Exploring thermal imaging variables for the detection of stress responses in grapevine under different irrigation regimes

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Abstract

Temperatures of leaves or canopies can be used as indicators of stomatal closure in response to soil water deficit. In 2 years of field experiments with grapevines (Vitis vinifera L., cvs Castelão and Aragónès), it was found that thermal imaging can distinguish between irrigated and non-irrigated canopies, and even between deficit irrigation treatments. Average canopy temperature was inversely correlated with stomatal conductance measured with a porometer. Variation of the distribution of temperatures within canopies was not found to be a reliable indicator of stress. A large degree of variation between images was found in reference ‘wet’ and ‘dry’ leaves used in the first year for the calculation of an index proportional to stomatal conductance. In the second year, fully irrigated (FI) (100% Etc) and non-irrigated (NI) canopies were used as alternatives to wet and dry leaves. A crop water stress index utilizing these FI and NI ‘references’, where stressed canopies have the highest values and non-stressed canopies have the lowest values, was found to be a suitable measure for detecting stress. It is suggested that the average temperatures of areas of canopies containing several leaves may be more useful for distinguishing between irrigation treatments than the temperatures of individual leaves. Average temperatures over several leaves per canopy may be expected to reduce the impact of variation in leaf angles. The results are discussed in relation to the application of thermal imaging to irrigation scheduling and monitoring crop performance.

Key words: Leaf angle, leaf temperature, partial rootzone drying, regulated deficit irrigation, stomatal conductance, thermography, Vitis vinifera, water deficit.

Introduction

Mean global temperatures are expected to rise over the next few decades, evaporation rates will increase, arid regions will expand, and thus water availability will be a major limitation to plant growth in the future (Houghton et al., 2001; European Environment Agency, 2004). As a result, irrigation will become an increasingly common practice. Since water availability is already limited, an increase in the area under irrigation will only be possible if the quantity of water used per unit area is reduced, i.e. if plant water use efficiency can be improved. Additionally, precise manipulation of plant–water relations can be very important for maximizing the quality of the product, particularly in viticulture. Excessive application of water can reduce colour and sugar content and produce acidity imbalances in the wine (Bravo et al., 1985; Esteban et al., 2001). Conversely, insufficient water reduces grape yield and can
also adversely affect quality (Reynolds and Naylor, 1994; dos Santos et al., 2003).

Deficit drip irrigation strategies have been used to save water in viticulture and simultaneously to improve wine quality. Regulated deficit irrigation (RDI) aims to manipulate grapevine vegetative and reproductive growth by withholding or applying less than the full vineyard water requirement or by manipulating grapevine vegetative and reproductive growth by withholding or applying less than the full vineyard water requirement. Partial rootzone drying (PRD) is an alternative to withholding or applying less than the full vineyard water requirement (Dry et al., 2001). Partial rootzone drying (PRD) is an alternative technique, currently of interest for a variety of crops (Davies et al., 2000; Grant et al., 2004) including grapevine (Dry et al., 2000; dos Santos et al., 2003), that allows control of vegetative growth and transpiration without the severe water stress periods that can occur in RDI (Loveys et al., 1999). In PRD, part of the root system is slowly dried and the remaining roots are exposed to wet soil. Roots of the watered side maintain a favourable plant water status, while dehydrating roots produce chemical signals that are transported to the shoots via the xylem. These signals are thought to control shoot vigour and stomatal aperture (Dry and Loveys, 1999).

Leaf or stem water potential is a standard indicator of stress, and is sometimes used in irrigation scheduling (Smart et al., 2004). This method, however, is destructive and time-consuming. Stomatal closure is known to be a sensitive response to soil water deficit, occurring even in the absence of any change in plant water status, as a result of root signalling (Davies et al., 2000). It has potential as an indicator of plant water stress and therefore could be used in irrigation scheduling. Monitoring stomatal conductance could be particularly useful to determine the timing of irrigation (for example in RDI or PRD systems) where a very precise regulation of water supply is required in the production of high quality fruits, including grapes for wine (Dry et al., 2001). However, the traditional methods of measuring stomatal conductance (using porometers or infrared gas analysers) are time-consuming, labour-intensive, and only give point measurements.

As stomata close under water deficits, leaf temperatures rise. Thus leaf or canopy temperatures can be used as an indicator of plant stress and stomatal closure. Thermal imaging systems allow rapid and non-invasive collection of data, integrated over the area of individual leaves or areas of canopies. They may reveal spatial heterogeneity within or between leaves, and can be used repeatedly on the same leaves to monitor responses over time, without affecting the natural behaviour of the leaves. The nature of grapevine trellises, with plentiful leaves that are close to vertical exposure, means that this crop may be particularly suited to monitoring with a thermal imager which can be carried along the rows.

The development of thermal imaging and the associated image analysis software has overcome the problems experienced by researchers using infrared thermometry with regard to the difficulty of separating leaf and non-leaf (soil, sky, bark, etc.) temperatures. While application of thermal imaging is more straightforward in the laboratory (Chaerle et al., 1999; Lindenthal et al., 2005), researchers have also applied the technique to the field (Jones et al., 2002; Cohen et al., 2005). Nonetheless, rigorous testing of thermal imaging against more traditional physiological techniques under field conditions is still required for different types of crops. Indices that relate leaf or canopy temperatures to the temperatures of selected reference surfaces allow for variation in air temperature, radiation, and wind speed, thus removing the effect of environmental variation so as to indicate increases or decreases in stomatal conductance (Jones, 1999). An alternative possibility for detecting stress in plant canopies is to analyse thermal variation within the canopy. Leaf orientation plays a greater role in the energy budget of leaves when stomatal aperture is smaller, which may result in greater variation in temperatures within canopies that are more stressed, with lower stomatal conductance, than in unstressed canopies with very open stomata (Fuchs, 1990). Variability in temperatures between plants in the same management treatment has been noted to increase with stress, for example, Gardner et al. (1981), probably as a result of variation in soil properties and root depth. Leaf orientation and canopy geometry (row orientation, row spacing, plant height) interact with environmental factors and stomatal conductance to determine the temperature of the plant canopy (Boissard et al., 1990). As yet there has been little attempt to analyse the impact of canopy architecture on the application of thermal imaging. Leaf drooping during wilting in stressed canopies, or altered leaf orientation or inclination, may reduce the impact of stomatal closure on leaf temperature.

The objectives of this work were to evaluate thermal imaging as a tool for distinguishing between stressed and unstressed plants, and to optimize thermal imaging for determining plant responses to water deficits in the field. Experiments were carried out to test whether thermal imaging can be used to distinguish between irrigated and water-limited grapevines, and between grapevines growing under different deficit irrigation systems. The relationship between canopy or leaf temperatures, or indices derived from these temperatures, and stomatal conductance as measured with a porometer were explored. The influence of leaf size, leaf orientation angle, and leaf inclination angle on leaf and canopy temperatures was also investigated.

Materials and methods

Thermal imaging and stomatal conductance

All thermal images were obtained with a thermal imager (IR Snapshot 525, Infrared Solutions, Minneapolis, MN, USA) that operates in the wavebands 8–12 μm, has a thermal resolution of 0.1 °C, and produces pictures with spatial resolution of 120×120 pixels. Images were analysed in SnapView Pro software (Infrared Solutions); all images...
were corrected for spatial calibration drift by subtracting corresponding reference images of an isothermal surface (Jones et al., 2002). For each series of measurements, the background temperature was determined as outlined in the imager manual as the temperature of a crumpled sheet of aluminium foil in a similar position to the leaves of interest. Emissivity for measurements of leaves and plant canopies was set at 0.96 (see review by Jones, 2004). The areas of interest for analysis in the imager’s software were outlined, manually, by comparing thermal and normal digital images (Fig. 1). All thermal images were taken with the thermal imager on a tripod perpendicular to the area being imaged. Images of canopies and individual leaves were taken ~1.5 and 0.9 m from the canopies and leaves, respectively, capturing areas of ~50 cm × 50 cm and 29 cm × 29 cm, respectively.

Where individual leaves were imaged in 2003, dry and wet references were used to mimic leaves with fully closed and fully open stomata, respectively (Jones et al., 2002). These references were grapevine leaves, cut from the canopy prior to measurements and placed close to the leaves of interest. Wet reference leaves were sprayed with water on both sides, regularly, to maintain their moisture. Dry reference leaves were covered in petroleum jelly (Vaseline) on both sides. The temperatures of these references were obtained (\(T_{DR}\) and \(T_{WR}\)) and used in conjunction with leaf temperatures to obtain thermal indices. Stomatal conductance \(g_s\) of the same leaves used in thermography was measured with a steady-state porometer (Li-Cor 1600, Li-Cor, Lincoln, NE, USA).

Where canopies rather than individual leaves were imaged, reference leaves were not included. In 2004, images of non-irrigated (NI) and fully irrigated (FI) canopies were used as indicators of low and high stomatal conductance, respectively.

**Experimental conditions**

Field measurements were made in 2003 and 2004, in two different commercial vineyards. Both are located in south-east Portugal, where the climate is Mediterranean, with hot, dry summers and cool, wet winters. Both of the cultivars of grapevine (*Vitis vinifera* L.) studied (Castelão and Aragoneș) are red varieties and were grafted on 1103 Paulsen rootstock, and trained on a bilateral Royat Cordon system. The main characteristics of the vineyards are described in Table 1. Crop evapotranspiration \(E_{Tr}\) was calculated from Class A pan evaporation and using the crop coefficients proposed by Prichard (1992). Irrigation was applied with drip emitters, two per vine, positioned 25 cm from the vine trunk, one either side of the row.

**Castelão 2003:** The cultivar Castelão was subjected to the following treatments: non-irrigated (rain-fed) (NI), partial rootzone drying (PRD) where 50% of \(E_{Tr}\) was supplied to only one side of the root system, alternating sides every 15 d; deficit irrigation (DI), where 50% of \(E_{Tr}\) was divided between the two sides of the row; and full irrigation (FI), corresponding to 100% \(E_{Tr}\). Each treatment was replicated in each of four experimental rows, in a Latin square design, with two guard rows between each pair of experimental rows.

Thermal images were taken and \(g_s\) of the same leaves measured in the morning and afternoon on different dates (Table 2). Four replicate plants were used per treatment, one in each experimental row. The porometer measurement was taken immediately after each thermal infrared image. Additionally, on one date (6 August), eight replicates were taken for thermal infrared images (two plants per treatment per row). Thermal infrared images were also taken of areas of leaf canopies (eight replicates per treatment, two plants per treatment per row), in the morning and afternoon on different dates. Plants were sampled along rows, so that the order of sampling of treatments was randomized.

**Aragoneș 2004:** In 2004, measurements were conducted near Estremoz using the cultivar Aragoneș. Three treatments were imposed: PRD, DI, and regulated deficit irrigation (RDI). RDI plants received more water than the other treatments at the start of the growing season and less later in the growing season, with irrigation of RDI plants being stopped on 10 August. Over the whole season, RDI plants thus received the same total amount of water as PRD and DI plants. Measurements were also conducted on adjacent NI vines and FI vines. Thermal infrared images were taken of three vines per treatment in each of three selected blocks and the same plants were used throughout. Before measurements on each block, thermal

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**Table 1. Characteristics of two vineyards in south-east Portugal where the experiments were conducted**

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Castelão</th>
<th>Aragoneș</th>
</tr>
</thead>
<tbody>
<tr>
<td>Year of experiment</td>
<td>2003</td>
<td>2004</td>
</tr>
<tr>
<td>First year of treatments</td>
<td>2000</td>
<td>2004</td>
</tr>
<tr>
<td>Year vines were grafted</td>
<td>1995</td>
<td>2000</td>
</tr>
<tr>
<td>Location</td>
<td>Centro Experimental de Pedães</td>
<td>Seis Reis, near Estremoz</td>
</tr>
<tr>
<td>Latitude</td>
<td>30°38’N</td>
<td>38°48’N</td>
</tr>
<tr>
<td>Longitude</td>
<td>8°39’W</td>
<td>7°29’W</td>
</tr>
<tr>
<td>Start date for irrigation</td>
<td>26 June 2003</td>
<td>15 June 2004</td>
</tr>
<tr>
<td>Experimental design</td>
<td>Latin square, 12 rows, 8 blocks, 9 rows per block</td>
<td>Latin square, 12 rows, 8 blocks, 9 rows per block</td>
</tr>
<tr>
<td>Orientation of rows</td>
<td>N-S</td>
<td>ENE-WSW</td>
</tr>
</tbody>
</table>

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**Fig. 1.** An example of a thermal image and the corresponding digital image. The area of interest on the thermal image is outlined.
infrared images were taken of one NI plant and one FI plant, to be used as references. A set of measurements took ~1 h.

The main lateral veins of each individual marked leaf and of five leaves in the selected sections of canopy were measured as an indication of leaf area (Lopes and Pinto, 2000). The inclination from horizontal of each individual marked leaf, and of five randomly sampled representative leaves in two of the selected sections of canopy per treatment per row were measured with a protractor attached to a level, and the azimuths of the central vein of the same leaves were measured relative to the orientation of the row with a protractor, and then converted to absolute azimuths, where leaves with the central blade facing directly north have an azimuth of 0°.

To test the hypothesis that the effect of leaf drooping in stressed grapevine canopies influences leaf temperature, leaves in the south–south-west-facing side of nine NI vines were forced to stay in their pre-stress position, using metal wire to hold the petiole and string to maintain the distance between the petiole and the row. In adjacent control plants, drooping of leaves was not prevented. Canopy thermal images were taken and stomatal conductance was recorded.

Data analyses and statistics

**Thermal indices:** The index I_G was calculated from leaf temperatures: I_G=(T_{dry}-T_{leaf})(T_{leaf}-T_{wet}). This index is theoretically proportional to stomatal conductance (g_s) (Jones, 1999). An index analogous to Idso’s (1982) crop water stress index (CWSI) was also calculated, where in this case CWSI=(T_{dry}-T_{leaf})/(T_{dry}-T_{wet}). Similar indices were used in 2004 with T_{dry} replacing T_{dry} and T_{FI} replacing T_{wet}. These indices are called CWSI_{NI/FI} and I_{NI/FI} to distinguish them from the more established indices CWSI and I_G.

**Temperature distribution in canopies:** Images of areas of canopies in SnapView Pro were exported to Excel, to obtain the temperature of every pixel in the image. Canopies were outlined and the frequency distributions of the temperatures of pixels in these areas were calculated, together with the mean temperature, variance, skewness (deviation of the distribution from symmetry), and kurtosis (deviation of the distribution from the normal peak) as reported by Guiliani and Flore (2000). A histogram-derived CWSI (HCWSI), based on the approach of Bryant and Moran (1999), was also calculated as a measure of the deviation of the shape of the histogram from a normal curve with the same mean and variance. The observed temperature frequency distribution was normalized by expressing the frequency in any 0.1 K temperature range as a fraction of the maximum frequency in any range to give f_r. The corresponding normal distribution for each range was calculated using the mean and variance, and again normalized by expressing as a fraction of the maximum to give dist_r. HCWSI was calculated as the sum of the absolute differences for each temperature range:

\[ HCWSI = \sum_{r=T_{min}}^{T_{max}} abs[(f_r - dist_r)] \]

where T_{max} and T_{min} are the maximum and minimum temperature values of pixels in the image.

Variance, skewness, and kurtosis of thermal distributions were calculated for images of canopies taken either year. Additionally, indices of variation within images were calculated from each image as (maximum temperature–minimum temperature)/maximum temperature, and were averaged for each treatment. For indices of variation within treatments, average canopy values were obtained for each image and the index was calculated as (maximum average temperature–minimum average temperature)/maximum average temperature.

**Statistical analyses**

Data were tested for normality and homogeneity of variances using Kolmogorov–Smirnov and Levene’s tests, respectively, in STATISTICA (1995). The significance of relationships between I_G and g_s was tested by Pearson-product or Spearman correlations. The effects of treatments were analysed by analysis of variance (ANOVA), using a Latin square design for 2003 data and two-factor ANOVA for the 2004 data, with the factors being treatment and block. Coefficients of variation (=100×SD/mean) were calculated for thermal measurements.

Results

**Castelão 2003**

In 2003, air temperatures in the vineyard were very high in July and August (Fig. 2); the average daily maximum temperature recorded between 30 July and 27 August was 38°C.

No significant differences were found between treatments in g_s, as measured with the porometer, on the four dates of measurement, perhaps due to small sample sizes as well as variability between treatments. As a result, differences between treatments in thermal variables might not have been expected. However, lack of variation between treatments in stomatal conductance was in contrast to predawn leaf water potential, which was significantly lower in NI and DI vines than in FI vines both at the end of July and in mid-August (Table 3). Stomatal closure may have occurred in all treatments at some time during the hot summer, but evidently not for sufficient lengths of time to prevent the development of differences in leaf water potentials.

Of the four dates on which the temperatures of individual leaves were measured, only on one was a significant effect of treatment observed (6 August; Fig. 3). The significant effects on this date probably relate to greater sample sizes (n=8 on 6 August compared with n=4 on the other dates).
rather than any meteorological or other factor that might differentiate this date from the others. Temperature differences were found both in the morning (in shaded leaves, \( P=0.019 \)) and in the afternoon (sunny, \( P=0.049 \)). At both times, FI leaves were cooler than NI leaves. Correspondingly, \( I_G \) was lower, both in the shade and in the sun, in NI compared with FI leaves (Fig. 3B). In the morning (shade), PRD canopies also showed significantly cooler temperatures and higher \( I_G \) than NI canopies. In the afternoon (sun), all the irrigated canopies had significantly higher \( I_G \) than NI.

Stomatal conductance as measured with the porometer and \( I_G \) showed significant correlations (\( P<0.02 \)) on 31 July am, 13 August pm, and 14 August am and pm (example in Fig. 4), indicating that individual vines with low leaf temperatures showed high \( g_s \), and vice versa. The correlation between \( g_s \) and \( I_G \) was not significant on 31 July pm or 13 August am. The significance of the correlations was not related to the range of conductances, nor to exposure. Some negative values of \( I_G \) were obtained, due to higher values for \( T_{leaf} \) than \( T_{dry} \).

When images of areas of canopies rather than individual leaves were taken, there were significant treatment effects on canopy temperature (\( P=0.001 \)), both in the morning and in the early afternoon (Fig. 5). FI canopies were cooler than NI or DI canopies, whether viewing the sunlit or shaded canopies. The HCWSI varied considerably within the same treatment, and even between two canopies of the same treatment imaged in quick succession. As a result, neither HCWSI nor the other measures of temperature variation varied significantly between treatments (Table 4). Thus no increase in temperature variance was detected with greater plant stress. In general, the frequency distributions of pixels in NI and FI canopies were fairly similar. Indices of variation within images of individual vines were fairly high, irrespective of treatment, with relatively low indices of variation within treatments (i.e. between images of different vines), when the maximum and minimum of mean image temperatures is used in this calculation (Table 5).

**Aragonês 2004**

Clear treatment effects on stomatal conductance (measured by porometry) were found on all occasions studied in August 2004 (\( P<0.03 \)), with conductances consistently increasing in the order: NI, RDI, FI, with the PRD and DI treatments often approaching or equalling the FI value (Fig. 6). RDI leaves had significantly lower predawn water potentials than PRD or DI leaves at this time (Table 4).

For the afternoon of 13 August, a highly significant effect of treatment was found on average canopy temperature (\( P=0.0001 \)), with post hoc tests showing that RDI canopies were significantly hotter than PRD canopies or DI canopies (Fig. 7C). Since in 2004 no reference wet and dry leaves were included in thermal infrared images, alternative reference temperatures were derived from the temperatures of FI and NI canopies imaged at intervals: values were extrapolated between the three measurements of FI and extrapolated to the time of the last measurement in any given session. The same was done for NI measurements. As a result, for every image of a PRD, DI, or RDI canopy, corresponding FI and NI temperatures were obtained. Some temperatures of PRD, DI, or RDI canopies fell outside the range of the corresponding NI and FI canopy temperatures. Canopy temperatures higher than the corresponding NI temperature result in negative values of \( I_{NI/FI} \) \((T_{NT}-T_{leaf} \) is negative\), but canopy temperatures cooler than the corresponding FI also result in negative values of \( I_{NI/FI} \) \((T_{leaf}-T_{FI} \) is negative\) (examples in Table 6). Thus, negative values of \( I_{NI/FI} \) could indicate either a very stressed or a completely unstressed canopy. With the modified CWSI, on the other hand, values outside the range

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**Table 3. Water potential and leaf area responses of the cultivar Castelão to different irrigation schedules**

<table>
<thead>
<tr>
<th>Property</th>
<th>Cultivar</th>
<th>Date</th>
<th>NI</th>
<th>PRD</th>
<th>DI</th>
<th>RDI</th>
<th>FI</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \Psi_{PD} ) (MPa)</td>
<td>Castelão</td>
<td>31/07/03</td>
<td>-0.49±0.03 c</td>
<td>-0.34±0.01 b</td>
<td>-0.36±0.02 b</td>
<td>-0.26±0.02 a</td>
<td></td>
</tr>
<tr>
<td>14/08/03</td>
<td></td>
<td>-0.50±0.04 b</td>
<td>-0.33±0.02 a</td>
<td>-0.49±0.02 b</td>
<td>-0.30±0.01 a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leaf area (m² vine⁻¹)</td>
<td>Castelão</td>
<td>19/08/03</td>
<td>5.48±0.52 b</td>
<td>8.46±1.07 ab</td>
<td>8.04±1.10 ab</td>
<td>10.09±1.46 a</td>
<td></td>
</tr>
</tbody>
</table>
0–1 are consistent with the idea of low values when canopies are not stressed and high values when they are stressed. Thus the temperatures of NI and FI canopies can be seen not as absolute limits of possible canopy temperatures, but as indicator temperatures.

For CWSI_{NI/FI}, there was a highly significant effect of treatment \((P < 0.0001)\), with RDI canopies showing higher values than canopies receiving the other treatments (Fig. 8A). Canopy temperature and CWSI_{NI/FI} were significantly negatively correlated with stomatal conductance \((P < 0.02, r^2=0.3; \text{Fig. 9})\); stomatal conductance, however, was measured for only one leaf within each canopy imaged.

A significant effect of treatment was also found on \(I_{NI/FI} (P=0.012)\) (Fig. 8B), with lower \(I_{NI/FI}\) values for RDI canopies than PRD canopies. The variance of the temperature distribution was correlated with the average canopy temperature \((r^2=0.37, P < 0.001, \text{Fig. 9C})\), but did not significantly differ between treatments. Neither the kurtosis nor skewness of the temperature of canopies was correlated with the average canopy temperature. The index of variation between canopies was highest for RDI (0.14), a little lower for PRD (0.13), and lowest for DI (0.11).

With respect to thermal images of individual leaves (rather than canopies) on 19 and 24 August, no significant differences between treatments were found in leaf temperature or CWSI_{NI/FI}. The only significant effect of treatment \((P=0.007)\) was found for \(I_{NI/FI}\) on the afternoon of 19 August, with the highest values being found for PRD leaves and the lowest for RDI leaves (Fig. 8C).

The lengths of the two main lateral veins of the grapevine leaves mostly fell between 6 cm and 15 cm (Fig. 10A). The most frequent leaf orientations were between 150° and 180°, where 0° faces north, i.e. approximately perpendicular to the direction of the row (Fig. 10B). Leaf inclination angles were mostly distributed between 40° and 80° from horizontal (Fig. 10C). No significant effect of treatment was found on the angle, orientation, or size (average length of the two main lateral veins) of the marked leaves. No significant correlation was found for these leaf properties and either temperatures or CWSI_{NI/FI}.
Table 4. Water potential and carbon isotope discrimination responses of the cultivar Aragones to different irrigation schedules

<table>
<thead>
<tr>
<th>Property</th>
<th>Cultivar</th>
<th>Date</th>
<th>Treatment</th>
<th>NI</th>
<th>PRD</th>
<th>DI</th>
<th>RDI</th>
<th>FI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ψ&lt;sub&gt;Ψ&lt;/sub&gt; (MPa)</td>
<td>Aragones</td>
<td>19/08/04</td>
<td>−0.39±0.02 a</td>
<td>−0.45±0.02 b</td>
<td>−0.64±0.03 c</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>δ&lt;sup&gt;13&lt;/sup&gt;C</td>
<td>Aragones</td>
<td>02/09/03</td>
<td>−27.25</td>
<td>−28.34±0.17 a</td>
<td>−28.42±0.16 a</td>
<td>−27.79±0.27 a</td>
<td>−28.89</td>
<td></td>
</tr>
</tbody>
</table>

Table 5. The histogram crop water stress index (HCWSI) and variance, skewness, and kurtosis of temperature distributions of canopies in different treatments, for Castelão vines on two dates

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Date</th>
<th>Distribution measures</th>
<th>Index of variation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>HCWSI</td>
<td>Variance</td>
</tr>
<tr>
<td>NI</td>
<td>5 Aug</td>
<td>53.47±8.02</td>
<td>1.44±0.28</td>
</tr>
<tr>
<td></td>
<td>26 Aug</td>
<td>67.32±6.51</td>
<td>2.47±0.39</td>
</tr>
<tr>
<td>PRD</td>
<td>5 Aug</td>
<td>66.08±5.15</td>
<td>2.35±0.34</td>
</tr>
<tr>
<td></td>
<td>26 Aug</td>
<td>72.48±6.70</td>
<td>3.04±0.44</td>
</tr>
<tr>
<td>DI</td>
<td>5 Aug</td>
<td>58.99±7.79</td>
<td>1.79±0.24</td>
</tr>
<tr>
<td></td>
<td>26 Aug</td>
<td>66.17±3.21</td>
<td>2.57±0.37</td>
</tr>
<tr>
<td>FI</td>
<td>5 Aug</td>
<td>57.48±5.09</td>
<td>1.76±0.13</td>
</tr>
<tr>
<td></td>
<td>26 Aug</td>
<td>64.24±4.29</td>
<td>3.37±0.38</td>
</tr>
</tbody>
</table>

Table 6. Examples of L<sub>G</sub> and CWSI derived from temperatures of the treatment canopies (T<sub>canopy</sub>) and interpolated NI and FI canopy temperatures to correspond to each T<sub>canopy</sub> measurement (T<sub>NN</sub> and T<sub>FI</sub>, respectively), for Aragones grapevines

<table>
<thead>
<tr>
<th>Time</th>
<th>T&lt;sub&gt;canopy&lt;/sub&gt;</th>
<th>T&lt;sub&gt;NN&lt;/sub&gt;</th>
<th>T&lt;sub&gt;FI&lt;/sub&gt;</th>
<th>L&lt;sub&gt;G&lt;/sub&gt;</th>
<th>CWSI</th>
</tr>
</thead>
<tbody>
<tr>
<td>08:39:36</td>
<td>19.06</td>
<td>20.43</td>
<td>18.06</td>
<td>1.37</td>
<td>0.42</td>
</tr>
<tr>
<td>08:40:56</td>
<td>20.63</td>
<td>20.53</td>
<td>18.29</td>
<td>−0.04</td>
<td>1.04</td>
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Discussion

Sensitivity to crop stress

While it is encouraging that thermal imaging could consistently distinguish between NI and FI canopies in the experiment in 2003, even more interesting is the ability to distinguish between different irrigation treatments, as seen in 2004. Thermal imaging distinguished RDI from the other two treatments, PRD and DI (as observed on 13 August 2004), as did porometry on several dates. RDI exhibited the highest temperature and lowest g<sub>s</sub>. Predawn water potential followed the same pattern. A similar trend was seen in carbon isotope discrimination of leaf material, with less negative values for leaves from RDI and NI vines, and more negative values in PRD, DI, and FI vines (Table 4). In 2004, the same number of replicates was used for leaf and canopy temperatures, so it is of interest that significant differences were found between treatments that year in canopy temperature but not in leaf temperature—more data would be needed, however, in order to ascertain whether canopy temperature is consistently more sensitive than the temperature of individual leaves. Similar patterns in both years between canopy thermal data and other indicators of crop stress (stomatal conductance, water potential, and carbon isotope discrimination) suggest that thermal imaging is an effective method of detecting crop stress.
Thermal indices

Significant differences between treatments in the absolute temperatures of areas of canopy suggest that this may be an effective method of distinguishing stressed from non-stressed plants. However, in other situations, where there are no randomized treatments to compare, such as monitoring a plant canopy over time for the purposes of irrigation scheduling, it can be difficult to distinguish increasing plant stress from an increase in air temperature. The use of references is designed to eliminate such a problem. Lower \( I_G \) values in NI than FI leaves on 6 August 2003 reflected greater stress in the NI vines. \( I_G \), however, often showed values below 0, resulting in an inability to distinguish canopies with extremely low conductance from canopies with very high conductance. This problem does not occur with CWSI, for which canopies with very high conductance should show very low values of CWSI and canopies with very low conductance would always show relatively high values of CWSI. Nonetheless, the individual wet and dry leaves used as references to calculate these indices may not be good references for whole canopies, whereas moving whole branches around the vineyard to act as more suitable references is not convenient. Different lengths of time between spraying the ‘wet’ leaves and taking the image are bound to lead to errors. Furthermore, previous work with grapevine (Jones et al., 2002) and cotton (unpublished) suggests a treatment effect on wet reference leaf temperatures, which would be possible if increased evaporation in well-irrigated canopies affects the measured temperatures of the wet references. For these reasons, it was decided to explore an alternative to the use of wet and dry reference leaves. Extrapolating between repeated measurements of NI and FI canopies, as done in 2004, allowed the use of indices similar to those currently used with reference leaves, but without the associated problems listed above.

It is suggested that this system may be preferable to the use of wet and dry leaves. This method allows easy detection of areas within a field where vines are stressed, and could be incorporated into vineyard management. It does not require any additional meteorological data.

Canopy architecture

Water loss can be minimized by closing stomata, but also by reducing light absorbance. Rolling leaves, wilted leaves or steep leaf angles, or reduced canopy leaf area through reduced growth and shedding of older leaves, are all involved in minimizing water loss from plants (Ludlow and Muchow, 1990; Chaves and Oliveira, 2004), and are
also important for preventing photoinhibition (Werner et al., 2002). If leaf movements occur after stomata close, they may contribute to canopy cooling, as intercepted irradiance changes. Thus, with thermal imaging, a canopy with closed stomata may not be distinguished from one with open stomata, but different architecture. Greater sensitivity of canopy temperatures than leaf temperatures to irrigation would occur if variation in the angle of individual leaves obscures differences relating to stomatal conductance. These masking effects of individual leaf angles may cancel out over whole canopies, if the distribution of leaf angles is similar in different canopies. It had been considered that leaf angle may vary measurably in different treatments, but did not find evidence to support this. Additionally, variation in leaf angle was not correlated with any temperature variables, and leaf drooping during wilting did not affect canopy temperature. Nonetheless, using a model to derive stomatal conductance from leaf temperature and vice versa (Leinonen et al., 2006), the range of orientations and angles found in the canopies measured would be expected to have a large influence on the relationship between conductance and temperature. The inverse correlation of canopy temperatures with \( g_s \) in 2004, but lack of correlation between leaf temperature and \( g_s \), suggests that individual leaf temperatures may bear less relationship to \( g_s \) than temperatures of areas of canopies.

In the data collected in 2003, the possibility that temperature differences between canopies might relate to canopy density rather than stomatal conductance alone cannot be ruled out. Irrigated plants had significantly greater leaf area than non-irrigated vines (Table 3). A reduced leaf area would result in a reduced area of
transpiring surface per area of canopy in a thermal image, which may lead to a lower estimate of conductance, even if the conductance per leaf is the same. Decreased leaf density in non-irrigated vines means that the average canopy temperature could be higher than the average of a similarly transpiring but denser canopy. This effect would accentuate differences between treatments in \( T_{\text{canopy}} \), but not in \( T_{\text{leaf}} \), and may partly explain why greater sensitivity of canopy temperatures than leaf temperatures to irrigation was found.

**Temperature variability within canopies**

It has been suggested that an alternative to using the absolute temperatures of canopies to determine stress is to use the variation in temperatures within a canopy (Gardner et al., 1981; Fuchs, 1990; Leinonen and Jones, 2004). No evidence was found to support this in Castelão and Aragonès, with no greater variation of thermal distribution within vines in stressed than well-irrigated canopies. This may relate to the non-random distribution of leaf angles in grapevine canopies, as the effect would only be expected in a canopy with random leaf orientation (Fuchs, 1990), and therefore should be investigated in other crops. Indeed, in grapevines, leaf angles could become more uniform as the vines become more stressed and leaves droop, with the effect that temperature variability within canopies could be greater in less stressed canopies. Furthermore, images of dense canopies may contain a greater diversity of leaf angles, which again could lead to greater temperature variance within non-stressed than within stressed canopies. However, temperature variability between rather than within canopies of grapevines may be a better indicator of stress, if some locations in a field become water deficient before others. In these experiments, indices of within-treatment variation were not consistently higher in stressed canopies than in well-irrigated canopies, but analysis of variation between canopies could aid in the detection of individual stressed plants or areas of poor soil or faults in irrigation systems.

**Conclusions**

It is suggested that the average temperatures of areas of canopies containing several leaves are perhaps more useful for distinguishing between irrigation treatments than the temperatures of individual leaves. Average temperatures over several leaves per canopy may be expected to reduce the impact of variation in leaf angles. The effect of the interaction of stomatal conductance and canopy architecture on canopy temperature needs further investigation, but it has been shown that thermal imaging can be a useful tool for distinguishing between stressed and unstressed vines. Temperature differences found between canopies under two different irrigation regimes are encouraging for the application of thermal imaging for irrigation scheduling. While an estimation of stomatal conductance requires additional meteorological data, the CWSI that was used here, which requires no additional information, may be sufficient for the detection of relative stress required for irrigation scheduling. This CWSI using NI and FI canopies as alternatives to wet and dry references removes problems associated with wet and dry reference leaves. Since it does not require any props or equipment other than the thermal camera, it is also a more rapid and convenient approach, and may be useful for commercial application. This needs to be tested in experiments in which scheduling is determined by different methods, one of these being thermal imaging alone.
Acknowledgements

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