Dependence of Sap Flow and Stem Diameter Fluctuation of Grapevines on Reference Evapotranspiration: An Event Coincidence Analysis

Abstract

Aim: A relationships between sap flow and stem diameter change with weather parameters included in ET0 calculation have been reported by numerous authors. Thus, we can assume that there is a statistical interdependency between a series of chronologically recorded of physiological indicators and a simultaneously time spaced ET0 data. To demonstrate our assumption and for quantification of the interrelationship between the series, we used time series of sap flow and diameter change of grapevines together with an ET0 series of the vineyard location to perform an event coincidence analysis.

Methods and results: Two sets of six grapevine each, one irrigated and the other rainfed, were fitted with dendrometer and sap flow needles and their fluxes of sap (SF) and their maximum daily shrinkage (MDS) of the trunk were recorded during 5 years from 1 June to 30 September. During the same period, an automatic weather station collected weather data to calculate the reference evapotranspiration (ET0). Stomatal conductance (gs) was measured at solar noon twice a month. Averages of SF, MDS and gs were significantly different between irrigated and rainfed grapevines. The correlations between SF, MDS and ET0 were significant and both high and low ET0 events triggered directly corresponding SF and MDS events but not both simultaneously as shown by a statistical procedure named event coincidence analysis.

Conclusions: SF and MDS are related to different physiological processes and have a differentiated response to the main weather parameters that drive the ET0. SF or MDS as single descriptor does not reflect the grapevine response to changing environmental conditions but their combination can provide more detailed information about its water needs. Significance and impact of the study: This study highlights the advantages and limitations of SF and MDS as descriptor of the grapevine water needs and their relationship with atmospheric environment.

Keywords: Event coincidence analysis; Stomatal conductance; Sap flow; Maximum daily shrinkage; Reference evapotranspiration; Vitis vinifera

Abbreviations: ECA: Event Coincidence Analysis; ET0: Reference Evapotranspiration; Gs: Stomatal Conductance; K: Observed Number Of Events; Ke: Expected Number of Events; MDS: Maximum Daily Shrinkage; Q1: First Quartile; Q4: Forth Quartile; SF: Sap Flow

Introduction

The use of plant-based water status indicators is today very common because the plant itself is the best indicator of its water status, the data acquisition at a high resolution across larger temporal scale and its analysis is now more affordable, and plant water status controls many physiological processes and crop productivity, thus this information has great practical interest for crop management, in particular the water management. Grapes are widely cultivated worldwide but they are concentrated in Mediterranean type climates where water is scarce and face increased competition with other stakeholders.

For these reasons but also because water influence vegetative growth and reproductive growth, yield and berry composition through their effects on berry size and on photosynthetic activity, vineyard water management has received great attention [1,2]. Vineyards managers try to maximize the water use efficiency at the cost of some reduction in crop yield that is not detrimental for the wine makers as the highest crop loads are linked to low grape quality, in fact, limitations to grape yield are a common practice for a market standard wine production and premium wines [3]. To determine the influence of environmental and cultural conditions on vine water use and the grapevine water status, and to manage water in the vineyard, one must use both a physiological indicator that integrates environmental conditions and tools to evaluate water use [4].

A common and widely use indicator of plant water status is stomatal conductance (gs) that can be a precise indicator in grapevines [3] but gs readings cannot be automated and a
very limited number of observations can be made at a given time and location. However, several authors have found a relationship between gs and other water status indicators, such as sap flow and changes on diameter of plant organs, and also to evapotranspiration [5-7]. For grapevines and fruit trees, indicators derived from measurements of sap flow rate and diameter changes in conductive organs, normally the trunk, are considered good predictors of plant water status [8]. These indicators can be continuously and automatically recorded with high resolution [9,10] and are reported to be closely related to climatic variables such as solar radiation [11], temperature [12], vapor pressure deficit [13] and evaporative demand of the atmosphere [2]. The evaporative demand of the atmosphere at a specific location is evaluated by the reference evapotranspiration (ET0) that integrates several weather parameters [14] that affect sap flow and diameter changes. Given the relationships between sap flow and diameter change with weather parameters included in ET0 calculation that have been reported by numerous authors, we can assume that there is a statistical interdependency between a series physiological indicators chronologically recorded and a simultaneously time spaced ET0 data. To demonstrate our assumption and for quantification of the interrelationship between the series, we used time series of sap flow and diameter change of grapevines together with an ET0 series of the vineyard location to perform an event coincidence analysis (ECA) [15]. This study is expected to highlight the advantages and limitations of SF and MDS as descriptor of the grapevine water needs and their relationship with atmospheric environment.

Materials and Methods

Field description

The experimental field was set up in 2011 in a vineyard at the campus of University in Vila Real, Portugal (41.285431N, -7.738142W). The 15 years old vineyard of Vitis vinifera L. (cv. Touriga Nacional) trained as vertically positioned shoots, it was commercially managed, it had a total area of 0.8 ha, it was planted in a gently sloping land (<2%) and it was spaced 2m between rows and 1m between adjacent plants. When the canopy was fully formed, the shoots were pruned to maintain the rows between rows and 1m between adjacent plants. The soil is a Cambisol dystric [16] with a regular depth of 0.8m. The weeds below the row were removed by shallow tillage when necessary and those between rows were mowed regularly.

Data collection

Two sets of six adjacent plants each, disease free and about the same size, were chose 12 meters apart. One set was rainfed and the other was irrigated weekly from June to September with 40% of the ET0 calculated for the previous week. The trunk of two vines on each set was fitted with a dendrometer (Ecomatik DD-L, ECOMATIK Muenchner Str. 22 D-85221 Dachau/Munich, Germany) 10cm above the grafting point and with a sap flow needles (Thermal Dissipation Probe, Dynamax Inc. Fallstone Ltd, 130 Low Road Burwell, Cambridge, CB25 0EJ, UK) that took readings every five minutes and recorded the average over 30 minutes intervals. The data was collected every year from 2013 to 2015 starting 1 June (DOY 121) to 30 September (DOY 273) that comprises the flowering stage (usually during the second week of June) and the period about two weeks before commercial harvest.

Reference evaportranspiration was calculated at daily intervals (mm day−1) as follows [14]

\[
ET_0 = \frac{0.408 (R_n - G) + \gamma \left( \frac{900}{T + 273} \right) \left( \delta + \frac{\gamma}{1 + 0.34 \gamma} \right)}{\delta - \left( \frac{(\text{relative humidity})}{100} \right)}
\]

where \(R_n\) is the net radiation at the surface (MJ m−2 day−1), \(G\) is the soil heat flux (MJ m−2 day−1), \(T\) is the mean air temperature at 2 meters height (°C), \(u_1\) is the wind speed at 2m height (m s−1), \(e_i\) is the saturation vapor pressure deficit (kPa), \(e_{-i}\) is the actual vapor pressure (kPa), \(\delta\) is the slope vapor pressure curve (kPa°C−1), and \(\gamma\) is the psychometric constant (kPa°C−1). Individual daily vine transpiration (L day−1) was calculated from the procedure described by Davis [17] and Dynamax [18]. Dendrometer raw data was used to derive the descriptors depicted in (Table 1).

During the first year, one grapevine apart from the others under observation was fitted with a dendrometer and irrigated weekly from May to September to 90% of ET0 calculated for the previous week and we considered this vine at non-limiting soil-water conditions. Higher amount of irrigation would waterlog the soil for too long time periods. The dendrometer raw data was used to obtain the reference values for each of the descriptors mentioned in (Table 1). The quality of the descriptor was defined by its sensitivity that assesses the usefulness of the water-stress indicator [19]

\[
\text{Sensitivity} = \frac{\text{Signal Intensity}}{CV}
\]

Where CV is the coefficient of variation and

\[
\text{Signal Intensity} = \frac{\text{Actual value of the predictor}}{\text{Reference value of the predictor}}
\]

The sensitivity of MDS (3.66) was higher than of any other indicator; thus, its time series was the only one to describe the trunk diameter variation of the grapevines. The time series for MDS and sap flow (SF) were obtained by averaging the data from two plants on each set. The series (MDS, SF, ET0) had a total of 765 observations each and a standard correlations test (two tailed Pearson) was performed to quantify the direction and significance of the linear association between the variables. The low and the high values of ET0 are of particular importance as they might drive larger differences on both MDS and SF in relation to their averages. Each series was subdivided in their quartiles and ECA
was performed only on the sub-series corresponding to the first (Q₁) and forth (Q₄) quartiles, like a procedure used by Siegmund [20]. The notation

Table 1: Descriptors derived from dendrometer raw data.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Definition</th>
<th>Calculation</th>
</tr>
</thead>
<tbody>
<tr>
<td>MXSD</td>
<td>24 hour maximum stem diameter</td>
<td>MXSD(i+1)-MXSD(i)</td>
</tr>
<tr>
<td>MNSD</td>
<td>24 hour minimum stem diameter</td>
<td></td>
</tr>
<tr>
<td>MDS</td>
<td>Maximum daily shrinkage</td>
<td>MXSD(i)-MNSD(i)</td>
</tr>
<tr>
<td>DR</td>
<td>Daily recovery</td>
<td>MXDS(i+1)-MNSD(i)</td>
</tr>
<tr>
<td>DG</td>
<td>Daily growth</td>
<td>MXSD(i+1)-MNSD(i)</td>
</tr>
<tr>
<td>SGR</td>
<td>Stem growth rate</td>
<td>MXSD(i)-MXSD(i-n)</td>
</tr>
</tbody>
</table>

For each series is as follows:

a. First quartile SF irrigated plants  
   \( Q_{1SF} \)

b. Forth quartile SF irrigated plants  
   \( Q_{4SF} \)

c. First quartile SF rainfed plants  
   \( Q_{1SFr} \)

d. Forth quartile SF rainfed plants  
   \( Q_{4SFr} \)

e. First quartile MDS irrigated plants  
   \( Q_{1MDS} \)

f. Forth quartile MDS irrigated plants  
   \( Q_{4MDS} \)

g. First quartile MDS rainfed plants  
   \( Q_{1MDSr} \)

h. Forth quartile MDS rainfed plants  
   \( Q_{4MDSr} \)

i. First quartile ET₀  
   \( Q_{1ET₀} \)

j. Forth quartile ET₀  
   \( Q_{4ET₀} \)

Every first and third week of each month from June to September on clear sky days, one adult leaf on the middle of a shoot per plant on each set was chosen to determinate stomatal conductance \( (gs) \) [AP4 parameter; Delta-T Devices Ltd, 130 Low Road Burwell, Cambridge, CB25 0EJ, UK] at solar noon. A total of 240 \( gs \) readings were recorded per set during the duration of the experiment. Pearson correlation was also calculated between \( gs \) and SF, MDS. Given the disparity on the number of observations among these variables, a single value for \( gs \) was obtained averaging the six readings on each set on a given date that was paired with the corresponding SF and MDS values for the same date.

Event coincidence analysis (ECA)

A detailed explanation of this procedure can be found in Donges [15] & Siegmund [20] that is concerned with quantifying the statistical interrelationships between pairs of event series where the event time series \( A \) and \( B \) are defined as two ordered sets \( t_{A1}, \ldots, t_{Aα} \) and \( t_{B1}, \ldots, t_{Bβ} \) with numbers of events \( N_A \) and \( N_B \) respectively, covering a time interval \( T \). The assumption to be tested and quantified is that events \( B \) precede events \( A \) because there is a cause-effect relationship between them (Figure 1).

Figure 1: Schematic illustration of event coincidence analysis for quantifying statistical interrelationships between two event time series \( A \) and \( B \) for the case of precursor coincidences [15].

An instantaneous coincidence occurs if two events at \( t_{Aα} t_{Bβ} \) are closer in time than a coincidence interval \( ΔT (t_{Aα} - t_{Bβ} ≤ ΔT) \). A lagged coincidence is an instantaneous coincidence between the time shifted event at \( t_{Aα} - τ \), where \( τ ≥ 0 \) is a time lag parameter, and the event at \( t_{Bβ} < t_{Aα} - τ \). Assuming that both \( A \) and \( B \) type events are generated by Poisson processes with event rates \( λ_A = N_A / T \) and \( λ_B = N_B / T \), respectively, both types are distributed randomly, independently and uniformly over the continuous time interval of length \( T \). The probability for a lagged precursor coincidence between an \( A \)-event and a preceding \( B \)-event is given by

\[
P = \frac{ΔT}{T - τ}
\]

The probability of a specific event \( A \) to coincide with at least of one event \( B \) is

\[
1 - (1 - p)^N_B
\]

The probability of \( K \) precursor coincidences are observed for a given realization of two Poisson processes is

\[
P(K; N_A, 1 - (1 - p)^N_B) = \frac{N_A^K}{K!} \left( \frac{ΔT}{T - τ} \right)^K \left( 1 - \left( \frac{ΔT}{T - τ} \right) \right)^{N_A - K}
\]

If the events type \( A \) are spaced much wider than the coincidence interval, i.e.

\[
ΔT ≪ \frac{T}{N_A}
\]

The expected value for \( K \) is then

\[
K = N_A \left( 1 - (1 - p)^N_B \right)
\]
And the standard deviation is

$$\sigma(K) = \sqrt{\frac{N_d(1-(1-p)^N_d)(1-p)^N_d}{N_d}}$$

(9)

Then the expected value of the precursor coincidence rate is

$$\langle r_p \rangle = \frac{K}{N_d}$$

(10)

And the standard deviation

$$\sigma(r_p) = \frac{\sigma(K)}{N_d}$$

(11)

The probability of obtaining a number of coincidences $K$ larger or equal to the empirically observed number $K_0$ is given by

$$P(K \geq K_0) = \sum_{K=K_0}^{N_d} P(K; N_d, 1 - (1 - p)^N_d)$$

(12)

To conform to equation (7), $\Delta T$ was set up at value 1, consequently $\tau=0$, testing that both SF and MDS events (Type A) were triggered by the closest $ET_i$ events (Type B). To transform the SF, MDS and $ET_i$ time series into event time series, we applied the first and the fourth quartile threshold creating series for values of those variables that were either smaller or larger than the thresholds and recorded as 1 (true) or 0 (false).

**Results and Discussion**

The predictors of the water status of grapevines ($gs$, SF, MDS) could clearly tell apart the ones that were irrigated from those that were rainfed. The overall average of stomatal conductance showed a highly significance ($P<0.01$) difference between the values read for the irrigated set ($gs=2.64$ cm$^3$ s$^{-1}$) and the rainfed set ($gs=1.89$ cm$^3$ s$^{-1}$). The trunk MDS and SF averages showed highly significant differences between both sets of plants, respectively, 57.6 $\mu$m and 4.2 L day$^{-1}$ plant$^{-1}$ for the irrigated and 61.8 $\mu$m and 2.7 L day$^{-1}$ plant$^{-1}$ for the rainfed. The overall average for crop load at harvest of irrigated plants was 2863 g per plant and the rainfed was 2054 g per plant. Reference evapotranspiration average was 1.62 mm day$^{-1}$ and no significant ($P>0.05$) correlation was found between $gs$ and $ET_0$. However, on Table 2 can be seen that the correlations between $ET_i$ and both MDS and SF were significant for both water regimes. The correlation between $gs$ and both SF and MDS, both water regimes, were not significative as shown in (Table 4). ECA analysis was performed only between variables that were significatively correlated. The results of ECA are shown in (Table 5 & 6) and they did not distinguished between rainfed (0) and irrigated (1) plants. The closest $ET_i$ event to SF or MDS always triggered an empirically observed number ($K$) for SF or MDS that was significatively higher than the expected coincidence ($K_e$) if the process was purely random. Lower values ($Q_i$) of $ET_i$ triggered lower values of both SF and MDS and, conversely, higher values ($Q_i$) of $ET_i$ triggered higher values of both SF and MDS (Table 5). The ECA showed that $ET_i$ events could not trigger simultaneusly either higher or lower values for SF and MDS as $K_e$ was always much lower than $K_i$ (Table 6).

Several authors have considered $gs$, SF and MDS as good predictors of the plant water status [9,7,21]. The results show these predictors could distinguish between irrigated and rainfed plants and they are in agreement with a large body of bibliographic references. The discrepancies appear when the relationships among these predictors and between them and environmental factors are established. Environmental conditions affect the physiological activity of plants altering their transpiration through stomatal control which must influence the sap flow rate [22] that should decrease under high atmospheric demand because augmented stomatal resistance reduces transpiration [5]. A close relationship between SF and $gs$ was found by Hernandez-Santana [21] if multiple readings of $gs$ from several leaves were taken during the day. The results of this work failed to establish a significant correlation between $gs$ and SF and the reason must reside on the measurement particularities of each variable.

**Table 2**: Pearson correlation and its significance (765 observations) between Sap Flow (SF), Maximum Daily Shrinkage (MDS), reference Evapotranspiration ($ET_o$), Vila Real 2011 to 2015.

<table>
<thead>
<tr>
<th>MDS Irrigated</th>
<th>$ET_o$</th>
</tr>
</thead>
<tbody>
<tr>
<td>SF irrigated</td>
<td>0.227**</td>
</tr>
<tr>
<td>MDS irrigated</td>
<td>0.181*</td>
</tr>
<tr>
<td>MDS rainfed</td>
<td>$ET_0$</td>
</tr>
<tr>
<td>SF rainfed</td>
<td>0.194**</td>
</tr>
<tr>
<td>MDS rainfed</td>
<td></td>
</tr>
</tbody>
</table>

**Correlation is significant at $\alpha<0.01$**

**Correlation is significant at $\alpha<0.05$**

**Abbreviations**: SF: Sap Flow; MDS: Maximum Daily Shrinkage; $ET_o$: Reference Evapotranspiration

<table>
<thead>
<tr>
<th>MDS Irrigated</th>
<th>$ET_o$</th>
</tr>
</thead>
<tbody>
<tr>
<td>SF irrigated</td>
<td>$V_1$</td>
</tr>
<tr>
<td>V1</td>
<td>0.155*</td>
</tr>
<tr>
<td>V2</td>
<td>0.460**</td>
</tr>
<tr>
<td>MDS irrigated</td>
<td>$V_1$</td>
</tr>
<tr>
<td>V1</td>
<td>0.149*</td>
</tr>
<tr>
<td>MDS rainfed</td>
<td>$V_1$</td>
</tr>
<tr>
<td>V1</td>
<td>0.197*</td>
</tr>
<tr>
<td>V2</td>
<td>0.386**</td>
</tr>
</tbody>
</table>

**Correlation is significant at $\alpha<0.01$**

**Correlation is significant at $\alpha<0.05$**

**Abbreviations**: ns: not significative; SF: Sap Flow; MDS: Maximum Daily Shrinkage; $gs$: Stomatal Conductance

SF integrates multiple readings taken and recorded automatically during 24 hour periods while gs represents a specific value on a given time, thus SF integrates constant changes of influential factors and gs represents the prevailing conditions of a given moment. There is no proved method of automatically measuring gs and to take multiple readings along the day is too taxing on resources. The relationship between MDS and gs, found by different authors, is sometimes contradictory. A significant, linear and direct relationship between MDS and gs was found in apple trees [28] and in olive trees [23] but they were inversely related in cherry trees [24]. It appears unquestionable that the stem diameter variation can be related to plant growth, water-use and status but any possible correlation with gs is likely to be indirect [3]. The lack of correlation between MDS and gs in this work is, again, on the specifics of their measurement process.

A large degree of agreement between daily reference evapotranspiration and SF has been found [25,26] and in olive trees the relationship was significant for both well-watered and water shorted plants [27]. A significant correlation was also found between MDS and ET₀ in apple trees [28] and in peach trees [29]. However, there is no full agreement on which weather meteor integrated in ET₀ calculation is the main underlying factor driving SF and MDS. Photosynthetically active radiation (PAR) and vapor pressure deficit (VPD) were the main drivers of SF in conifers [13] and in a desert shrub [26], while solar radiation and air temperature were found to be main driving forces in olive trees [30]. In vineyards [31], daily SF rate increased linearly with solar radiation and it showed an exponential increase with VPD or, for [30]. In vineyards [31], daily SF rate increased linearly with solar temperature were found to be main driving forces in olive trees [13] and in a desert shrub [26], while solar radiation and air pressure deficit (VPD) were the main drivers of SF in conifers [32].

Other factors besides weather conditions, such as soil water availability, species, tree age, phenological period and crop load, affect SF and MDS and a single or a couple of weather variables do not correlate well with SF or MDS in all circumstances. The results show a significant correlation of MDS and SF with ET₀ but it was independent of the phenological period (Table 3) or the irrigation regimen (Table 2). Given that the crop load was larger in irrigated plants than in rainfed, we can assume that the correlation was also independent from the crop load and Intrigliolo and Castel [34] also found that the crop load did not affect the variation in trunk diameter. The soil water content affected the dimension of MDS because in rainfed plants it averaged 61.8µm while in irrigated plants it was 57.6µm; the difference must be due to a larger availability of soil water that irrigated plants can draw and it prevents a strong shrinkage of inner tissues and also helps the full recovery of the tissues hydration when transpiration is low. The roots of plant with more limited water supply might not be able to meet the transpiration needs and the internal water storage pool is depleted resulting in a larger shrinkage of the plant organs [35].

SF reflects the flow of water from the soil to the plant and to the atmosphere thru the stomata. This flow is largely controlled by stomatal conductance [7] that the results show to be systematically higher in irrigated vines, thus they can experience higher values of SF as it was found by Hernandez-Santana [21].

The correlation (Tables 2 & 3) show a direct relationship between MDS and SF. Other factors besides weather conditions, such as soil water availability, species, tree age, phenological period and crop load, affect SF and MDS and a single or a couple of weather variables do not correlate well with SF or MDS in all circumstances. The results show a significant correlation of MDS and SF with ET₀ but it was independent of the phenological period (Table 3) or the irrigation regimen (Table 2). Given that the crop load was larger in irrigated plants than in rainfed, we can assume that the correlation was also independent from the crop load and Intrigliolo and Castel [34] also found that the crop load did not affect the variation in trunk diameter. The soil water content affected the dimension of MDS because in rainfed plants it averaged 61.8µm while in irrigated plants it was 57.6µm; the difference must be due to a larger availability of soil water that irrigated plants can draw and it prevents a strong shrinkage of inner tissues and also helps the full recovery of the tissues hydration when transpiration is low. The roots of plant with more limited water supply might not be able to meet the transpiration needs and the internal water storage pool is depleted resulting in a larger shrinkage of the plant organs [35].

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**Table 4:** Pearson correlation and its significance (40 observations) between Sap Flow (SF), Maximum Daily Shrinkage (MDS) and Stomatal Conductance (gs), Vila Real 2011 to 2015.

<table>
<thead>
<tr>
<th></th>
<th>gs Irrigated</th>
<th>gs Rainfed</th>
</tr>
</thead>
<tbody>
<tr>
<td>SF irrigation</td>
<td>0.122**</td>
<td>-0.050**</td>
</tr>
<tr>
<td>MDS irrigation</td>
<td>-0.064**</td>
<td>-0.027**</td>
</tr>
</tbody>
</table>

**Abbreviations:** ns: not significative; SF: Sap Flow; MDS: Maximum Daily Shrinkage; gs: Stomatal Conductance

**Table 5:** Expected (Ke) and empirically observed (K) coincidences between Type A (SF and MDS) and Type B (ET₀) events paired by column. Vila Real 2011-2015.

<table>
<thead>
<tr>
<th>Type A</th>
<th>Q, SFI (Nₐ=191)</th>
<th>Q, SFᵢ (Nₐ=191)</th>
<th>Q, SFᵢ (Nₐ=191)</th>
<th>Q, SFᵢ (Nₐ=190)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type B</td>
<td>Q, ET₀ (Nₐ=169)</td>
<td>Q, ET₀ (Nₐ=169)</td>
<td>Q, ET₀ (Nₐ=163)</td>
<td>Q, ET₀ (Nₐ=163)</td>
</tr>
<tr>
<td>Ke</td>
<td>37.9</td>
<td>37.9</td>
<td>36.7</td>
<td>36.5</td>
</tr>
<tr>
<td>K</td>
<td>51*</td>
<td>49*</td>
<td>49*</td>
<td>46*</td>
</tr>
<tr>
<td>Type A</td>
<td>Q, MDSₐ (Nₐ=192)</td>
<td>Q, MDSₐ (Nₐ=192)</td>
<td>Q, MDSₐ (Nₐ=191)</td>
<td>Q, MDSₐ (Nₐ=191)</td>
</tr>
<tr>
<td>Type B</td>
<td>Q, ET₀ (Nₐ=169)</td>
<td>Q, ET₀ (Nₐ=169)</td>
<td>Q, ET₀ (Nₐ=163)</td>
<td>Q, ET₀ (Nₐ=163)</td>
</tr>
</tbody>
</table>
All three types of stomata (sunken, same level and raised above epidermis) are present on the leaf lower face of Touriga Nacional grapevines, but same level and raised are dominant (about 54 and 47%, respectively) and they are, in comparison with other red varieties, longer (2.4 to 30 µm) and wider (17 to 18 µm) that might contribute to the enhanced plasticity of stomatal conductance under higher temperatures [37]. Touriga Nacional has high capacity for heat dissipation, it withstands high light intensities, and in the outer xylem, while SF depends essentially on the level of hydration of the whole sapwood [40].

An ET₀ event, either low or high value, does not trigger, simultaneously, a corresponding SF and MDS event (Table 6). It means that SF and MDS respond differently to weather conditions. Similar values of ET₀ can be reached with different combinations of its main driving factors: solar radiation, vapor pressure deficit and temperature. ECA does not explain the underlying mechanisms between ET₀ and SF and MDS, rather establishes a stochastic relationship of casualty, but given the conclusions draw by other authors mentioned above, we might assume that the trigger ET₀ event was due predominately by one of its main driving factors that affects preferentially either SF or MDS. We must keep in mind that SF and MDS are related to different physiological processes [19]. The variation of trunk diameter is, predominantly, the result of dynamics in water transport between tissues and depends mainly on the water stored in the phloem and in the outer xylem, while SF depends essentially on the level of hydration of the whole sapwood [40].

SF is a transpiration driven phenomenon and as transpiration progresses the hydraulic resistance throughout the plant increases causing the xylem water potential to decrease, influencing the water potential gradient between xylem and storage tissues, which has a direct effect on the radial water flow between these tissues [10,41]. This higher gradient decreases the radial conductance to water transport between the xylem and the storage tissue enhances the time lag between transpiration and diameter variation dynamics [40]. The relationship between ET₀ and the plant indicators might not follow the same pattern being linear with MDS and curvilinear with SF [5], resulting also in a time lag between MDS and SF. SF and MDS are good predictors of the plant water status [9] but the results show that a single indicator, either SF or MDS, does not reflect the grapevine response to changing environmental conditions but their combination can provide more detailed information about its water needs. The same observation was made by Fernández [8] regarding the water needs of mature olive orchards.

**Conclusion**

The predictors of the water status of grapevines (gs, SF, MDS) could clearly tell apart the ones that were irrigated from the ones that were rainfed. However, reference evapotranspiration had no significant correlation with gs but it was significant between ET₀ and both MDS and SF for both water regimens. The results of ECA did not distinguished between rainfed and irrigated plants. The closest ET₀ event to SF or MDS always triggered an empirically observed number for SF or MDS that was significatively higher than the expected coincidence if the process was purely random. Lower values of ET₀ triggered lower values of both SF and MDS and, conversely, higher values of ET₀ triggered higher values of both SF and MDS. The ECA showed that ET₀ events could not trigger simultaneously either higher or lower values for SF and MDS. A single indicator, either SF or MDS, does not reflect the grapevine response to changing environmental conditions but their combination can provide more detailed information about its water needs.

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**Conflict of Interest**

We declare that there are no conflicts of interest of any kind.

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**Table 6:** Expected (Ke) and empirically observed (K) coincidences between Type A (simultaneous SF and MDS) and Type B (ET₀) events paired by column. Vila Real 2011-2015.

<table>
<thead>
<tr>
<th>Type</th>
<th>Q₁SF + Q₁MDS</th>
<th>Q₂SF + Q₂MDS</th>
<th>Q₃SF + Q₃MDS</th>
<th>Q₄SF + Q₄MDS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(Nᵢ=321)</td>
<td>(Nᵢ=313)</td>
<td>(Nᵢ=341)</td>
<td>(Nᵢ=356)</td>
</tr>
<tr>
<td>Type A</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type B</td>
<td>Q₁ET₀ (Nᵢ=169)</td>
<td>Q₂ET₀ (Nᵢ=169)</td>
<td>Q₃ET₀ (Nᵢ=163)</td>
<td>Q₄ET₀ (Nᵢ=163)</td>
</tr>
<tr>
<td>Ke</td>
<td>38.1</td>
<td>38.7</td>
<td>36.7</td>
<td>36.7</td>
</tr>
<tr>
<td>K</td>
<td>49*</td>
<td>50*</td>
<td>51*</td>
<td>47*</td>
</tr>
</tbody>
</table>

*Significant difference at α=0.05

**Abbreviations:** Q₁: First Quartile; Q₄: Forth Quartile; SF: Sap Flow; MDS: Maximum Daily Shrinkage; ET₀: Reference Evapotranspiration; K: Observed Number of Events, Ke: Expected Number of Events

**Abbreviations:** ns: not significative; Q₁: First Quartile; Q₄: Forth Quartile; SF: Sap Flow; MDS: Maximum Daily Shrinkage; ET₀: Reference Evapotranspiration; K: Observed Number of Events, Ke: Expected Number of Events

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References


