

Calibration of sap flow estimated by the compensation heat pulse method in olive, plum and orange trees: relationships with xylem anatomy

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Summary The compensation heat pulse method is widely used to estimate sap flow in conducting organs of woody plants. Being an invasive technique, calibration is crucial to derive correction factors for accurately estimating the sap flow value from the measured heat pulse velocity. We compared the results of excision and perfusion calibration experiments made with mature olive (*Olea europaea* L. ‘Manzanilla de Sevilla’), plum (*Prunus domestica* L. ‘Songal’) and orange (*Citrus sinensis* (L.) Osbeck. ‘Cadenero’) trees. The calibration experiments were designed according to current knowledge on the application of the technique and the analysis of measured heat pulse velocities. Data on xylem characteristics were obtained from the experimental trees and related to the results of the calibration experiments. The most accurate sap flow values were obtained by assuming a wound width of 2.0 mm for olive and 2.4 mm for plum and orange. Although the three possible methods of integrating the sap velocity profiles produced similar results for all three species, the best results were obtained by calculating sap flow as the weighted sum of the product of sap velocity and the associated sapwood area across the four sensors of the heat-pulse-velocity probes. Anatomical observations showed that the xylem of the studied species can be considered thermally homogeneous. Vessel lumen diameter in orange trees was about twice that in the olive and plum, but vessel density was less than half. Total vessel lumen area per transverse section of xylem tissue was greater in plum than in the other species. These and other anatomical and hydraulic differences may account for the different calibration results obtained for each species.

Keywords: Mediterranean crop trees, transpiration, water consumption, wood characteristics.

Introduction

Sap flow measurements in conductive organs of plants are widely used to determine both water consumption and the dy-

namics of transpiration and water uptake by roots. Several methods for determining sap flow have been developed (Wullschleger et al. 1998, Kucera 2002, Čermák et al. 2004), their potentialities and limitations have been evaluated (Jones et al. 1988, Smith and Allen 1996, Köstner et al. 1998) and a unified nomenclature for sap flow measurements has been proposed (Edwards et al. 1996). Most of the methods fall within one of two categories: heat balance methods, which rely on measurements of the components of heat transport from a continuous heat input, and heat pulse methods, which use heat pulses for determining the sap velocity.

Among the heat pulse methods, the compensation heat-pulse (CHP) method—originally conceived by Huber and Schmidt (1937) and later refined (Marshall 1958, Swanson and Whitfield 1981, Green 1998)—has been used in several fruit tree species, including kiwifruit (Green et al. 1989), apple (Green et al. 1989, 2003), pear (Caspari et al. 1993), apricot (Alarcon et al. 2000, 2003, Nicolás et al. 2005), lemon (Ortuño et al. 2004) and olive (Moreno et al. 1996, Fernández et al. 2001, 2003, Giorio and Giorio 2003, Williams et al. 2004). The CHP method is invasive, requiring the insertion of two temperature probes and a heater into the xylem at the location where the sap flow is to be estimated. This disrupts the sap stream and alters the thermal homogeneity of the surrounding sapwood, causing a systematic underestimation in the measured heat pulse velocity (Cohen et al. 1981, Green and Clothier 1988). Consequently, correction factors relating the measured heat pulse velocity (v_h) to actual sap flow (Q) are needed to account for the influences of probe thermal properties and flow blockage.

The correction factors depend mainly on the material used to build the probes, on the wound width and on the distances from the heater to the probes inserted downstream and upstream. Anatomical investigations in rain forest and eucalypt forest species, led Barrett et al. (1995) to conclude that total wound width extends to about 0.3 mm on either side of the drill hole. The actual wound effect, however, depends on the tissue

thermal characteristics, which vary among species (Green and Clothier 1988, Swanson 1994). As a consequence, it is impossible to predict the actual wound size for a particular species, although it must be greater than the diameter of the drill hole. In addition, the reaction to wounding and heating is species dependent and exhibits temporal variation (Swanson and Whitfield 1981, Marshall et al. 1989). Swanson (1983) and Green and Clothier (1988) reported that wound width was related to the diameter and density of the xylem vessels.

Previously, we calibrated the CHP method for olive (*Olea europaea* L., 'Manzanilla de Sevilla') (Fernández et al. 2001) using the heat pulse velocity (HPV) system designed by Green (1998), which consists of Teflon temperature probes inserted upstream at distance $x' = 5$ mm and downstream at distance $x = 15$ mm from the heater. To estimate sap flows from heat pulse velocity measurements, Fernández et al. (2001) used the correction factors of Green and Clothier (1988). In 2003, Green et al. published new correction factors for x distances of 10 and 15 mm. Because x is one of the main parameters considered when choosing the right correction factors, the calibration results obtained by Fernández et al. (2001) in olive need validation.

The first aim of this study was to calibrate the CHP method for olive (*Olea europaea* 'Manzanilla de Sevilla'), plum (*Prunus domestica* L. 'Songal') and orange (*Citrus sinensis* (L.) Osbeck. 'Cadenero') based both on the latest HPV system developed by Green and the new correction factors of Green et al. (2003) and for $x' = 5$ mm and $x = 10$ mm. The only other published study we have found on the calibration of the CHP method for plum and orange was conducted with young potted trees of cultivars different from those studied here (namely, *Prunus salicina* Lindl. 'Santa Rosa' and *Citrus sinensis* 'Mm. Vinous') with the corrections factors of Green and Clothier (1988) (Zreik et al. 2003). The second aim of our study was to obtain information on the sapwood characteristics of the study species in an attempt to account for the different calibrations results found for each.

Materials and methods

Calibration experiments

In the spring of 2004, a set of experiments was performed to calibrate the CHP method, as described by Green and Clothier (1988) and Green et al. (2003), for olive, plum and orange trees. An excision experiment and a forced-flow perfusion experiment, similar to those described by Fernández et al. (2001), were carried out for each species (Table 1). Each probe set used for the heat pulse velocity measurements consisted of two 1.70-mm-diameter Teflon temperature probes and one 1.63-mm-diameter stainless steel heater with a $5 \Omega \text{ m}^{-1}$ nichrome resistance wire inside. Each temperature probe had four thermocouple sensors that were installed at depths of 5, 15, 25 and 40 mm below the cambium. The x' and x distances were 5 and 10 mm, respectively. We used the drill bits (1.8 mm diameter) and jig provided by the maker and confirmed that the drill holes were parallel. A heat pulse of 0.75 s was fired

every time sap flow was to be recorded. We used the latest version of the probes and associated electronics and software (Environmental and Risk Management Group, HortResearch, Palmerston North, New Zealand). The system was controlled by a Campbell CR10X data logger and powered by a 12 V battery.

Excision experiments were made on one tree per study species (Table 1), which had been well irrigated for 15 days before the start of the experiment. Two hours before dawn of the first day of the experiment, we cut one tree at ground level. The butt was placed in a reservoir of water and the trunk recut under water 0.15 m from the butt to remove air emboli created after the first cutting. The tree was kept in an upright position by a metal support with the cut end submerged in 0.05 mol l^{-1} KCl solution made with distilled water and to which was added 50 ml of 3% safranin that had been filtered through a $0.45 \mu\text{m}$ membrane. The total solution volume was 120 l. Just before dawn, we inserted three sets of probes equally spaced around the azimuth of the trunk. Heat pulses were fired every 30 min and the time delay (t) for the temperatures at points x and x' to become equal were registered. The t values were used to calculate v_i at the four measurement points of each probe set, following Swanson (1962). We added vegetable oil to the reservoir to avoid water loss by evaporation and refilled the reservoir to a constant level immediately following each heat pulse. Thus, the volume added to the reservoir was equal to that of the water taken up by the tree (E) during each 30-min period. The system was maintained for 48 h for each of the studied trees. One day after excision, however, the trees exhibited severe stress symptoms (cf. Green and Clothier 1988, Fernández et al. 2003) and water uptake decreased markedly. Onset of stress is believed to be a result of clogging of the xylem vessels at the cut stem base caused by phloem exudates and particles in the KCl solution. At the end of each excision experiment, the trunk was cut transversally at the probe locations and sapwood area (S , m^2) estimated by planimetry of the safranin-stained area.

Before the trees were felled, water status and gas exchange measurements were determined. Leaf water potential (Ψ_l , MPa) was assumed to equal the xylem pressure potential at the petiole, as measured with a pressure chamber (Soilmoisture Equipment Corp., Santa Barbara, CA). Measurements of Ψ_l were made every 2 h from dawn to sunset, in 16 healthy current-year leaves located at positions all around the canopy. During the Ψ_l measurements, we used a portable photosynthesis system (LI-6400, LI-COR, Lincoln, NE) to determine stomatal conductance (g_s , $\text{mol}^{-2} \text{ s}^{-1}$) in 20 healthy current-year leaves at positions all around the canopy.

The perfusion experiments were made with 0.3-m-long trunk sections from one tree of each species, of similar characteristics to those used in the excision experiment (Table 1). After removing the bark, the trunk section was attached to the aluminum box of the high-pressure device used by Fernández et al. (2001) to force the perfusion solution through the section. The trunk section was sealed to the aluminum box with epoxy resin and silicon rubber, taking care not to contaminate the xylem (Fernández et al. 2001). The 0.05 mol l^{-1} KCl perfu-

Table 1. Details of the excision and perfusion experiments. Potential evapotranspiration (ET_o) was estimated with the FAO56 Penman-Monteith equation from meteorological measurements taken locally. Leaf water potentials are mean values for 16 leaves distributed around the canopy, measured at predawn (Ψ_{pd}) and noon (Ψ_{md}). Leaf area (LA, one side) of the trees and the sapwood area were estimated as described in the text. Abbreviation: DOY = day of year.

Experiment	<i>Olea europaea</i> 'Manzanilla'	<i>Prunus domestica</i> 'Songal'	<i>Citrus sinensis</i> 'Cadenero'
<i>Excision experiment</i>	DOY 111	DOY 167	DOY 175
Daily ET_o (mm)	3.9	6.8	6.5
Ψ_{pd} (MPa)	-0.19	-0.17	-0.11
Ψ_{md} (MPa)	-1.07	-0.58	-0.79
Tree age (year)	15	5	16
Tree height (m)	2.60	2.18	3.2
Canopy diameter (m)	2.50	1.65	3.5
LA (m ²)	12.76	16.41	28.71
Xylem radius ¹ (mm)	49	35	52
Height of measurement ² (m)	0.77	0.40	0.80
Sapwood area (m ²)	0.00572	0.00334	0.00795
<i>Perfusion experiment</i>	DOY 89	DOY 128	DOY 106
Length of trunk section (m)	0.3	0.3	0.3
Xylem radius ¹ (mm)	41	40	39
Sapwood area (m ²)	0.00449	0.00410	0.00481
Height of measurement ² (m)	0.80	0.40	0.40

¹ Radius of the trunk without the bark at the probe locations.

² Mean height above the ground of the portion of the trunk where the probes were located, measured before cutting each tree.

sion solution containing 3% safranin, identical to that used in the excision experiments, was pressurized to 0.14 MPa for olive and 0.13 MPa for orange. For plum, sap flow reached the maximum limit measurable for the system at the pressure of 0.05 MPa. Once a steady flow rate at each selected pressure was achieved, we fired a heat pulse and the t values measured with three sets of probes installed at equal spacings around the azimuth of the trunk were recorded. The actual flow through the trunk section (F) was collected at the distal end, which was vented to the atmosphere. At the end of each perfusion experiment, S was determined following the procedure described for the excision experiments. The volume fractions of wood (F_M) and water (F_L) were measured as described by Green (1998), in wood samples (5 mm diameter, 40 mm length) taken with an increment borer in the vicinity of the probe sets. The wood samples were taken at the end of each perfusion experiment and at noon on each day of the excision experiments.

Values of t were analyzed as described by Green et al. (2003). Sap flux density (J_s , mm h⁻¹) data were calculated as in Edwards and Warwick (1984):

$$J_s = (kF_M + F_L)v_c \quad (1)$$

where k is a factor related to the thermal properties of the woody matrix and is assumed to have a constant value of 0.441 (Becker and Edwards 1999), and v_c (mm h⁻¹) is v_h corrected for wounding. Values of Q (l h⁻¹) were calculated by integrating the measured sap-velocity profile, $J(r)$, over the area of conducting sapwood (Green and Clothier 1988):

$$Q = \int_H^R 2\pi r J(r) dr \quad (2)$$

where R (m) is sapwood radius, H (m) is radius of the heartwood, and r (m) is radial distance from the center of the stem. In practice, this integral must be approximated with a least-squares regression fit to the measured velocity profile. The analysis software developed by Green et al. (2003) allows the user to choose a wound width, from 1.6 to 3.4, and produces three output files (*.vel, *.flx, *.sum) corresponding to the three methods of integrating the measured sap velocity profiles over the area of conducting sapwood (Green 1998):

In Method 1 (*.vel output file), Q is calculated by integrating the fitted profile of $\langle J(r) \rangle$:

$$Q = 2\pi \int_H^R r \langle J(r) \rangle dr \quad (3)$$

where the operator is used to represent the regression fit.

In Method 2 (*.flx output file), Q is calculated by integrating the fitted profile of $\langle rJ(r) \rangle$:

$$Q = 2\pi \int_H^R \langle rJ(r) \rangle dr \quad (4)$$

In Method 3 (*.sum output file), Q is calculated as the weighted sum of the product of sap velocity, v_{ei} , and the associated sapwood area, A_i across the n sensors:

$$Q = \sum_{i=1}^n A_i v_{ci} \quad (5)$$

For each experiment, we compared the volumes of solution flowing through the trunk with the three output files corresponding to Equations 3–5 for each set of correction factors (Green et al. 2003) for wound widths ranging from 1.8 to 2.8 mm. This comparison allowed us to establish which combination of wound width and integration method gave the best agreement to the actual values. That is, we did not measure the actual wound width, but selected a virtual wound width corresponding to correction factors that produced the closest fit between sap-flow-derived and empirical measurements of water use.

Xylem anatomical measurements

Wood samples were collected from one tree per species growing next to the excised trees, at a height similar to that at which the probes were located (Table 1). We used a 5-mm-diameter increment borer to take a 40-mm-long core from the south side of each trunk. Three to five transverse sections of 0.6 mm were taken from each core, both at 5 and 25 mm below the cambium. Because plant anatomy is influenced by ambient conditions (Schweingruber 1990), we investigated the characteristics of the xylem tissue at the two depths monitored by the probes. The wood samples were dehydrated in an acetone series and embedded in SPURR resin. Sections were cut with an ultramicrotome with a 6-mm-wide 45° angle glass blade, stained with toluidine blue and photographed with a camera attached to a confocal microscope. Vessel dimensions (lumen diameter and wall thickness) and distance between groups of vessels were determined with Leica Confocal Software (Leica Microsystems, Wetzlar, Germany). The distribution of the resulting sets of data was assessed for normality, kurtosis and skewness by the Kolmogorov-Smirnov test. A Student's *t* test was used to assess differences between mean values. Statistical analyses were carried out with the software program SPSS 11.5 for Windows (SPSS, Chicago, IL).

Results

Excision experiments

Mean minimum leaf water potentials recorded on the first day of the excision experiments were -1.07 MPa at 1145 h GMT for olive, -0.59 MPa at 1710 h GMT for plum and -0.79 at 1435 h GMT for orange, indicating that none of the trees suffered from excessive water stress on the first day following excision. However, the excised trees exhibited symptoms of severe water stress after 30–32 h; therefore, only the data obtained on the first day of the excision experiments were used to compare actual and estimated water consumption (Figures 1–3). Table 2 shows the actual amount of water taken up from dawn to sunset (E , l) on day of year (DOY) 111 for olive, DOY 167 for plum and DOY 175 for orange. Table 2 also shows sap flow (Q , l) estimated from the heat-pulse measurements for the

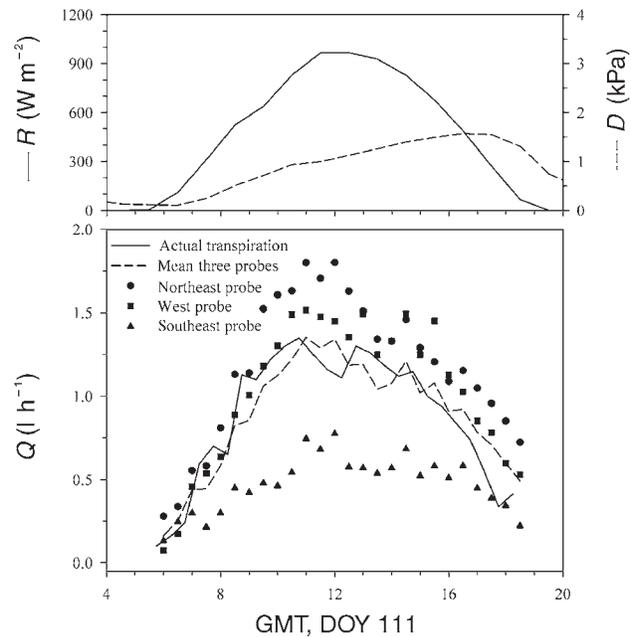


Figure 1. Solar irradiance (R), vapor pressure deficit of the air (D) and diurnal trace of water uptake by the olive tree during the first day of the excision experiment, measured as water consumed. Also shown are the sap flows (Q) estimated from the heat pulse measurements made by the three probe sets installed in the trunk, as well as the mean of the three sets. We used the correction factors derived by Green et al. (2003) for a wound width of 2.0 mm and integration Method 3 (Equation 5). Abbreviations: GMT = Greenwich mean time; and DOY = day of year.

same period for the three wound widths that gave the most illustrative results and for the three integration methods. The Q values are expressed as percentages of the corresponding E values. For olive, the best agreement between Q and E was obtained with a wound width of 2.0 mm and integration Method 3. For plum and orange the best integration method was 3, but the best wound width was 2.4 mm. The differences between integration methods were small for olive (< 5%) and plum (< 5%) and greater for orange (up to 28%).

Diurnal water uptake by the excised olive, plum and orange trees (Figures 1–3, respectively) did not show abnormal behavior. The Q values were estimated with the wound width and integration method most appropriate for each species (Table 2). Water uptake from the reservoir showed that, for the olive tree, E from dawn to sunset on DOY 111 was 22.29 l, with a maximum rate of 1.30 l h⁻¹ at 1030 h GMT (Figure 1). This low maximum value was probably associated with both the low atmospheric demand on that day and the small size of the tree (Table 1). For the plum tree, E was 31.55 l from dawn to sunset on DOY 167, with a maximum rate of 1.70 l h⁻¹ at 1600 h GMT (Figure 2). For the orange tree, diurnal E on DOY 175 was 36.25 l, with a peak value of 2.16 l h⁻¹ at 1200 h GMT (Figure 3).

The finding that maximum E in olive occurred early in the morning (Figure 1) is explained by the marked midday sto-

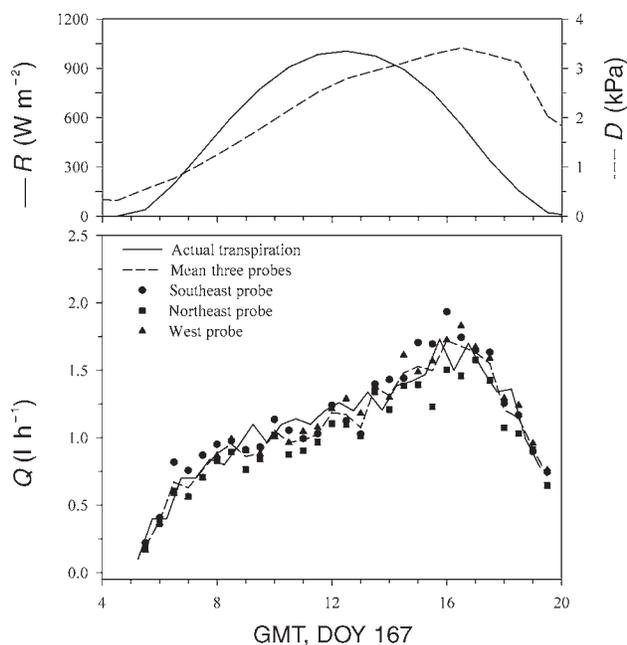


Figure 2. Solar irradiance (R), vapor pressure deficit (D) and diurnal trace of water uptake by the plum tree during the first day of the excision experiment, measured as water consumed. Also shown are the sap flows (Q) estimated from the heat pulse measurements made by the three probe sets installed in the trunk, as well as the mean of the three sets. We used the corrections factors derived by Green et al. (2003) for a wound width of 2.4 mm and integration Method 3 (Equation 5). Abbreviations: GMT = Greenwich mean time; and DOY = day of year.

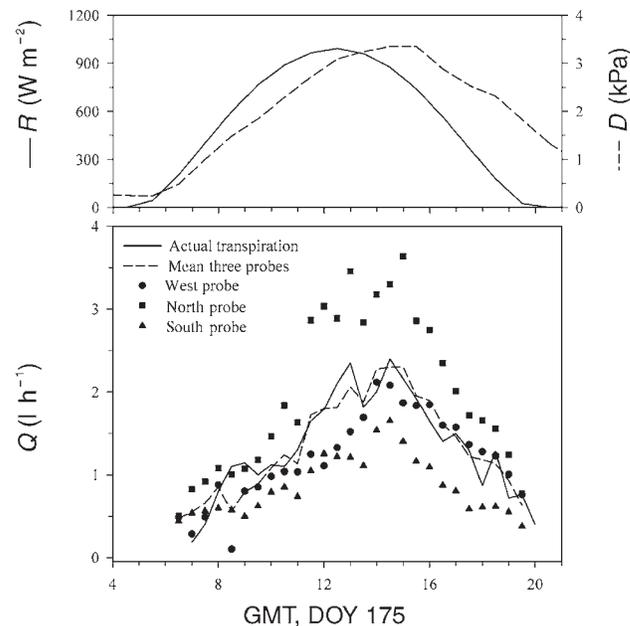


Figure 3. Solar irradiance (R), vapor pressure deficit of the air (D) and diurnal trace of water uptake by the orange tree during the first day of the excision experiment, measured as water consumed. Also shown are the sap flows (Q) estimated from the heat pulse measurements made by the three probe sets installed in the trunk, as well as the mean of the three sets. We used the corrections factors derived by Green et al. (2003) for a wound width of 2.4 mm and integration Method 3 (Equation 5). Abbreviations: GMT = Greenwich mean time; and DOY = day of year.

metal closure in this species (Fernández et al. 1997). Mean maximum g_s ($0.155 \text{ mol m}^{-2} \text{ s}^{-1}$) was recorded around 1000 h GMT, when the leaf-to-air vapor pressure deficit (D_l) was still below 1.50 kPa. The plum tree showed no evidence of stomatal closure (Figure 2) and mean maximum g_s increased throughout the day, with peak values (about $0.073 \text{ mol m}^{-2} \text{ s}^{-1}$) recorded at 1920 h GMT. At around 1600 h GMT, when maximum E values were registered for the plum tree, g_s was slightly lower than late in the evening, ($0.067 \text{ mol m}^{-2} \text{ s}^{-1}$), but D_l was greater (3.65 kPa at 1600 h GMT versus 2.20 kPa at 1920 h GMT). Mean maximum g_s in orange ($0.039 \text{ mol m}^{-2} \text{ s}^{-1}$) was recorded at 1415 h GMT, when mean D_l was 4.27 kPa. Peak E values for the orange tree were also recorded at 1415 h GMT (Figure 3).

A marked effect due to probe location, already mentioned by other authors (Fernández et al. 2001, Alarcón et al. 2003), was clearly evident in olive (Figure 1) and orange (Figure 3). There was less variability between probe sets in plum than in the other species (Figure 2).

Figure 4 shows the J_s profiles estimated with the probe set installed in each excised tree that showed the best agreement with the E values. The low sap flow recorded at 40 mm below the cambium confirms that our probes reached the deepest limits of the sapwood in each of the three monitored trees. The shapes of the profiles shown in Figure 4 provide additional information on the hydraulic behavior of each tree. In both the

plum and orange trees, higher sap flow values were always recorded in the outer part of the sapwood, close to the cambium. In the olive tree, however, this pattern occurred early in the

Table 2. Sap flow of each excised tree from dawn to sunset on the first day of the excision experiments, derived from the heat pulse measurements, calculated for different wound widths and with the three integration methods described in the Materials and methods. Data are expressed as a percentage of actual water uptake (E) in the same periods, measured as mass of water consumed.

Integration method	Wound width (mm)		
	2.0	2.4	2.8
<i>Olive</i> ($E = 22.29 \text{ l}$)			
Method 1	105.3	115.4	131.4
Method 2	108.0	118.5	135.2
Method 3	103.9	113.8	129.6
<i>Plum</i> ($E = 31.55 \text{ l}$)			
Method 1	89.1	101.9	122.3
Method 2	90.6	103.7	124.5
Method 3	87.2	99.6	119.4
<i>Orange</i> ($E = 36.25 \text{ l}$)			
Method 1	93.1	105.8	127.0
Method 2	106.4	122.1	147.9
Method 3	88.7	100.4	120.0

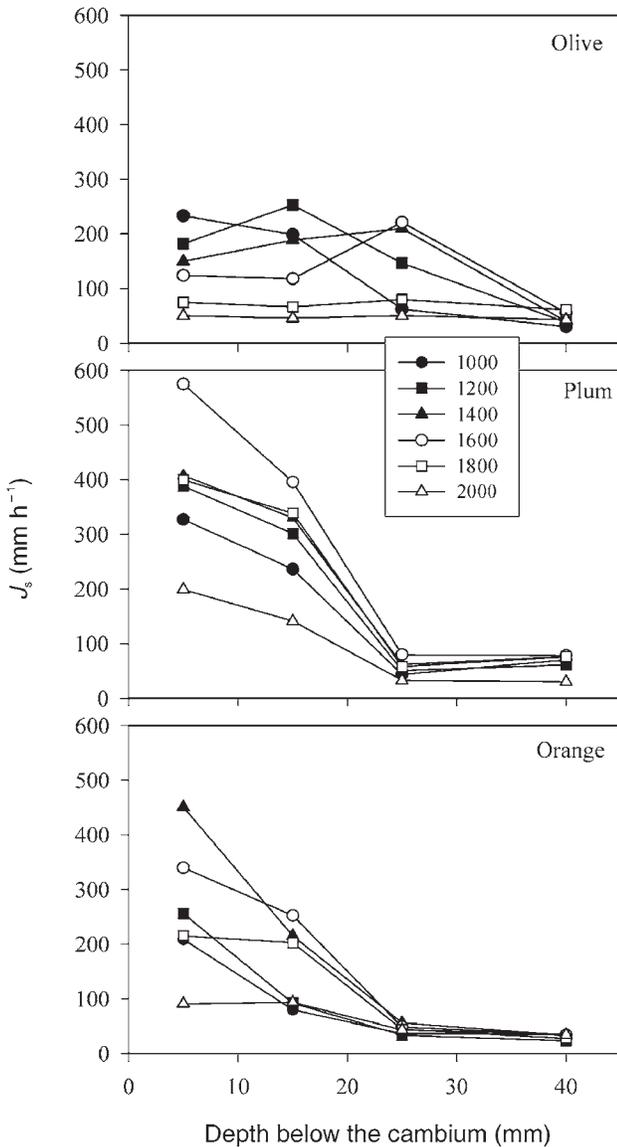


Figure 4. Profiles of sap flux density (J_s) for a single probe set in each excised tree, recorded at different times on the first day of the excision experiment. For each species, we chose the probe set for which estimated sap flow agreed best with actual water uptake, which was the west probe in all cases. See Table 1 for details on trunk diameters and probe locations.

morning only; at noon and thereafter, higher sap flow values were recorded in the inner part of the cambium, at 1200 h GMT and at 25 mm at 1400 and 1600 h GMT.

Perfusion experiments

For the perfusion experiments, the closest fits between the actual flows and the Q values derived from the sap flow measurements (Figure 5) were obtained for the same wound widths and integration method as identified in the excision experiments. The perfusion experiments yielded peak values of sap flow closer to values observed in mature trees in the field,

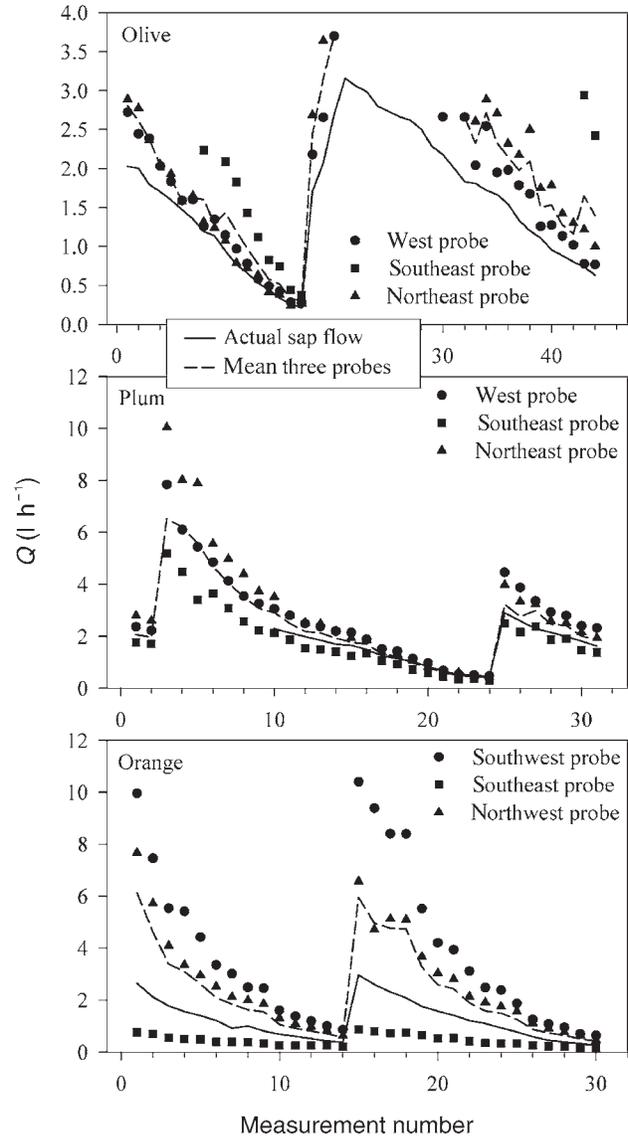


Figure 5. Comparison of actual sap flow (Q) with sap flow estimated by the heat pulse measurements during the perfusion experiment with excised trunk sections from the olive, plum and orange trees. Results with the three probe sets are shown, as well as the mean from the three sets. Actual sap flow through the trunk section was measured by collecting the perfusion solution from the distal end. The measurements were 10–15 min apart, each corresponding to a different pressure of the perfusion solution (see text for details). For each species, we used the correction factors and integration methods shown in Figures 1–3.

where sap flow of more than 6 l h^{-1} can easily be reached by mature olive trees (Fernández et al. 2001, 2003) and presumably more by plum and orange trees, which use water less parsimoniously. The agreement between actual flows and those estimated from the heat pulse velocity measurements was reasonably good for plum. For olive and orange, however, the heat pulse velocity system clearly overestimated sap flow, especially at high perfusion rates. It was in these two species that we found variability associated with probe location, which may account for the observed discrepancy.

Xylem anatomy

Both olive and orange are diffuse-porous trees, i.e., early- and latewood pores show little variation in size or density. The plum tree is semi-ring- to diffuse-porous (Schweingruber 1990). Representative transverse sections of the xylem tissue at 5 and 25 mm below the cambium of the studied species are shown in Figure 6. In olive, xylem vessels are rarely solitary and generally occur in multiples of 2–4. The xylem vessel contains numerous fibers, little parenchyma and the rays have short squarish cells. In plum, the vessels are generally in irregular, small groups. In orange, the vessels are solitary or in radial files of 2–4 vessels; the parenchyma is vasicentric and in more or less continuous, bi- to 5-seriate tangential bands. In olive and plum, the vessels are of small diameter and uniformly distributed, with short distances between vessels (Table 3). In orange, both lumen diameter and distances between vessels are greater than in the other species, but small enough to assume that the xylem is thermally homogeneous, a requirement of the theory underlying the HPV technique. Mean lumen diameter of vessels was slightly but significantly greater ($P < 0.01$) in plum than in olive, at both 5 and 25 mm below the cambium. In addition, a greater density of vessels was observed in plum than in olive. Orange xylem vessels were about twice as large in diameter as those of the other species, but vessel density was much lower. Some differences were observed with depth, e.g., in both olive and plum, vessel diameter was significantly greater ($P < 0.001$) at 5 mm than at 25 mm below the cambium.

Discussion

In the excision experiments, the measured E values cannot be compared directly with the Q values estimated by the CHP method, because the E values represent the total amount of water taken up by the trees every 30 min, whereas the Q values correspond to transpiration occurring for a few minutes after the firing of the heat pulse. In addition, high variability between the results from the three probe sets installed in the same tree was found in some of the experiments, which can be attributed to xylem variability. Thus, analyses of the stained area adjacent to the location in which the probes were inserted and of the J_s profiles showed that both the southeast probe in the excised olive tree (Figure 1) and the south probe in the excised

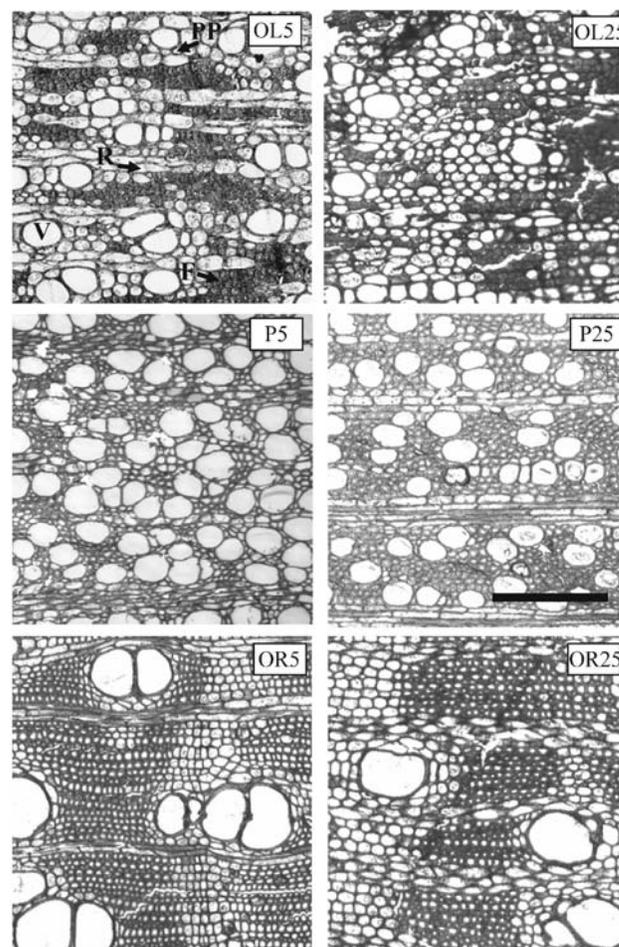


Figure 6. Transverse sections of olive (OL), plum (P) and orange (OR) wood at 5 and 25 mm below the cambium. Samples were taken from the south side of the trunk of one tree of each species growing next to the excised tree, and at the same height at which the heat pulse velocity probes were located (Table 1). Abbreviations: F = fiber; PP = paratracheal parenchyma; R = ray; and V = xylem vessel. Scale bar = 200 μm .

orange tree (Figure 3) were located where sapwood depth was abnormally limited (< 20 mm). Fernández et al. (2001) attributed differences in Q among different probe sets located in the same trunk to differences in sapwood depth between probe lo-

Table 3. Size, distribution and wall thickness of xylem vessels in the trunk of the studied olive, plum and orange trees. Wood samples were taken as explained in Figure 8. Number of replicates ranged from 30 to 55.

Species	Depth (mm)	Lumen diameter ($\pm S$, μm)	Distance between groups of vessels ($\pm S$, μm)	Wall thickness ($\pm S$, μm)
Olive	5	40.3 \pm 4.5	102.9 \pm 33.1	1.0 \pm 0.15
	25	36.9 \pm 4.6	126.9 \pm 36.8	1.0 \pm 0.15
Plum	5	45.4 \pm 4.9	81.9 \pm 21.5	1.0 \pm 0.31
	25	44.8 \pm 2.9	80.1 \pm 20.3	1.7 \pm 0.27
Orange	5	83.9 \pm 13.6	280.1 \pm 60.3	3.2 \pm 0.8
	25	79.5 \pm 11.4	340.7 \pm 133.8	3.1 \pm 0.9

cations. Other authors have reported that differences in sap flow at different trunk locations are related to different branch patterns along the trunk (Moreschet et al. 1990, Alarcón et al. 2003). Radial and or circumferential variation in sap velocity is an error source with the HPV technique (Hatton et al. 1995, Wullschlegel and King 2000). Despite these sources of variability there was agreement between both the Q values, estimated from dawn to sunset after taking the mean of the three probe sets installed in each tree, and the E values measured as water uptake (Table 2).

In previous calibration experiments, Fernández et al. (2001) reported that a 2-mm wound width and integration Method 1 was the best for 'Manzanilla' olive trees, although differences among the three integration methods were small. In our study with olive trees of the same cultivar and from the same orchard, we also found that the assumption of a wound width of 2 mm was best, but integration Method 3 yielded optimal results; however, as reported by Fernández et al. (2001), the differences among results of the three integration methods were small (Table 2). The small discrepancy between our results and those of Fernández et al. (2001) could be associated with differences in the correction factors (they used those of Green and Clothier 1988, whereas we used those of Green et al. 2003) and the k values (they used 0.505, whereas we used 0.441).

Among the integration methods tested, integration Method 3 gave the best results for the plum and orange trees. This method corresponds to an integration scheme in which Q is calculated as the weighted sum of the product of sap velocity and the associated sapwood area across the n sensors of the heat pulse velocity probe. This integration scheme was considered by Hatton et al. (1990) to be the best method for analyzing velocity profiles exhibiting strong curvature owing to more rapid flow close to the cambium. As shown in Figure 4, the velocity profiles of our study species were curved.

The change in the J_s profiles observed in olive around midday on the first day of the excision experiment (Figure 4) agrees with the early stomatal closure recorded in the tree on that day (Figure 1). Fernández et al. (2001) found a similar behavior in their excision experiments with olive and postulated that stomatal control in the younger leaves, which are linked to the outer xylem vessels, plays a role in reducing sap flow in that area of the xylem. They also speculated that the decrease in flow close to the cambium reflected embolism in large-diameter vessels as a result of water stress. We have no evidence to support either of these suggestions; however, it seems unlikely that the mild water stress suffered by our olive tree on DOY 111 was sufficient to cause cavitation in a substantial number of vessels. In well-watered trees, greater sap flows at 5 mm below the cambium than deeper in the sapwood have also been recorded in olive by Fernández et al. (2001) and in plum and orange trees by Zreik et al. (2003).

Swanson (1983) concluded that accurate sap flow values could be calculated from sap flow velocity measurements based on a wound width of 2 mm if the distance between xylem vessels is $< 400 \mu\text{m}$. This conclusion was supported by the findings of Fernández et al. (2001) in olive and those of Green

and Clothier (1988) in apple: both species have closely spaced xylem vessels. For kiwifruit, however, Green and Clothier (1988) found that the best results were obtained assuming a wound width of 3.6 mm. They reported that the diameter of the xylem vessels in this species ranges from 0.1 to 0.5 mm, and the distance between vessels varies from 0.1 to 0.8 mm. They concluded that large vessels and substantial interstitial area of woody matrix affect the thermal homogeneity of the xylem of kiwifruit, which, in turn, affects the transmission and measurements of heat pulse in this species.

Both Figure 6 and Table 3 show that the xylem of our study species can be considered to be thermally homogeneous. In the orange tree, although vessel diameter was about twice that in the olive tree, vessel density was less than half. The differences in vessel size and distribution may account for the virtual wound width being greater for orange (2.4 mm) than for olive (2.0 mm). The virtual wound width was also 2.4 mm for the plum tree, which had vessels that were slightly greater in diameter and more closely packed than those of olive (Table 3).

A study by Zreik et al. (2003) is the only one we have found in which the CHP method has been calibrated for plum and orange trees; however, both their experimental and analytical conditions differ slightly from those used in this study. In their calibration experiments, they compared the estimated Q values with mass losses, recorded by an automated balance, in potted trees of different cultivars to those that we used (namely, *Prunus salicina* 'Santa Rosa' and 'Golden Japan' for plum and *Citrus sinensis* 'Mm. Vinous' for orange). In addition, they used the correction coefficients of Swanson and Whitfield (1981) and the equation for J_s was that of Marshall (1958). Under those conditions, they found that integration Method 1 gave the best results for both plum and orange trees. With respect to wound width, Zreik et al. (2003) reported different values depending on the length of the heat pulse velocity probes and the calibrated tree, ranging from 2.4 to 3.6 for plum trees and 2.8 to 3.2 for orange trees. This contrasts with the single wound width value for a species usually reported by other authors (e.g., Green and Clothier 1988, Smith and Allen 1996, Vertessy et al. 1997, Fernández et al. 2001, Green et al. 2003). Zreik et al. (2003) also reported that the wound effect changed with time, both for plum and orange, as a result of reaction wood formation in response to probe insertion.

The lowest Q values determined from the heat pulse velocity measurements during the perfusion experiments showed good agreement with the corresponding E values. In plum, Q was 0.404 l h^{-1} for a minimum measured E value of 0.439 l h^{-1} . The corresponding heat pulse velocities measured with the three probe sets were 5.7, 5.8 and 6.0 cm h^{-1} . Green et al. (2003) reported that the lowest velocities that can be accurately measured with the CHP method are close to 2 cm h^{-1} , whereas Becker (1998) and Bleby et al. (2004) mentioned a threshold velocity of $3\text{--}4 \text{ cm h}^{-1}$.

In conclusion, we confirm that reliable data on transpiration can be obtained with the CHP method in species with thermally homogeneous wood. The capacity of the CHP method to measure sap flow at different depths in the sapwood enables accurate determination of sap flow in water-stressed trees. We

also confirmed that results from calibration experiments cannot be extrapolated to species with different wood characteristics. Accurate values of sap flow were derived from the measured HPVs based on a virtual wound width of 2.0 mm for olive and 2.4 mm for plum and orange and the corresponding correction factors derived by Green et al. (2003). For all species, best results were obtained when sap velocity profiles were integrated as the weighted sum of the product of sap velocity and the associated sapwood area across the four sensors of the HPV probes.

In olive, we observed a decrease in sap flow in the outer sapwood area in agreement with midday stomatal closure, although embolism of the xylem vessels close to the cambium may have contributed to the decrease. In plum and orange, species in which stomatal closure is less apparent, sap velocities were always highest close to the cambium. Xylem vessels in orange had lumen diameters more than twice those in olive, but vessel density in orange was less than half that in olive. Plum had the greatest proportion of lumen area per cross section of xylem tissue of the species studied.

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