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From leaf to whole-plant water use efficiency (WUE) in complex canopies: Limitations of leaf WUE as a selection target



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ABSTRACT

Plant water use efficiency (WUE) is becoming a key issue in semiarid areas, where crop production relies on the use of large volumes of water. Improving WUE is necessary for securing environmental sustainability of food production in these areas. Given that climate change predictions include increases in temperature and drought in semiarid regions, improving crop WUE is mandatory for global food production. WUE is commonly measured at the leaf level, because portable equipment for measuring leaf gas exchange rates facilitates the simultaneous measurement of photosynthesis and transpiration. However, when those measurements are compared with daily integrals or whole-plant estimates of WUE, the two sometimes do not agree. Scaling up from single-leaf to whole-plant WUE was tested in grapevines in different experiments by comparison of daily integrals of instantaneous water use efficiency [ratio between CO₂ assimilation (A_N) and transpiration (E); A_N/E] with midday A_N/E measurements, showing a low correlation, being worse with increasing water stress. We sought to evaluate the importance of spatial and temporal variation in carbon and water balances at the leaf and plant levels. The leaf position (governing average light interception) in the canopy showed a marked effect on instantaneous and daily integrals of leaf WUE. Night transpiration and respiration rates were also evaluated, as well as respiration contributions to total carbon balance. Two main components were identified as filling the gap between leaf and whole plant WUE: the large effect of leaf position on daily carbon gain and water loss and the large flux of carbon losses by dark respiration. These results show that WUE evaluation among genotypes or treatments needs to be revised.

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1. Introduction

Water use efficiency (WUE) is an important subject in agriculture in semiarid regions, because of the increasing areas under irrigation and the high water requirements of crops (which consume around 70% of water available to humans). The scarcity of water resources is leading to increasing controversy about the use of water resources by agriculture and industry, for direct human consumption, and for other purposes. Such controversy could be alleviated by increasing crop water use efficiency, so that improving WUE of crops is becoming a main goal for agriculture and food security goals [1–5]. Moreover, climate change predictions show clear increases in temperatures (and concomitant increase in potential evapotranspiration) and more frequent episodes of climatic anomalies, such as droughts and heat waves [6,7]. All of these climate change phenomena are prevalent in most semiarid areas [8]. Consequently, the optimization of water use for crops by improvement of WUE is a challenge for securing agricultural sustainability in semiarid areas. In response to this challenge, a large volume of applied and fundamental research has been focused on optimization of crop water use.

The water issue is crucial for environmental sustainability of viticulture, because 60% of vineyards are located in semiarid areas and regular water applications are necessary to complete the growth cycle of grapevines. Grapes growth and mature during the driest months, making irrigation scheduling and timing critical [9–11]. Consequently, scientific interest in research on grapevine WUE has focused on the evaluation of new irrigation techniques [12–15] and on genetic variation in WUE in grapevine rootstocks or cultivars [16–18] and reflect the social interest and necessity of optimizing water use in viticulture. Fortunately, in most winegrowing regions, the main concern for farmers is not high grape yield but quality. Fruit quality is negatively correlated with high

yield [19,20], so that it can be said that high quality yield is generally achieved under suboptimal crop conditions. For this reason, water stress has become a management target to secure high fruit quality, increasing the sustainability of water use by favoring crop quality over quantity.

WUE can be measured at different scales, ranging from instantaneous measurements on the leaf to more integrative ones at the plant and crop levels (Fig. 1). The pros and cons of those different ways to estimate WUE have been discussed elsewhere [21,22], and the decision on the most appropriate way depends on the capacity, facilities, and scale of the specific study. Most studies of WUE are performed on the basis of instantaneous measurements of leaf photosynthesis and transpiration, on the assumption that they are representative of whole-plant WUE, although only a few reports have evaluated WUE at the whole-plant level [18,23–25]. Comparison between instantaneous and whole-plant values sometimes reveals a clear relationship [10], but often does not. This lack of correspondence is an important limitation to the applicability of the research conducted in this field. Its causes need to be clarified for scaling from single to whole-plant estimates of WUE.

In the present work we analyze data from multiple experiments identifying sources of environmentally induced leaf WUE variations, showing the importance of both the light environment and dark respiration, often neglected, to whole-plant carbon balance and in turn to whole-plant WUE.

2. Materials and methods

2.1. Plant material and treatments

2.1.1. Field-grown plants

A field experiment was conducted in the experimental field of the University of Balearic Islands (Majorca, Spain) on grapevines of the cultivars Tempranillo and Grenache during

MEASUREMENT LEVELS OF GRAPEVINE WATER USE EFFICIENCY

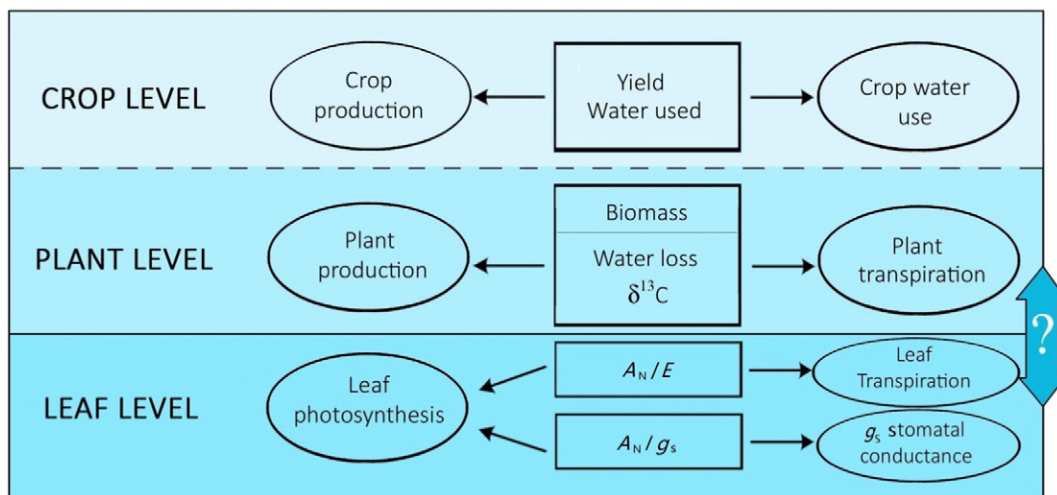


Fig. 1 – Different complexity levels for water use efficiency measurements. From leaf to crop level, as from instantaneous to growth-season measurements, there is a progressive integration of different crop production processes and water expenses with different measurement techniques. The double arrows indicate the difficulties in scaling up from leaf to plant level.

summer 2012, as described by Martorell et al. [26]. Plants were five years old (planted in 2007) grafted onto 110-Richter rootstock and planted 1 m apart in rows 2.5 m apart. They were trained in a bilateral double cordon having between 10 and 12 canes per plant in 2012. Two irrigation treatments were applied: well watered (WW), consisting of approximately 9 L day⁻¹ plant⁻¹, and water stress (WS), consisting of withholding irrigation for the entire summer. Predawn leaf water potential (ψ_{pd}) was used as a stress indicator. ψ_{pd} was measured monthly (June, July and August) with a Scholander pressure chamber (Soil Moisture Equipment Corp., Santa Barbara, CA). Four replicates per treatment and cultivar were measured. The WW treatment maintained ψ_{pd} between -0.16 and -0.27 MPa in Grenache and between -0.16 and -0.30 MPa in Tempranillo. The WS treatment reduced ψ_{pd} in August to a minimum of -0.85 MPa in Grenache and -0.53 MPa in Tempranillo.

2.1.2. Potted plants

Seven different cultivars of grapevine subjected to well-watered and water-stressed conditions were studied in three different experiments performed in three consecutive years (summer 2008, 2009, and 2010) at the University of Balearic Islands (Majorca, Spain), as described in Tomás et al. [18]. Briefly, ungrafted plants were grown outdoors in 15-L pots in a mixture of organic substrate and perlite (3:1). The cultivars Malvasia of Banyalbufar, Cabernet Sauvignon, Grenache, and Tempranillo were studied in all three years. Escursac, Manto Negro, and Pinot Noir were studied only in 2010.

Environmental conditions were recorded during the experiment using a meteorological station (Meteodata 3000, Geónica SA, Madrid, Spain). In general, the climatic variables were very similar in the three experimental years with small differences in mean air temperature, which ranged from 25.7 °C in 2008 to 24.2 °C in 2010. Total daily potential evapotranspiration was not significantly different among the three experimental periods (5.3–5.6 L m⁻² day⁻¹). Well-watered plants were irrigated to field capacity throughout the experiment.

Moderate water stress level was sustained for three weeks to maintain leaf maximum daily stomatal conductance values (g_s), around 0.05 mol CO₂ m⁻² s⁻¹. Once the desired level of water stress was reached, plants were maintained under constant water stress for three weeks by daily replacement of the exact amount of water consumed, determined by pot weight. The imposition of water stress treatment produced large reductions in soil water content (SWC calculated as follows: (pot weight – minimum pot weight) / (maximum pot weight – minimum pot weight) × 100), from 70–90% (well irrigated) down to 13–22%.

An additional experiment was performed in September of 2010 in the same location, as described in Escalona et al. [27]. Ten-year-old grapevine plants (cv. Tempranillo) grafted onto 110-Richter rootstock, were grown outside in 60-L containers in a mix of sand, Prohumin (Projar SA, Valencia, Spain) horticultural substrate, and perlite (1:1:1). The surfaces of containers were covered with a thin layer of perlite and sealed with plastic film (held with a rubber band around the edge of each container) to minimize water losses by direct evaporation. Two treatments were imposed: (i) five plants were maintained at field capacity throughout the experiment by daily irrigation and (ii) five plants were subjected to progressive

drought stress by withholding irrigation. Stem water potential (ψ_{stem}) was used as a stress indicator. Leaves were sealed in a plastic bag and covered with aluminum foil. After 1 h, ψ_{stem} was measured using a Scholander pressure chamber (see above). Different levels of water stress were obtained with time; moderate drought stress was achieved by day 4, when plants showed ψ_{stem} values of -0.8 MPa, and severe stress by day 7, when ψ_{stem} reached -1.34 MPa. The plants were maintained outside during the growing season. At the outset of the experiment, plants showed 8 shoots of 1.5 m length with about 20 leaves per shoot.

2.2. Gas exchange measurements

Instantaneous gas exchange measurements were made on four to six recently fully expanded leaves in the upper part of the canopy for each variety and treatment between 10:00 and 12:00 h using an open gas exchange system (LI-6400; LI-COR, Inc., Lincoln, Nebraska USA).

Measurements of net CO₂ assimilation (A_N), g_s , and transpiration (E) were performed at saturating red light (1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$) achieved with the red LED lamp of the system, with an additional 10% of blue light to maximize stomatal opening, and 400 $\mu\text{mol CO}_2 \text{mol}^{-1}$ in the cuvette. Air temperature and humidity in the chamber was set to match environmental conditions, in consequence of which leaf temperature ranged between 28 and 34 °C depending on leaf water status.

Gas exchange measurements were made in leaves located at 14 different positions in the canopy (lower, medium and upper parts of east and west sides and internal leaves) on August 23, 2012 in five-year-old Tempranillo plants (in the field experiment) using the same (LI-6400) open-flow gas exchange system equipped with a clear chamber (LI-6400-08). Air temperature and humidity in the chamber was set to match ambient and CO₂ concentration was set at 400 mol mol⁻¹.

Intrinsic water use efficiency (A_N/g_s) was calculated as the ratio between A_N and g_s , and instantaneous water use efficiency (A_N/E) between A_N and E .

2.3. Night transpiration and respiration rates

Night transpiration was measured on Tempranillo potted plants as described in Escalona et al. [27]. Briefly, gas exchange measurements were performed using the LI-6400 instrument equipped with a 6 cm² chamber. Measurements were performed at 400 mol CO₂ mol⁻¹ of air and at low airflow rates (150 mol air s⁻¹) on three leaves per plant (15 replicates per treatment) every 2 h during the entire nighttime period, starting 1 h after sunset (19:30 solar time) and finishing 1 h before dawn (05:30 solar time).

Respiration rates of plant organs and plant carbon balance estimation were performed in potted plants of Tempranillo and Grenache cultivars during summer 2010 as described in Escalona et al. [28].

2.4. Whole plant water use efficiency and carbon isotope composition

In potted plants of seven cultivars (see [Plant material and treatments](#)), four plants per cultivar were harvested to

determine initial whole-plant biomass. Similarly, four plants per cultivar and treatment were harvested at the end of the experiment. Leaves, shoots and roots per plant were separated and dried in an oven at 60 °C to obtain dry weight. The total biomass increase during the experiment was estimated as the difference between the whole-plant dry weights at the beginning and end of the experiment.

Plant water consumed over the three-week period was estimated from the sum of the daily water consumption as previously described.

Whole plant WUE was determined as follows:

$$WUE_{WP} \left(g L^{-1} \right) = \frac{\text{(dry weight of final biomass - dry weight of initial biomass)}}{\text{total water consumed.}}$$

For carbon isotope composition, six young leaves per cultivar and treatment from different plants, developed after the outset of the stress treatment, were sampled at the end of the experiment. They were dried for 48 h at 60 °C and ground into powder. Subsamples of 2 mg were analyzed for isotope ratio ($\delta^{13}C$) as a long-term indicator of WUE. The samples were combusted in an elemental analyzer (Carlo-Erba, Rodano, Italy), CO_2 was separated by chromatography and directly injected into a continuous-flow isotope ratio mass spectrometer (Thermo Finnigan Delta Plus, Bremen, Germany). Peach leaf standards (NIST 1547) were run every eight samples. $\delta^{13}C$ was calculated as follows:

$$\delta^{13}C_{\text{sample}} (\text{‰}) = \left\{ \left[\frac{^{13}C/^{12}C_{\text{sample}}}{^{13}C/^{12}C_{\text{standard}}} \right] - 1 \right\} \times 1000.$$

$\delta^{13}C$ values were referenced to a Pee Dee Belemnite standard.

3. Results and discussion

3.1. Variation in WUE over time

A literature survey of plant WUE shows that WUE determinations rely on direct measurement of instantaneous gas exchange rates (photosynthesis and transpiration) at the leaf level with portable equipment. Usually, such measurements are taken on recently fully expanded leaves, well light-exposed, around midmorning because in most cases this time yields the highest values for A_N , g_s , and E . However, as Fig. 2 shows, there is large variation in “intrinsic” water use efficiency (estimated as A_N/g_s) throughout the day, as measured under field conditions. The figure shows that at the typical measurement time (midmorning) A_N/g_s values range from 50 to 70 $\mu\text{mol } CO_2 \text{ mol}^{-1} \text{ H}_2\text{O}$, but that afterwards A_N/g_s values were higher or lower and that these daily changes are even higher under water stress. The evidence of these daily time changes calls into question the widely accepted principle of optimization of resources by the plant, showing how daily variations in environmental and leaf conditions correspond to large changes in physiological parameters. The extent to which the typically measured values are representative of the whole day A_N/g_s is not under discussion, although obviously integration over the full day would more accurately represent the actual leaf WUE. Measurement limitations always influence the decision between greater numbers of more comparable measurements

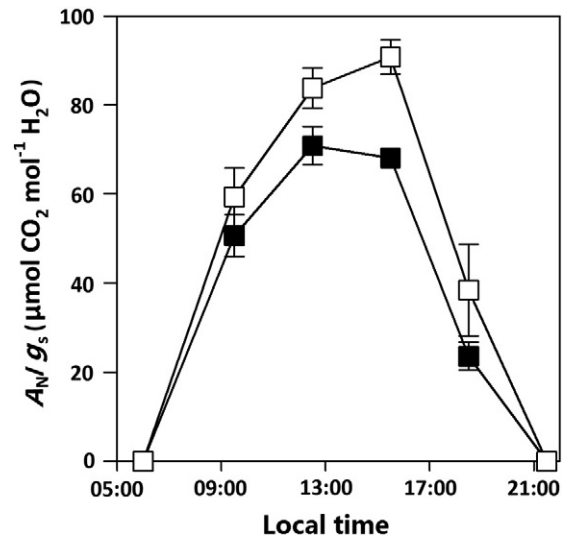


Fig. 2 – Diurnal time variation in intrinsic WUE (as A_N/g_s). Diurnal time cycles of grapevine (cultivar Tempranillo) under irrigation (filled symbols) and moderate water-stress conditions (open symbols). Plants were grown outdoors in the field during summer in Mallorca (Balearic Islands, Spain). Values are averages of 5 replicates \pm SE.

and more accurate but restrictive measurements. The limitation imposed by daily variation in WUE has been shown by Medrano et al. [29] with plots of midmorning values of A_N/g_s against whole-day integrals (as $\mu\text{mol } CO_2 \text{ mol}^{-1} \text{ H}_2\text{O day}^{-1} \text{ m}^{-2}$) for different grapevine genotypes. The correspondences were high or low, depending on the experiment.

Along with diurnal time effects, there is seasonal variation in leaf WUE as a consequence of both changing environmental conditions and the physiological changes expected with leaf aging, which modifies leaf photosynthesis and transpiration. Fig. 3 shows how these changes in grapevine leaves modify intrinsic WUE from early growth to harvest. Under irrigation, the midmorning values of A_N/g_s , measured in field-grown Grenache and Tempranillo plants, changed from 40 to 80 $\mu\text{mol } CO_2 \text{ mol}^{-1} \text{ H}_2\text{O}$, similar in the two varieties. However, the increase in A_N/g_s in response to moderate water stress is greater for Grenache than for Tempranillo from veraison to ripening and harvest time, and the reputation of Grenache as a more drought-resistant variety is more clearly corroborated in the latter growth periods.

3.2. Spatial variation in leaf WUE in complex canopies: the case of grapevine

In complex canopies, the light intercepted by an individual leaf is highly dependent on the leaf position and the canopy geometry. In the grapevine, the trellis system and row orientation provide differential light exposure for different leaf positions in the canopy, corresponding to differences in microclimate that clearly affect the daily time course of leaf gas exchange rates. The effect of leaf position on integral daily carbon gain was reported by Escalona et al. [30], showing large variation from top layers of the canopy receiving 100% of

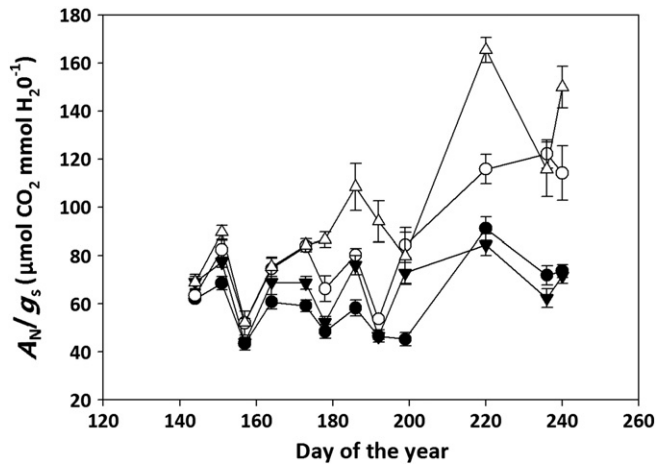


Fig. 3 – Variation of intrinsic WUE (as A_N/g_s) with phenology. Intrinsic WUE variation throughout the growing period in two different grapevine cultivars grown in the field from bloom to harvest period. Black symbols, well-watered plants; white symbols, water-stressed plants. Cultivars are Tempranillo (squares) and Grenache (triangles). Values are averages of 5 replicates \pm SE.

incoming light to lower positions receiving only 25%, and showing that inner shaded leaves received only around 5% of incoming light. This differential light and microclimate environment caused large changes in the daily time courses of photosynthesis and transpiration but also in daily and seasonal integrals, leading to large variation in carbon gain and water consumption among different positions of the canopy. Concerning leaf WUE, reanalyzing these data, Medrano et al. [29], showed that both instantaneous and daily integrals of leaf WUE (as integrals of A_N/g_s or A_N/E values) were also highly dependent on the microclimate environment of each leaf position and that WUE values of upper locations were double those of lower ones. These variations were similar or even higher under moderate and severe water stress. In fact, daily leaf WUE proved to be highly determined by the daily intercepted light at each leaf position (with a R^2 of 0.98 for irrigated plants). Fig. 4 shows the effect of leaf position on WUE at multiple positions in the canopy (14). The average values of typical midmorning measurements WUE (as A_N/g_s) for well-irrigated vines showed a similar tendency, with clear differentiation (expressed as values three to four times higher) between the east and west sides of the canopy.

Those results show large spatial variation of WUE in the canopy as well as the importance of this complexity for the evaluation of plant WUE on the basis of instantaneous measurements of typical fully exposed leaves in specific locations. These results also provide an interesting example of the fine responses of leaves to environmental variation, showing that leaf capacity to regulate photosynthesis and transpiration results in large variation in WUE, calling into question the leaf gas-exchange rate optimization theory [31]. Although comparative studies on the basis of WUE measurements in a single leaf are useful and provide an affordable way to compare genotypes and agronomic practices, the relationships among these standard values and whole-plant

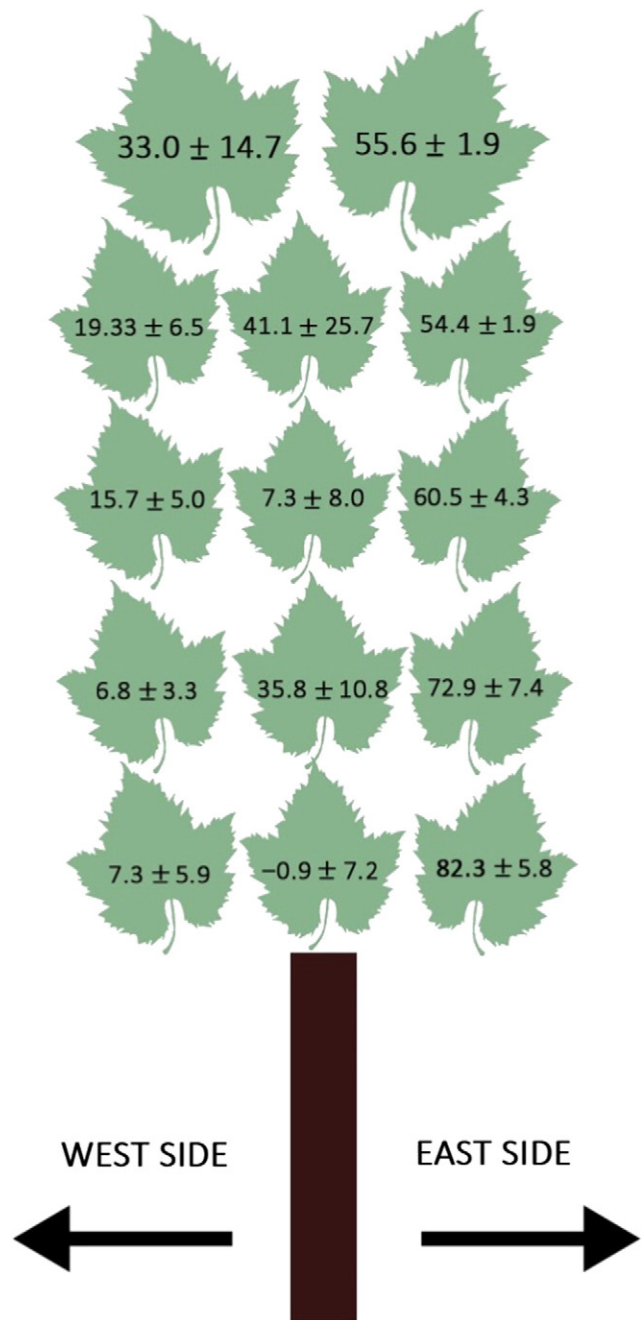


Fig. 4 – Variation in intrinsic WUE (as A_N/g_s) with leaf position in the canopy. Intrinsic WUE (A_N/g_s , $\mu\text{mol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$) measured at 14 positions throughout the canopy at midday in five-year-old grapevines of cv. Tempranillo under field conditions. Values are means of four replicates \pm SE measured in August 2012.

values are not simple, because of the complexity of the canopy and the differential responses of the leaf to cumulative daily irradiance.

3.3. From leaf to whole plant WUE: effects of night transpiration and respiration rate

Besides the difficulties of extrapolating to whole-plant photosynthesis and transpiration from instantaneous single-leaf

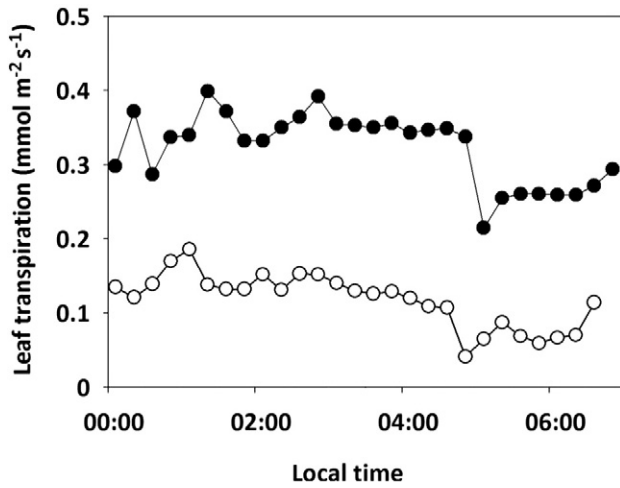


Fig. 5 – Night transpiration rates in irrigated and water-stressed grapevines. Leaf night transpiration rates during a typical dark period in 10-year-old potted Tempranillo grapevines grown under irrigation (black symbols) and water stress (white symbols).

measurement, whole-plant WUE measured as biomass increase with water used is strongly dependent on other physiological processes determining WUE: respiration losses and night transpiration.

Night transpiration has recently been reviewed and measured, proving to be non-negligible and possibly markedly reduced under water stress. Also, under certain circumstances nighttime transpiration can account for 10% of daily transpiration losses [27,32]. Fig. 5 shows, as an example, nighttime leaf transpiration rates of irrigated and water-stressed 10-year-old potted Tempranillo plants, showing rates of around 10% of daily transpiration (data not shown) and threefold higher in irrigated than in water-stressed grapevines. As already reported by our group [27], these differences between treatments cannot be explained by epicuticular changes. Using whole-plant mini-lysimeters, we also showed that on very humid nights these losses are nearly compensated by dew income [27]. In any case, these water losses reduce the expected daily WUE of the whole plant.

With respect to carbon losses by respiratory processes, there is complexity derived from respiration rate variation with environmental conditions and plant growth and development. A main limitation to evaluating the effect on carbon balance of variation in respiration rate is the paucity of studies on dark

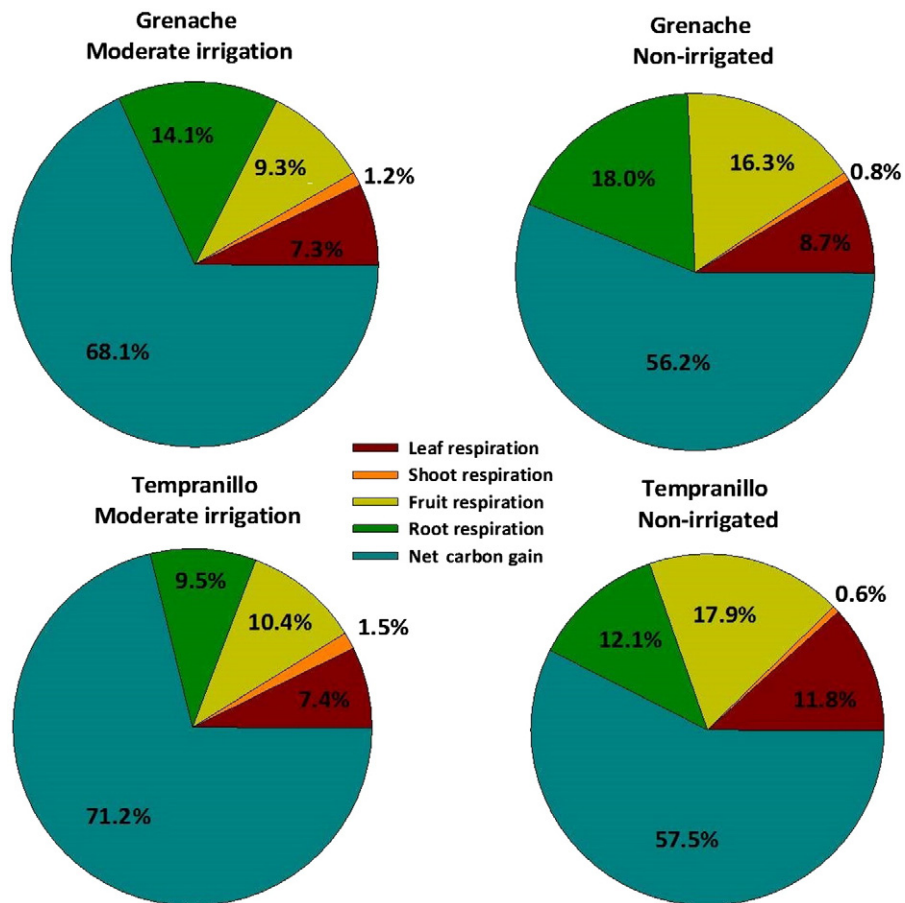


Fig. 6 – Carbon balance as affected by respiration components. Contribution as percentage of total carbon gain of several respiratory components of grapevines (cultivars Grenache and Tempranillo). Net carbon gain is expressed as the remaining percentage.

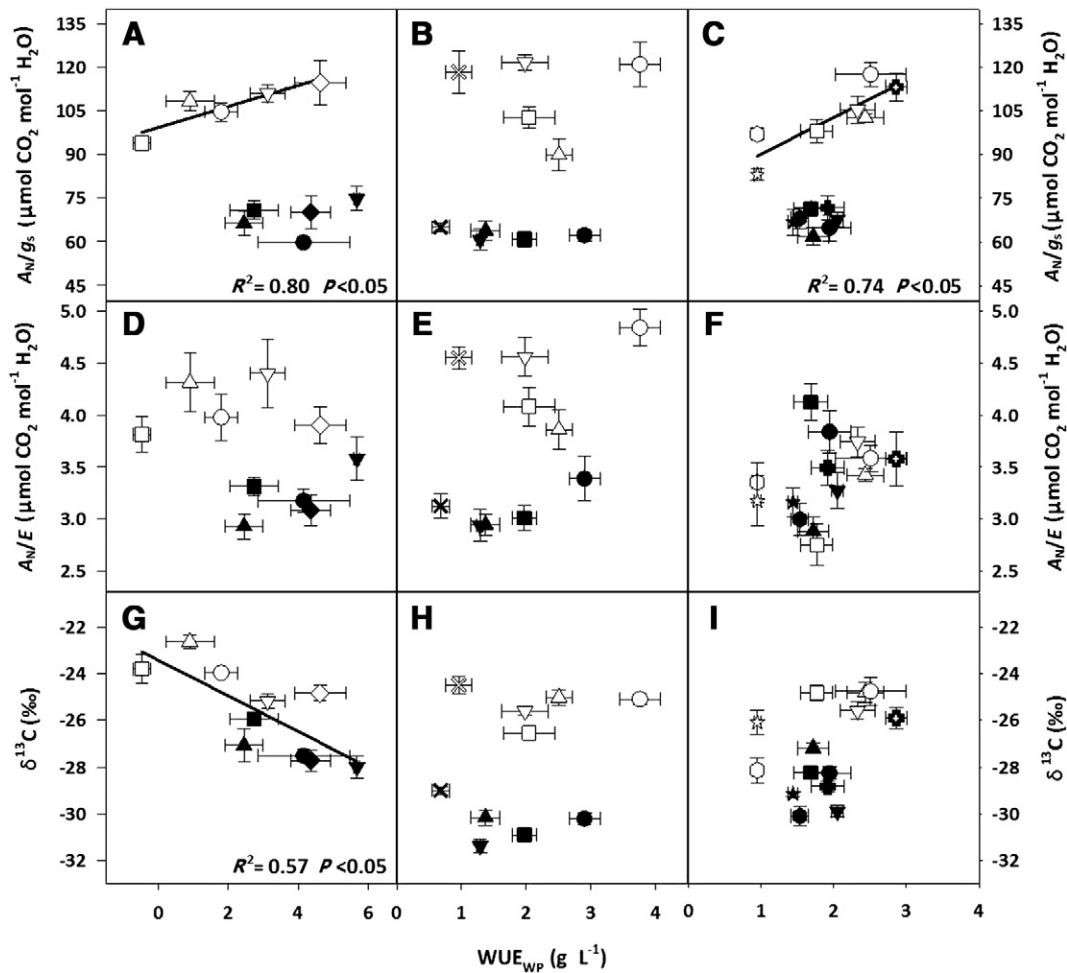


Fig. 7 – Relationships between water use efficiency (WUE) measured at leaf and whole-plant levels. Relationships between intrinsic WUE (A_N/g_s) and whole-plant WUE (WUE_{WP}) in 2008 (A), 2009 (B), and 2010 (C). Relationships between instantaneous WUE (A_N/E) and WUE_{WP} measured in 2008 (D), 2009 (E), and 2010 (F). Relationship between leaf $\delta^{13}C$ and WUE_{WP} from values obtained in three different experiments, 2008 (G), 2009 (H), and 2010 (I). A_N/g_s and A_N/E were measured in midmorning during the experimental periods and leaf $\delta^{13}C$ was measured at the end of the experimental periods. Black symbols, control plants; white symbols, water-stressed plants. Cultivars are represented as follows: Tempranillo (square), Malvasia of Banyalbufar (circle), Grenache (inverted triangle), Cabernet Sauvignon (upright triangle), Callet (diamond), Richter-110 (cross), Escursac plus, Manto Negro (hexagon), and Pinot Noir (star). Values are means \pm SE of six replicates. WUE_{WP} was measured at the end of each experiment. Values are averages of 4 replicates \pm SE.

respiration. Estimates of plant respiration are often obtained by measurement in organs such as leaves, shoots, and roots, but most reports have focused on leaf respiration. However, it is well known that the largest respiratory losses come from the root system, presenting great difficulty for accurate determination under field conditions. Still, for grapevines there are some reports on root respiration rate [28,33–35] based on calculation for pot- and field-grown plants and assuming specific soil respiration activities for the latter. Similarly to other parameters, leaf respiration rates showed large variation with canopy position (for leaves), age (for shoots and fruits), and plant water status [28]. The relative magnitudes of respiration losses are shown in Fig. 6, showing that, in Tempranillo and Grenache potted plants, respiration losses represent around 33% of total carbon gain for irrigated plants and around 45% for water-stressed plants. Among those respiration losses, there is clear variation linked to water status and also differences

between the two sampled varieties. In general, root respiration losses seem to be the main losses, followed closely by fruit and leaf respiration losses.

Overall, these results confirmed the importance of respiratory losses for understanding plant carbon balance, but also for better understanding dark respiration as the largest unknown factor relating leaf instantaneous and whole-plant water use efficiency. Certainly, as Fig. 6 shows, respiration rates are not constant but show wide variation with water status and variety.

3.4. The missing key: identifying a more representative indicator of WUE

As described above, there are different sources of variation in carbon gain and water loss, from the single leaf to the whole-plant, which can affect the correspondence between

leaf and whole plant determinations of WUE. We have shown the large variation of photosynthesis and transpiration with leaf diurnal time courses and seasonal variation, but there is also marked variation with leaf position and a large effect of respiratory losses. As shown by Tomás et al. [36], the relative importance of this canopy complexity and plant respiration of grapevines can be weighted on the basis of collected data for irrigated Tempranillo vines. For this analysis the effect of canopy complexity [29,30] was assessed, considering the potential maximum values of A_N , E , and whole plant WUE as those that would be achieved by a plant if all of its leaves were fully exposed to the sun throughout the day. These theoretical carbon gains (A_N) and water losses (E) were defined as 100%, so that the theoretical maximum A_N/E ratio is 1. Taking into account the canopy location effect, the potential carbon gain by unit of leaf area falls to around 47%. With respect to transpiration, the canopy effect reduces water losses by around 36%. Consequently, the plant WUE would be expected to be around 0.75 of the “potential” whole-plant WUE. Introducing the effect of plant respiration, the carbon losses from respiration in roots, fruits, leaves, and shoots represent from 30% to 45% of the carbon fixed. Finally, considering the night transpiration component [27], transpiration increases, so that recalculating net carbon gain and transpiration losses yields a final WUE of the plant around 33% of the theoretically estimated WUE. Fig. 7 shows the general lack of relationship between leaf-level estimates of WUE (A_N/g_s , A_N/E , or $\delta^{13}C$), with whole-plant biomass-based WUE (WUE_{WP}), using data from seven grapevine cultivars over three consecutive years and two water-availability conditions. Among all of these studied combinations, only a few showed a clear correspondence between single-leaf and whole-plant measurements (Fig. 7A, C, G). For instance, in two of the three experimental periods, A_N/g_s was significantly and positively correlated with WUE_{WP} in non-irrigated plants, but the correlation was lost when irrigated plants were also considered. For $\delta^{13}C$, a single significant negative correlation with WUE_{WP} was observed when all data in the first of the three experiments were pooled, but no such correlation was observed in the other two experiments. Moreover, while water stress results in increased leaf-level WUE in all cases, its effects on WUE_{WP} are variable, from decreases in most cultivars in 2008 and some in 2010, to no changes or increases, depending on the cultivar, in 2009 and 2010. Genetic variability in WUE at different levels was recently reviewed [36,37] and the predicted causes of discrepancies between WUE values at leaf level and WUE_{WP} [10,29] were associated with all of the components analyzed in the present study: the complexity of light interception, night transpiration, and respiratory losses. Thus, these are major limitations to choosing a single selection criterion to rate the WUE of a given genotype. This difficulty is a serious handicap to conducting selection programs for this character.

4. Conclusion

The reported lack of correspondence among leaf gas exchange parameters and whole plant carbon and water balances imposes a severe limitation on the accurate measurement of treatment and/or genotype effects on whole-plant WUE under

field conditions. It is thus necessary to fill the gaps in scaling from single-leaf to whole-plant estimates of WUE to better understand the underlying processes leading to the variety of responses. This variety illustrates the diversity of single-leaf vs. whole-plant WUE relationships. The reported data and discussion clearly show that a more intensive and extensive research effort is needed to improve the representativeness of typical sampling procedures in evaluating whole-plant WUE.

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