COMPARISON OF HEAT BALANCE AND GAS EXCHANGE METHODS TO MEASURE TRANSPERSION IN IRRIGATED AND WATER STRESSED GRAPEVINES

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Abstract

Heat balance and leaf gas exchange methods were compared to measure plant transpiration rate in grapevine potted plants in different environmental conditions. Diurnal time courses of last fully expanded leaf gas exchange rates, and sap flow rates were compared in sunny and cloudy days, in well watered and water-stressed plants. Total plant transpiration rates were derived from instantaneous leaf gas exchange and plant leaf area measurements. In sunny and cloudy situations, different transpiration rates were observed. Rate of transpiration showed to be completely dependent of the irradiance. Any change in the amount of irradiance causes an immediate response in transpiration rate, measured in the whole plant with heat balance method. Similar response was observed also in an individual leaf, measured with gas exchange method. The sap flow measurements carried out by heat balance method and the plant transpiration rates derived from leaf gas exchange rates showed quite high correlation in irrigated plants ($r^2=0.96$). When plants were under water stress ($\Psi_{pd} = -0.58$ MPa), this correlation didn't stabilize, even total daily rates of plant transpiration and sap flow were quite similar.

These results show the high interest of sap flow measurements for ecophysiological field studies, showing that in high transpiratory flux conditions it is possible to estimate transpiration rate variations continuously from the sap flow measurements obtained with the heat balance method. However, for permanent lower transpiratory fluxes., as recorded for water stressed plants, the sap flow measurements can only reflect, with certain accuracy, the total daily transpiration rate, but not the variations along the day.

1. Introduction

The effect of soil water deficit on leaf transpiration is commonly measured monitoring leaf gas exchange rates in a last fully expanded leaf of a certain hoot. The complexity of grapevine canopy architecture and the leaf movements along the day make difficulties to establish the expected correspondence between single leaf gas exchange measurements and total plant transpiration (Smart 1974). Porometry measurements of grapevine leaf transpiration clearly reflects the environmental effects (Chaves et al., 1987; Winkel and Rambal, 1993; Delgado et al. 1995), but these results usually come from single last fully expanded leaf measurements in the upper part of the canopy. To extrapolate the whole plant behaviour from single leaf measurements is therefore difficult to achieve. In addition there are some uncertainties derived from the differences between the environment maintained in the cuvettes for gas exchange measurements and the free air surrounding the plant both when we use cuvettes for single leaf or whole plant or canopy measurements (Ansley et al.,1994; Goulden and Field, 1994; Smith and Allen, 1996). The use of lysimeters is not a practical method to measure transpiration in
grapevines because the extensiveness of root systems of field grown plants and estimated values show an extremely wide range of variation between 150 to 800 mm year⁻¹ (Smart and Coombe, 1983; Williams and Matthews, 1990; Evans et al., 1990). Irrigation dosage is usually derived from pan evaporation but crop coefficients are empirically estimated and there is a very large crop and environment induced variation which limits the effectiveness of this index. These considerations are important for grapevine crop because fruit quality is proved to be largely dependent on water availability (Matthews et al., 1990).

To overcome these limitations, sap flow methods are being recommended as useful tools to quantify water flow throughout the plant in different crops (Smith and Allen, 1996). Measurements of water flow in the xylem can be achieved applying sap flow meters to grapevine shoots which according with the reported for grapevine (Lascano et al., 1992), and other crops (Chandra et al., 1994; Smith and Allen, 1996) give reliable, direct estimates of plant or shoot water loss without disturbing the environmental conditions of the grapevine leaves.

Heat balance is a method used for measuring continuously mass flow through the xylem. The method works by applying a known amount of heat to a small segment of the stem from a thin flexible heater that surrounds the stem and is itself encircled by foam isolation. In steady state, heat input from the heater is balanced by the heat fluxes out of the stem. Thus, the energy balance of the heated stem is given by \( P_{in} = Q_r + Q_v + Q_s + Q_{flow} \). The sap flow rate \( F \) (g h⁻¹) is calculated as \( F = \frac{Q_{flow}}{c_p \Delta T_{sap}} \) (Sakuratan, 1981; Steinberg et al., 1989; Steinberg et al., 1990; Smith and Allen, 1996).

In previous studies we compared soil water deficit effects on grapevine leaf transpiration and photosynthesis rates (Delgado et al., 1995). It seems difficult to scale up at the plant and crop level from such single leaf measurements and sap flow measurements seemed to be an interesting approach to contrast environmental effects on transpiration at the leaf and plant levels.

The objective of the present study is to compare the transpiration rates obtained with a portable leaf gas exchange equipment (Licor 6400) with those derived from the sap flow meter (heat balance method) in order to obtain a first assessment on the correspondence between leaf and plant transpiration rates in different environmental (diurnal time variations and sunny and cloudy conditions) and physiological (irrigation / water stress) conditions.

2. Materials and method

2.1. Plant material and water treatments

Four one year old plants of *Vitis vinifera* cv. Tempranillo were grown under favourable conditions to achieve a maximum development: they were planted in big pots (60 dm³) in a mix of organic substrate (20%) and sandy-loam soil (80%) maintained at field capacity using nutritive solution (Hoagland 50%) during the three months previous to starting measurements. A 2 cm layer of perlite was extended over each pot to reduce the effect of direct soil evaporation. Plants were growing in a sunny place outside. The experiment was conducted in an open greenhouse attached to the UIB Plant Physiology lab, where environmental conditions were similar with those outside, but rainfall risk was avoided. When experiments started (July 1996), the plant shoots where about 1.5 m large, and their total leaf area averaged 0.487 ± 0.045 m².

During the growing period, the plants were irrigated with Hoagland 's 50% at approximately 2.5 dm³ pot⁻¹ day⁻¹. The days 16th and 18th July (198 and 200 DOY) were considered as a duplicate of the 'Irrigation' (I) treatment. These days correspond to sunny days. July 17th (199 DOY) was a cloudy day. After 200 DOY, watering was immediately stopped. A progressive soil water deficit was developed. By August 2nd, plants were considered under drought.
2.2. Environmental conditions and plant water status

Leaf temperature and photosynthetically photon flux density (PPFD) incident on leaf surface were measured respectively with thermopar and quantum sensor incorporated in a portable PAM-2000 fluorimeter (Walz, Effeltrich, Germany). PFD incident over a horizontal surface was measured with the external quantum sensor of Li-6400 infrared gas-exchange analyzer (Li-Cor Inc., Nebraska, USA). The accuracy and repeatability of both sensors were previously tested. Leaf-to-air Vapour Pressure Deficit (VPD) was measured with the Li-6400.

Leaf water potential was measured with a Scholander chamber (Soil moisture Equipment Corp., USA) at predawn (6:00h) and midday (between 13:00h and 14:00h). Values are averages of two different leaves of the same age than those used for gas-exchange measurements randomly chosen. Soil water content (SWC) was measured with Time Domain Reflectometry (TDR TRIME-SYSTEM, IMKO, Switzerland), previously calibrated for the soil used in this experience.

2.3. Leaf area

Leaf area of each one of the four test plants was measured non-destructively, five times during the experiment. The procedure used was to measure the maximum length (x) of every leaf on each one of the plants. These measurements were converted to the equivalent leaf area units by using calibration equation obtained previously (leaf area \( \text{cm}^2 = 2.94 - 1.78x + 1.52x^2 \)). The total leaf area of each plant was obtained adding up all individual leaf areas.

2.4. Sap flow and gas-exchange measurements

Sap flow was determined by heat balance method. Direct measurements of the water flux were made with commercially available sister (Dinagauge; Dinamax INC., Huston, Texas, USA) described by Sakuratani (1981). We only used original components including gauges suitable for stem diameters from 0.9-1.1cm (type SG 10). Installation and operation of the equipment was carried out strictly following the Dinagauge manual (1994). Gauges were applied 20 cm above the ground to the shoot basis (previously cleaned to favour proper fit of the foam) in the four plants. Before the first measurements, all installed gauges were tested for proper operation and instantaneous measurements compared with some leaf gas exchange and gravimetric measurements in order to secure the proper operation of sap flow. The power input to the heater was set at values between the recommended and no damage was observed in the shoot during the measurements. The value used for thermal conductivity of the stem, \( K_s \), was 0.42 W/m°C as recommended by Steinberg et al., 1990. Sheath conductance, \( K_{sh} \) (Wm⁻¹ K⁻¹), was calculated for each gauge in predawn, assuming zero sap flow. Measurements were developed following the specifications of Dynamax Flow32 manual. A CR10 datalogger and an AM416 multiplexer (Campbell Scientific, Logan, UT.) were used to log and process gauge signals. Signals were logged every 10 seconds and averaged over 30 min. Sap flux measurements were also calculated per unit leaf area.

Leaf gas exchange measurements were taken from attached last fully expanded leaves (usually 6-8th from the top) using a portable equipment (LICOR 6400) operating in open circuit and continuous recording mode averaging every 30 min in coincidence with sap flow intervals, Leaf chamber (6 cm²) was maintained at 25°C during measurements. Leaf and chamber temperatures as well as irradiance and vapour pressure deficit were continuously recorded during measurements.
3. Results and discussion

3.1. Environmental conditions and plant water status

Environmental conditions were typical of Mediterranean summer and can be considered as rather similar for all sampling times. Fig. 1A represents the mean diurnal time course of PPFD incident on the leaf in which photosynthesis was monitored. The unusual shape of the morning light increase on this leaf was due to the relative position of the plants in respect to the greenhouse structure. Only diffuse light reached the plants until about 12:00h, when direct sunlight started to fall upon the leaves. In both drought and irrigated treatments, diurnal time courses were very similar. Higher values were registered between 12h and 16h (more than 1200 μmol photons m$^{-2}$s$^{-1}$). During the cloudy day, PPFD was maintained bellow 600 μmol photons m$^{-2}$s$^{-1}$ all day, except at 15h in which a short sun fleck of around 1300 μmol photons m$^{-2}$s$^{-1}$ arrived to the leaves.

Leaf to air Vapour Pressure deficit (VPD) changes along the day (Fig.1B) achieved midday values as high as 3 KPa in drought treatment. Significant differences between treatments could be due to the higher (more than 4°C) leaf temperature of drought plants in respect to irrigated ones (Fig. 1C).

Measurements were achieved at two different leaf water status, Irrigation (I, at soil field capacity, 21% SWC), and Drought (D, at around 11% of SWC). In response to soil drying $\Psi_{PD}$ and RWC showed a clear decrease (Table 1). The $\Psi_{MD}$, however, showed similar values despite the treatment indicating that transpiration rates of irrigated plants were not fully compensated with sap flow to the leaves, thus leading to midday $\Psi$ lower than the D ones. This is in agreement with field observations in the same cultivar. According with the recently reported by Schultz, (1996) and Winkel & Rambal, (1993) these characteristics corresponds with an isohydric behaviour and is coincident with the reported by these authors for other Mediterranean cultivars (Germache). Grapevine leaves showed a great capacity of $\Psi$ recovery, because after irrigation at 22:00h by night, in the following morning (only 6h later) $\Psi_{PD}$ reached values almost as high as I ones.

3.2. Leaf area

Plant leaf area was measured on four plants different times long the growing period. When experiment started, plant leaf was 0.77 m$^2$, achieving 1.0 m$^2$ at the end of the experiments (just after drought measurements finished). A linear increase in plant leaf area was maintained even during soil water depletion period (induction of drought treatment) suggesting that water relations grapevine requirements for leaf growth maintenance could be lower than in other plants.

3.3. Gas Exchange parameters

During the sunny day, both A and g diurnal time courses (Fig. 2A) clearly followed irradiance changes in I leaves. Midday depression and afternoon rec very, previously reported by Chaves et al. (1987) and Correia et al. (1990) were not present even though the low $\Psi_{MD}$ recorded for these leaves. During the cloudy day, a peak was coincident with sun fleck, but, previous to this peak, gradually, sustained A increases were observe corresponding to much moderate increases in irradiance. Soil water deficit strongly reduce A at midday time (Fig. 2B). However, morning values (lower irradiance) showed to be even higher than the corresponding for well swatered plants. A certain afternoon recovery was also observed.

As previously reported for other plants, (Socias et al., 1997; Vadell et al., 1995), as well as for grape vine (Downton et al., 1987; Chamont et al., 1997) stomatal conductance showed daily trends close to the A ones for all three diurnal time courses. From these diurnal trends A seems to be rather determined by g. However, this correspondence seems to be only valid for well watered plants because under drought, morning A results much
higher than expected on the basis of g variations, the short afternoon A recovery does not correspond with any g changes, and the dramatic fall around midday is not well justified by the corresponding g changes at this time. As recently reported by Correia et al. (1995), other environmental changes as photosynthetic photon flux density increases and vapour pressure changes should be affecting g and A in such a different manner so that the expected correspondence is not achieved.

Nevertheless the different light regime, adding up the carbon flux along the day, the carbon fixed per day was very similar in sunny and cloudy days for I plants (Table 2). The transpiration flux was 20% lower during the cloudy day, so that water use efficiency was higher for the cloudy day. Under drought, carbon fixation was 40% but transpired water 13% therefore much higher WUE were achieved.

3.4. Sap Flow versus leaf gas exchange: Transpiration measurements

Sap flow measurements of transpiration daily time courses showed similar patterns to gas exchange ones for I plants, even though transpiratory flux was recorded in the whole plant (heat balance method) and leaf gas exchange rates were measured in a single, last fully expanded and south orientated leaf with a maximum light exposition later recalculated for all plant leaf area (Figure 3). Certain differences were found between both measurements. In the sunny day, maximum transpiration values were reordered between 2 and 14 hours (280 and 230 g h⁻¹ m⁻² registered with licor and Dinamax system respectively), but gas exchange estimated flux was always higher than heat balance in the morning. An average of 45 minutes delay in data monitored with heat balance method was observed in respect to gas exchange data. In cloudy conditions, transpiration rates decreased considerably in respect to sunny conditions for both methods. Both fluxes remained below 180 g h⁻¹ m⁻² except for the short sun fleck interval in which transpiration rates equaled to sunny conditions. As Fig. 3 shows, the gas exchange measurements immediately responded to the light and temperature changes of the sun fleck. Also in these conditions the response appeared but with some delay when measured with sap flow. In previous works, Sap Flow was mainly tested for accuracy to measure transpiratory water flux in some days or daily intervals (Lascano et al., 1992; Schmid and Braun, 1997). The present data supports the interest of Sap Flow approach for ecophysiological measurements, because plant response pattern to environmental changes is clearly reflected and easily followed. The observed delay in the response, could be expected because the distance between leaf and stem basis and the complexity of the xylem flow throughout the stem, petiole, and leaf apoplast cause a delay in the leaf water supply by the stem as is reflected in the low \( \Psi_{MD} \) recorded.

For water stressed plants, transpiration rates were very low, (around 15 g h⁻¹ m⁻²) in respect to the I ones (around 250 15 g h⁻¹ m⁻²) for the same hours. The morning and midday maximums of T recorded from Leaf gas exchange rates were not present in the sap flow pattern which showed a gradual but sustained increase in sap flow during all day. These two pick are likely due to temporary leaf temperature increases because they do not correspond with any changes in g. Later in the evening even though leaf transpiration is completely stopped, sap flow remains quite high, showing the capacity of the leaf water deficit to maintain the flux. It is obvious that this flux really exits because \( \Psi_{MD} \) increases during afternoon-evening until the moderate values of the next morning. Nevertheless the important differences in the daily pattern, the total daily flux showed a 12% difference between the two methods.

3.5. Heat balance and gas exchange correlation

When leaf gas exchange data were plotted with respect to the corresponding sap flow ones a good correlation \( (r^2 = 0.878) \) between daily course transpiration monitored with heat balance and gas exchange methods was obtained in irrigated plants. Considering morning and afternoon data as separate plots, morning values showed a very high
correlation ($r^2 = 0.985$) meanwhile for the afternoon data, the highest regression coefficient ($r^2 = 0.987$) was achieved adjusting the data to a 2\textsuperscript{nd} grade curve (Fig. 4A). Heat balance data showed a lightly delay in respect to gas exchange dates. When such delay (approximately 45 minutes) was eliminated a much higher correspondence was achieved ($r^2 = 0.959$) (Fig. 4B). For cloudy day data (Fig. 4C), the correspondence was also very high ($r^2 = 0.895$), but for drought treatment, relationship between both plots was minimal (Fig. 4C).

The high correspondence between leaf gas exchange and sap flow data, is nevertheless surprising because the complexity of grapevine canopy (Schultz, 1995; Dokooolian and Kliewater, 1995), and the variations in micrometeorological conditions in the plant surroundings. Otherwise, leaf age effects could also be present. Nevertheless, a very high correspondence was achieved showing that, in some way, a certain compensatory flux should be occurring among the canopy leaves which leads to an average flux in some way coincident with the corresponding to the measured in the last fully expanded leaf. These data also support that such leaf measurements are representative of the whole plant flux, and this could be important to asses in a more extensive way for modelling purposes. Obviously, under field conditions with a much more complex environmental variations and canopy structure, the observed correlations might not be present, but it will be interesting to test sap flow and leaf gas exchange correspondences in order to complete the assessment on the interest of sap flow measurements for ecophysiological studies in grapevines.

4. Conclusions

Sap flow measurements by Heat Balance methods can be used to estimate grapevine transpiration. Daily balance transpiration estimated by the two methods (sap flow and gas exchange) present a deviation below 4\% under irrigation conditions. Under hard drought conditions (evapotranspiration<10\% respect irrigation condition), single leaf transpiration changes are not representatives of the whole plant transpiration. Nevertheless, total single leaf daily transpiration was nearer to sap flow values. Sap flow techniques (Heat Balance method) can be promising tools for ecophysiological studies in grapevine.

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Table 1. Mean Soil Water Content (SWC), Water Potential (Ψ) and Relative Water Content (RWC) of leaves at predawn and midday during the two treatments. SWC are mean values of four plots; T and RWC values are mean of two equivalent leaves.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>SWC (%)</th>
<th>ΨPD (MPa)</th>
<th>ΨMD (MPa)</th>
<th>RWC_PD (%)</th>
<th>RWC_MD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Irrigation</td>
<td>21.2 ± 0.4</td>
<td>-0.13</td>
<td>-1.17</td>
<td>96.0</td>
<td>91.2</td>
</tr>
<tr>
<td>Drought</td>
<td>10.9 ± 0.2</td>
<td>-1.25</td>
<td>-1.25</td>
<td>91.2</td>
<td>90.8</td>
</tr>
</tbody>
</table>

Table 2. Daily balance of CO₂ assimilation rate (A), transpiration rate (E), stomatal conductance (g) and estimated daily instantaneous water use efficiency on the basis of gas-exchange measurements. Daily balance was calculated from values of all sampling times.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>A (mmol m⁻²day⁻¹)</th>
<th>E (mol m⁻²day⁻¹)</th>
<th>A/E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Irrigation sunny</td>
<td>329.08</td>
<td>102.06</td>
<td>3.22</td>
</tr>
<tr>
<td>Irrigation cloudy</td>
<td>330.51</td>
<td>71.49</td>
<td>4.62</td>
</tr>
<tr>
<td>Drought</td>
<td>134.50</td>
<td>14.05</td>
<td>9.57</td>
</tr>
</tbody>
</table>

Table 3. Global daily transpiration balance estimated by heat balance and gas exchange methods and refereed to square meters of leaf.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Heat Balance (g m⁻²day⁻¹)</th>
<th>Gas Exchange (g m⁻²day⁻¹)</th>
<th>(GE – HB) × 100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Irrigation sunny</td>
<td>329.08</td>
<td>102.06</td>
<td>3.22</td>
</tr>
<tr>
<td>Irrigation cloudy</td>
<td>330.51</td>
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<tr>
<td>Drought sunny</td>
<td>134.50</td>
<td>14.05</td>
<td>9.57</td>
</tr>
</tbody>
</table>
Figure 1. Daily time courses of photosynthetic photon flux density (A), leaf to air vapour pressure deficit (B) and temperature (C) of the leaf.
Figure 2. CO₂ assimilation rate (A) and stomatal conductance (B) of the leaf measured by gas exchange in irrigated (sunny and cloudy days) and water stressed plants.
Figure 3. Daily time courses of transpiration (g h\(^{-1}\) m\(^{-2}\)) monitored by Heat Balance and Gas Exchange methods in different environmental and soil water availability conditions.
Figure 4. Correlations between Heat Balance and Gas Exchange transpiration values of leaves under different conditions: irrigated, separate regressions for morning and afternoon data (A); irrigated, but after correction of the 45 min. delay of sap flow data (B); irrigated, but on a cloudy day (C); under drought (D)