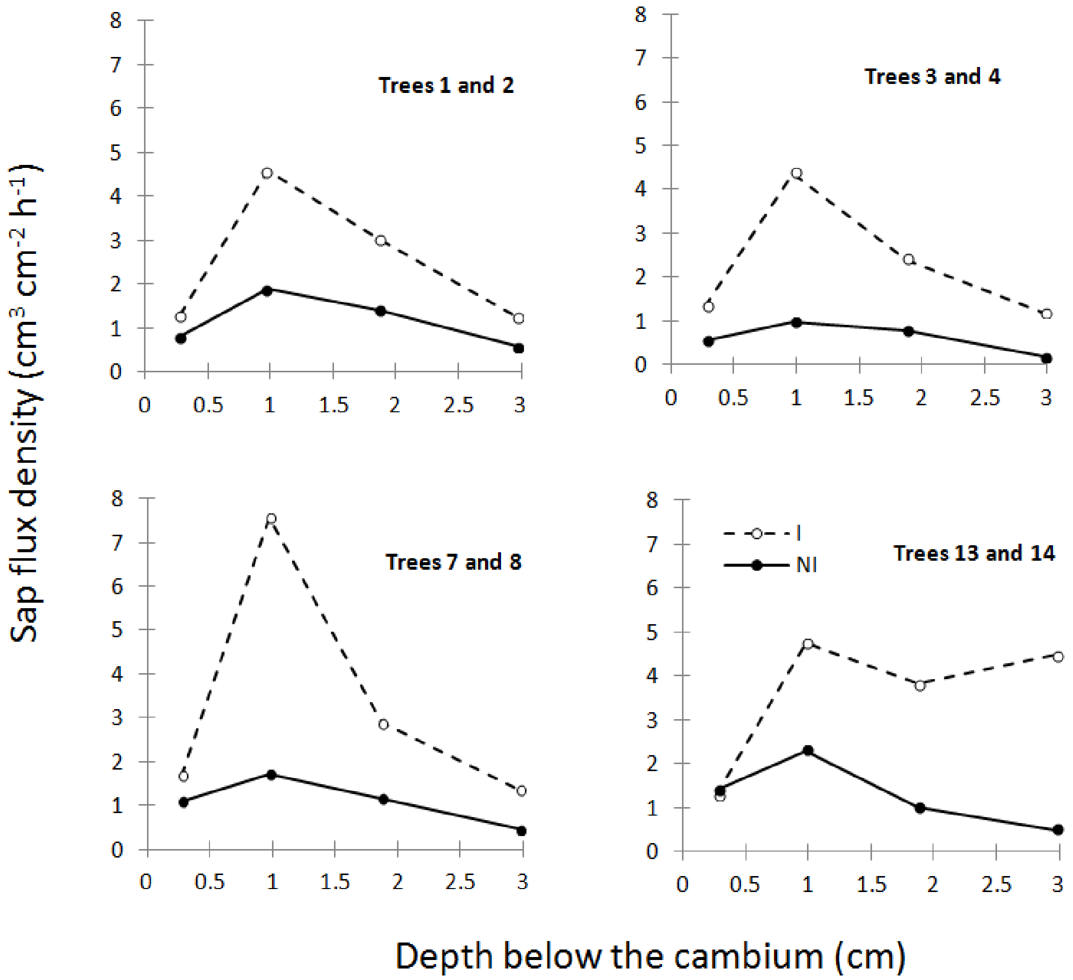

Figure 3. Normalized sap flux density (over the measurement at 1.2 cm from bark) in the irrigated (I) and the non-irrigated trees (NI). Tree characteristics are shown in Table 1.

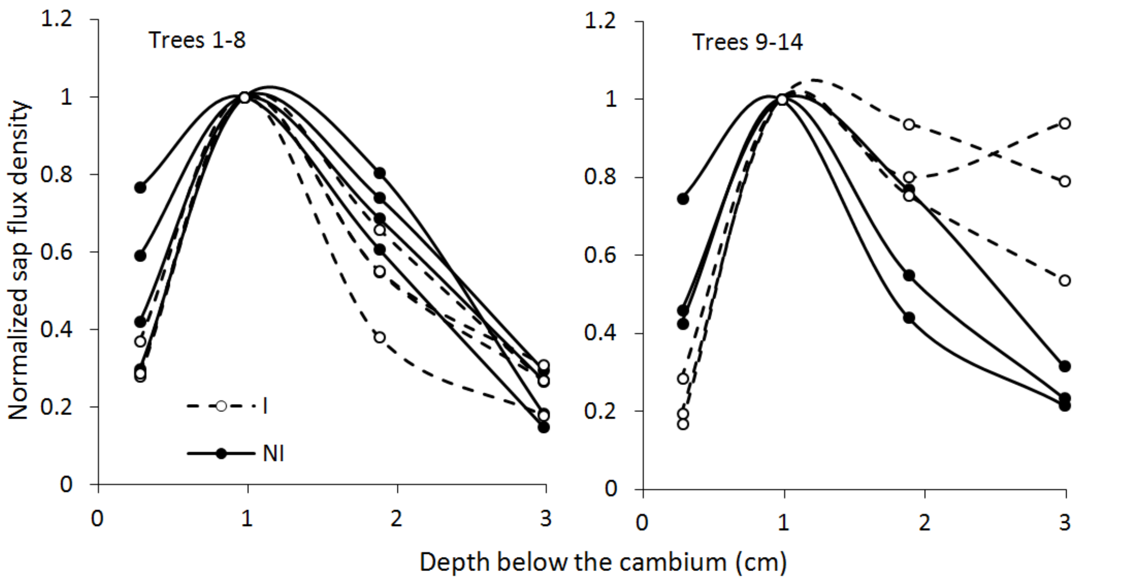

Figure 4. Raw data (heat pulse velocity) from two CHPM sap flow sensors during 1 day of measurements in two trees (one measurement each 30 minutes) at four depths from cambium.

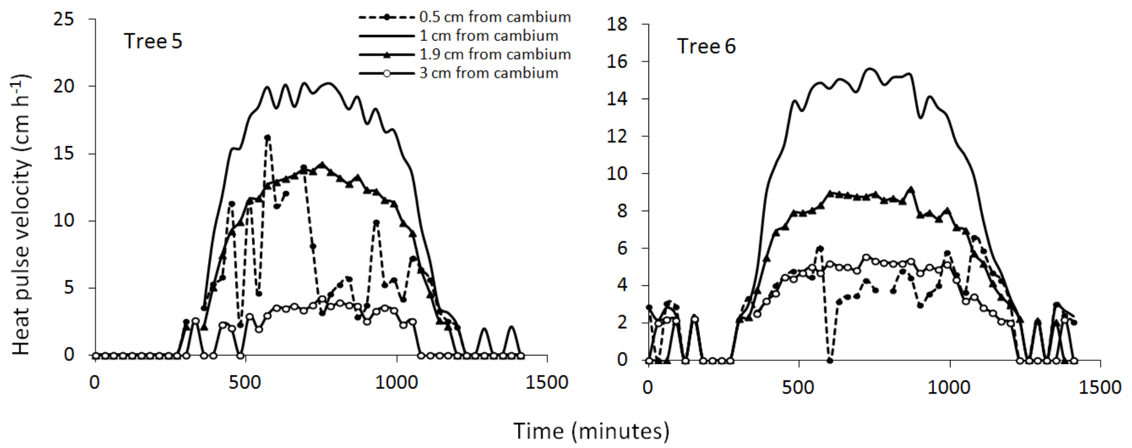

Figure 5. Radial profiles of volumetric water content and basic density (kg m^{-3}) in the irrigated (open symbols) and the non-irrigated trees (closed symbols). Only R^2 values from significant linear regressions were presented.

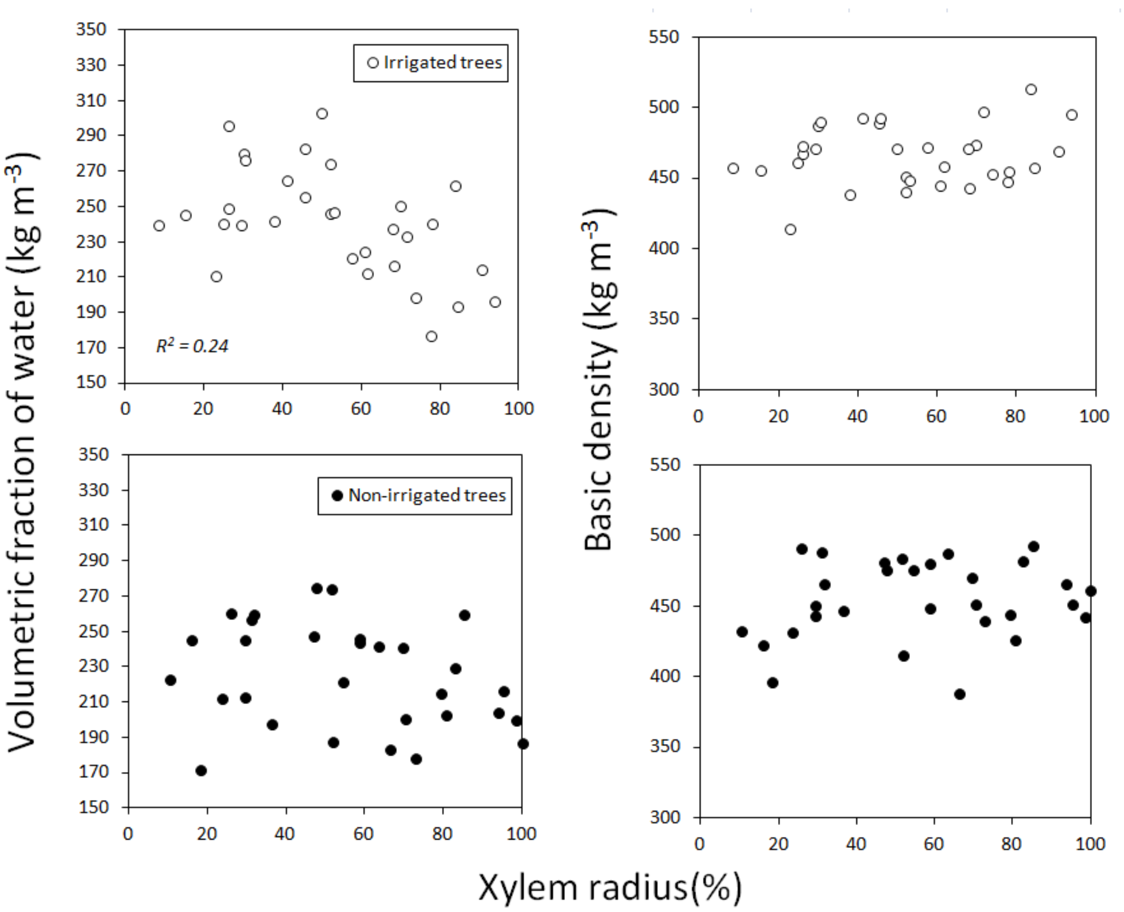

Figure 6. Relationship between trunk diameter (cm) and heartwood diameter (cm) for the irrigated and the non-irrigated trees. Quadratic functions ($y=a\ x^2+b\ x$) were fitted and forced through the origin.

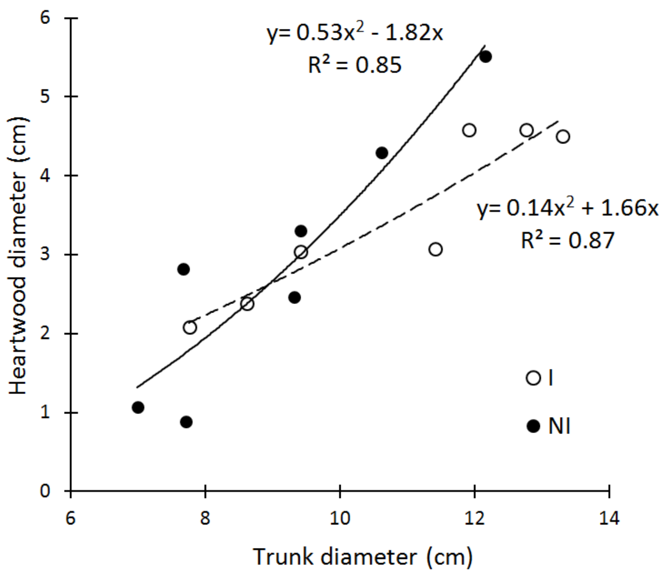

Figure 7. Relative differences over the mean (average and standard deviation values, %) for each study tree and for the both studied periods, the irrigation and non irrigation ones.

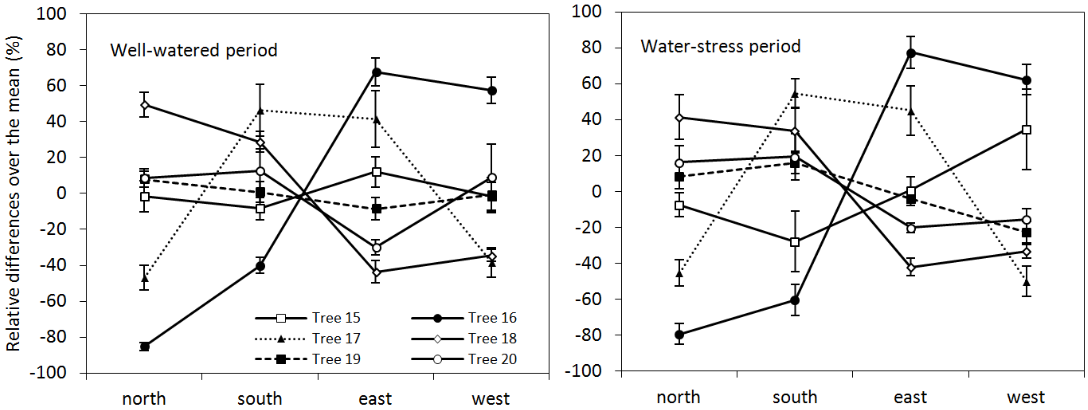

Table 1. Diameter at breast height (DBH) and sapwood depth, estimated from colour change in wood cores (VD_m, see text for details), of the studied trees in the radial experiment. Drip irrigated (I) and non-irrigated trees (NI).

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

Table 2. Diameter at breast height (DBH) and sapwood depth, estimated from the relationship with diameter obtained with the VD_m (see text for details), of the studied trees in the azimuthal experiment. I: Irrigation period, NI: water stress period (see 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)

Table 3. Sapwood depth relative to xylem radius determined from: visual differentiation based on colour change (VD_m), dye injection (DI_m) and radial profile of sap flux density (SF_r). Note that for trees 11, 12 and 14 linear regressions were not fitted to the sap flow data. Different letters indicate significant mean differences in the paired 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*