Use of infrared thermography for monitoring stomatal closure in the field: application to grapevine

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Abstract

This paper reviews and discusses strategies for the use of thermal imaging for studies of stomatal conductance in the field and compares techniques for image collection and analysis. Measurements were taken under a range of environmental conditions and on sunlit and shaded canopies to illustrate the variability of temperatures and derived stress indices. A simple procedure is presented for correcting for calibration drift within the images from the low-cost thermal imager used (SnapShot 225, Infrared Solutions, Inc.). The use of wet and dry reference surfaces as thresholds to eliminate the inclusion of non-leaf material in the analysis of canopy temperature is discussed. An index that is proportional to stomatal conductance was compared with stomatal measurements with a porometer. The advantages and disadvantages of a possible new approach to the use of thermal imagery for the detection of stomatal closure in grapevine canopies, based on an analysis of the temperature of shaded leaves, rather than sunlit leaves, are discussed. Evidence is presented that the temperature of reference surfaces exposed within the canopy can be affected by the canopy water status.

Key words: Energy balance, infrared thermography, infrared thermometry, leaf temperature, thermal imaging, stomatal conductance, Vitis vinifera.

Introduction

Most crops are highly sensitive to water status with small changes in water availability having large impacts on both productivity and crop quality (Salter and Goode, 1967; Hsiao, 1973). There is extensive evidence that water is a major factor limiting and regulating both quality and productivity in grapevine (Vitis vinifera L.) with photosynthesis being primarily affected through the effects on stomatal closure (Escalona et al., 1999). Since the precise regulation of water supply is critical for the effective regulation of grape quality for winemaking (Dry et al., 2001) there is a real need for sensitive and robust techniques for detection of plant water ‘stress’. This paper investigates the potential of infrared thermography as a tool for irrigation scheduling.

There has been interest for many years in using infrared measurement of canopy temperature as an indicator of ‘crop stress’, canopy conductance or canopy transpiration, usually for irrigation scheduling purposes (Jackson et al., 1981; Idso, 1982; Jackson, 1982). The recent development of portable thermal imagers has greatly extended the opportunities for analysis of the thermal properties of plant canopies and widened the information available relating to the growth and condition of plants (Boissard et al., 1990; Jones, 1999b). Following early developments (Tanner, 1963), the approach was put on a rigorous footing for irrigation purposes by Idso and colleagues in the early 1980s (Jackson et al., 1981; Idso, 1982; Jackson, 1982) who defined a ‘Crop Water Stress Index’, CWSI, as the difference between the canopy temperature ($T_c$) and a

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non-water-stressed baseline’ temperature for a similar but well-watered crop \( (T_{\text{nws}}) \), divided by the difference between the temperatures of a non-transpiring crop \( (T_d) \) and the \( T_{\text{nws}} \).

There are, however, a number of factors that limit the general application of the approach developed by Idso and colleagues. Firstly, although the approach corrected for variation in environmental conditions relating to atmospheric humidity, it has not been found to be sensitive enough for routine application in many more humid climates, where the absolute temperature ranges are small, as it is known to be sensitive to factors such as wind speed and irradiance (Hipps et al., 1985; Jones et al., 1997). A second problem with the CWSI approach has been that the infrared thermometers available until recently limited measurements to an average over a single target area, which could inadvertently include soil, trunk or sky in the sensed area, with consequent errors in estimated canopy temperature (Moran et al., 1994). In sparse canopies in particular, the infrared thermal images can be affected by the temperature of the background soil (Inoue et al., 1994).

The recent development of field-portable thermal imaging systems opens up the opportunity to study not only the average temperatures over a defined area but also to obtain frequency distributions of temperature over the area and, if necessary, to include only areas which are known to be the canopy of interest.

More recently, approaches have been developed in attempts to improve the sensitivity of infrared estimation of crop stress indices by the use of either dry (Qiu et al., 1996) or wet and dry (Jones et al., 1997; Jones 1999a) reference surfaces. Among a number of indices derived in the latter work was one, \( I_G \) (referred to in what follows as \( I_G \)), which is proportional to the leaf conductance to water vapour transfer \( (g_{W}) \): \[
I_G = (T_{\text{dry}}-T_l)/(T_l-T_{\text{wet}}) = g_{W}(r_a + \gamma s)\gamma r_{HR} \] (1)

where \( T_l \) is the temperature of the transpiring surface, \( T_{\text{wet}} \) is the temperature of a corresponding wet surface, \( T_{\text{dry}} \) is the temperature of a similar but non-transpiring surface, \( r_a \) is the boundary layer resistance to water vapour, \( r_{HR} \) is the parallel resistance to heat and radiative transfer (Jones, 1992; p. 108), \( \gamma \) is the psychrometric constant, and \( s \) is the slope of the curve relating saturation vapour pressure to temperature. Use of equation (1) to estimate leaf conductance requires an independent estimate of \( r_a \). Possible approaches to the estimation include the use of heated model leaves (Dixon and Grace, 1983; Brough et al., 1986), or from measurement/estimation of net radiation absorption by leaves (Brough et al., 1986).

The main advantage of the approach based on reference surfaces is that it allows an appropriate scaling of the leaf or canopy temperature measurements for the current environmental conditions. Nevertheless, the use of reference surfaces to obtain the data for equation (1), or other analogous Water Stress Indices (Jones, 1999a) involves the assumption that the radiative and boundary-layer mass transfer properties of models and real leaves are similar, and that their orientations relative to the sun (and hence radiation absorption) are similar. Even small differences in solar radiation absorption can significantly alter the energy balance. Although the use of wetted or petroleum jelly-covered leaves (Jones, 1999a) can largely overcome problems of ensuring equivalent radiative properties, there remains the problem of ensuring that all leaves are similarly exposed to the sun.

An alternative approach to the use of leaf temperature for the estimation of stomatal conductances (Aston and van Bavel, 1972; Fuchs, 1990) who pointed out that the variation in temperature within a typical canopy as conductance changes would be expected to increase as stomata closed. Indeed Fuchs’ analysis concluded that in many situations an assessment of the variance in leaf temperature could be a more sensitive measure of mean leaf conductance than was mean temperature itself. This arises because the magnitude of the variation in leaf temperature between leaves (as a result of differing orientation/shading) increases as stomata close, because in this situation the radiative component of the leaf energy balance becomes increasingly important. Thus the temperature variance within an image potentially provides an index of stomatal opening. Until recently the lack of suitable instrumentation to obtain information on the variation of leaf temperature within a particular field of view has limited the application of this theory. In practice, application of this approach is likely to be limited both by the assumption of random leaf orientations and by the fact that any image is likely to include non-transpiring tissues such as twigs and branches, as well as extraneous surfaces such as soil or even sky with their widely differing temperatures. Effective testing of this principle has only become possible since the introduction of portable thermal imagers. The only applications known have tended to be rather empirical (Boissard et al., 1990; Bryant and Moran, 1999; Giuliani and Flore, 2000). Constraints imposed by the inclusion of soil or other background within the image, have led to the need to apply arbitrary thresholds or the installation of background screens, thus limiting the application of the original theory (Giuliani and Flore, 2000).

This paper discusses the application of thermal imaging to the study of stomatal conductance in the field and reports the results of an investigation into methods of applying thermal imaging to the detection of stomatal closure in grapevine canopies growing in Portugal. This study concentrates on an evaluation of the consistency and repeatability of measurements made under a range of environmental conditions. A new approach to the use of thermal data when images are available is also proposed, which involves the analysis of data for shaded leaves rather
than for areas fully exposed to the sun as are more commonly studied.

Materials and methods

Field experiments

The field measurements were made at the Portuguese Ministry of Agriculture Research Station at Pegões, Portugal (8°40′ W; 38°58′30″ N) in July 2000 and July 2001. Times are presented in local time (for reference, solar noon for 20 July is at approximately 13:40 h). Detailed measurements were made in 2001 between 17 July and 28 July, with some additional measurements during July and August of 2000. Measurements were made on mature grapevines (Vitis vinifera, cvs Moscatel and Castelão (= Periquita)) growing on a deep sandy soil at 1 m spacing within the row and 2.5 m between rows. Each variety was grown in a different area of the field with a similar experimental design. There were four blocks of four irrigation treatments for each variety, with a single experimental row and two guard rows. The treatments were: NI (no irrigation), FI (100% of ETC supplied through two trickle lines placed 20 cm each side of the row), HI (50% of ETC supplied through two trickle lines placed 20 cm from the row), and PRD (50% of ETC supplied through one trickle line at one side of the row, this side alternating each two weeks). ETC was estimated from pan evaporation corrected using the appropriate crop coefficient according to Allen et al. (1999) and treatments commenced on 12 June 2001.

Thermal imaging

Thermal images were obtained with an Infrared Solutions SnapShot 225 long-wave (8–12 µm) thermal imager with a 20 mm (17.2°) lens (supplied by Alpine Components, Oban Road, St Leonards-on-Sea, East Sussex, UK). The camera is a line-scan imager producing images of 120×120 pixels at 14 bit dynamic resolution, with corrections for object emissivity and background temperature. Images were manipulated using the SnapView 2.1 software supplied or exported to ScionImage or Microsoft Excel for further analysis. The background temperature required for calculation of object temperatures was estimated as the radiative temperature of a crumpled aluminium foil sheet placed in as similar as possible a position as the object being viewed, with emissivity set at 1.0; emissivity was set at 0.95 for viewing leaves. The standard deviation of readings for individual pixels when measuring a constant temperature black background at room temperature was ≤0.35 °C. The field of view (FOV) is given by 2Dtan(θ/2), where D is the camera-object distance, so the ifOV or pixel size at closest focus (0.25 m) is 0.63 mm, increasing to 25.2 mm at 10 m. It was found that there was slight drift during any one series of measurements in the overall mean calibration of the camera, and more importantly spatially across the image, as the electronics warmed when the camera was used continuously. (In newer versions of this camera this problem has apparently been reduced significantly, J Thames, Infrared Solutions Inc., personal communication.) These effects were minimized by subtracting from each observed image appropriate correction images of a constant temperature background (the lens cap) obtained at intervals during the measurements, as indicated in Fig. 1.

Reference surfaces

Various types of reference surface were compared. Natural references were actual vine leaves (either attached in their natural position within the canopy, or detached and hung on a frame) which were either sprayed on both sides with water containing a small quantity of detergent as a wetting agent approximately 1 min before the imaging (T_wet) or covered in petroleum jelly (Vaseline) on both sides (T_m). As an alternative, filter paper (Whatman No. 3) models of different sizes were used which were either maintained wet via a wick attached to a reservoir or kept dry. For different experiments different sized references were used; some of these are illustrated in Fig. 3. In addition, the use of white filter paper references was compared with filter paper stained green in an attempt to match the spectral properties of leaves.

Other measurements

Stomatal conductances were obtained in 2000 using a Li-Cor 6400 gas-exchange system (Li-Cor, Lincoln, Nebraska, USA). In 2001 contemporaneous stomatal data were obtained using either a Li-Cor 1600 steady-state porometer (Li-Cor, Lincoln, Nebraska, USA) or an AP3 transit time porometer (Delta-T Devices, Burwell, Cambridge, UK). Although no rigorous comparison of the two instruments was conducted, results were broadly comparable (Table 4 and unpublished data). Measurements of stomatal conductance (g_s) were made on sun-exposed and recently fully-expanded leaves, with a minimum of four replicates per irrigation treatment. Leaf water potential (Ψ_s) was measured with a Scholander-type pressure chamber (PMS) in similar leaves, also on four leaves per treatment.

Energy balance modelling

The equations describing the energy balance of plant leaves have been discussed extensively (Jackson et al., 1981; Monteith and Unsworth, 1990; Jones, 1992). A standard rearrangement of the Penman–Monteith equation for evaporation has been used here (Jones, 1992; equation 9.6):
Table 1. Temperature of Moscatel and Castelão canopies, 20 July 2001 pm

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Treatment</th>
<th>Sun</th>
<th>Shade</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>σ_B</td>
<td>σ_W</td>
</tr>
<tr>
<td>Moscatel</td>
<td>FI</td>
<td>35.1</td>
<td>0.70</td>
</tr>
<tr>
<td></td>
<td>NI</td>
<td>36.5</td>
<td></td>
</tr>
<tr>
<td>Castelão</td>
<td>FI</td>
<td>36.3</td>
<td>1.76</td>
</tr>
<tr>
<td></td>
<td>NI</td>
<td>40.1</td>
<td></td>
</tr>
</tbody>
</table>

\[
T_1 - T_a = \frac{r_{HR}(r_{aw} + r_{lw})\gamma R_{ai}}{\rho \gamma (r_{aw} + r_{lw}) + sr_{HR}} - \frac{r_{HR}\delta e}{\gamma (r_{aw} + r_{lw}) + sr_{HR}} \tag{2}
\]

where \(T_1 - T_a\) is the leaf-to-air temperature difference, \(r_{lw}\) is the leaf resistance to water vapour transfer (assumed to be largely determined by the stomatal resistance), \(r_{aw}\) is the boundary layer resistance to water vapour, \(R_{ai}\) is the net isothermal radiation (the net radiation that would be received by an equivalent surface at air temperature), \(\delta e\) air water vapour pressure deficit, \(r_{HR}\) is the parallel resistance to heat and radiative transfer (Jones, 1992; p. 108), \(\gamma\) is the psychrometric constant, \(p\) is the density of air, \(c_p\) is the specific heat capacity of air, and \(s\) is the slope of the curve relating saturation vapour pressure to temperature.

The following stress indices (Jones, 1999a) were calculated from the measured mean canopy temperature (\(T_{canopy}\): a modified crop stress index (CWSI), given by

\[
CWSI = \frac{(T_{canopy} - T_{wet})}{(T_{dry} - T_{wet})} \tag{3}
\]

and an index, \(I_G\), that is proportional to stomatal conductance

\[
I_G = \frac{(T_{dry} - T_{canopy})}{(T_{canopy} - T_{wet})} = g_{lw} (r_{aw} + (s/\gamma)r_{HR}) \tag{4}
\]

Results and discussion

Image manipulation and sample selection

Until recently, thermal studies of plant canopies have used infrared thermometers which measure an average temperature over a single target area, which can inadvertently include soil, trunk or sky in the sensed area with consequent errors in estimated canopy temperature. Furthermore, the conventional approach to the use of infrared thermometry or thermography makes use of the average canopy temperature, but this is made up of a wide range of leaf temperatures, with sunlit leaves having much higher temperatures than do shaded leaves. With thermal imagers, however, it is feasible to select an area from each image which does not include sky, soil, grapes or other non-leaf components, alternatively it is possible to select shaded or sunlit areas as required. Figure 2 illustrates the effect of selecting specific areas of the image on the thermal frequency distribution. The larger area, outlined in black in the thermal image, includes sky and soil, this gives a wide frequency distribution for temperature as illustrated in Fig. 2c.

A further possibility with imaging is to use the temperatures of reference surfaces within the image to eliminate extraneous surfaces such as soil or sky. For this the temperature of wet and dry leaves or models are used as ‘thresholds’ and any pixels in the image which are outside of the dry-wet threshold range are excluded from analysis. The use of filter paper and leaf references was compared for this purpose. The choice of reference may affect the value of the mean temperature, and the frequency distribution of temperatures obtained. This approach allows the semi-automated analysis of a large area of canopy that includes, for example, some sky and/or soil in the image. Figure 2 shows how the use of thresholds can lead to the retrieval of similar mean temperatures as can a more careful selection of areas of leaf. The same area, when temperatures outside the range of filter paper references (dry (in red) = 28.8 °C and wet (in blue) = 24.3 °C) are excluded, gives the distribution shown in Fig. 2d, while exclusion of temperatures outside the range of wet and dry leaf references (dry leaf (in orange) = 30.4 °C and wet leaf (in purple) = 30.8 °C) gives the distribution in Fig. 2e, which is narrower with a lower mean. This agrees closely with that obtained, if only the smaller area, A2, is analysed (Fig. 2f).

Temperature variation within images and effects of sun/shade

Whichever technique is used for selecting areas of interest, portions of images can then be analysed to give either average temperatures or the frequency distribution of temperatures over the selected area. Such analysis for images has been taken both face-on to a canopy (Fig. 3a, b), and along a row of plants (Fig. 3c). These examples illustrate the different temperature distributions between sunlit and shaded canopies, with sunlit canopies displaying a far wider range of temperature variation, whether viewed down the rows or normal to the rows. The frequency distributions were clearly different with the sunlit side tending to have a greater variance of temperature. Typical results for a sunny day are summarized in Table 1, which shows that, on average, the mean temperatures were c. 3.0 °C higher for the sunlit than for the shaded sides. In addition to the differences in mean temperature, there were clear differences in the temperature variability within images, with the within image (pixel to pixel) standard deviation (\(\sigma_W\)) being between 25% and 42% greater for the
sunlit sides of the canopy. It is notable from Fig. 3 that there was significant overlap in the temperature ranges of sunlit and shaded sides of rows; this is at least partly related to the fact that there are always a few sunlit leaves on the shaded side and some shaded leaves visible on the sunlit side. On other occasions even greater differences in frequency distributions were apparent; for example, for Moscatel around 3 h before solar noon on 20 July 2001 the standard deviation averaged 1.81 °C for the sunlit side and 0.93 °C for the shaded side, with corresponding values for the temperature range being 11.4 °C and 8.4 °C, and for the kurtosis being −0.65 and 0.97, respectively. The negative kurtosis for the sunlit side indicates that the distribution is flattened in comparison with a normal distribution.

For canopies with randomly oriented leaves, the information on temperature frequency distributions obtained from imagers would allow application of Fuchs (1990) method for detecting stomatal closure. Similar variation to that presented in Fig. 3 in the radiative temperature frequency histograms for differently oriented canopies has previously been shown in maize (Boissard et al., 1990). Studies of variability, however, are dependent on the scale

**Fig. 2.** Frequency distributions obtained when analysing a thermal image of a section of grapevine canopy. The photograph (a) and thermal image (b) include sky and soil as well as leaf canopy and illustrates the use of both small filter-paper references (centre-left) or larger filter-paper rectangles and also the use of detached vine leaves either wetted or covered with petroleum jelly. The larger area selected in the thermal image therefore also includes sky and soil, and gives a wide frequency distribution as shown in (c). The other histograms represent frequency distributions after excluding temperatures outside the range of the filter paper references (d) and after excluding temperatures outside the range of the leaf references (e), while (f) is the distribution for the smaller area, A2. The wet (purple) and dry (blue) reference filter papers (rectangles) and leaves (ovals) are outlined in the thermal image. The wet and dry reference temperatures are marked on the frequency distributions. For further explanation, see text.
of viewing, with pixel size being likely to affect the variance observed. Too large a pixel will average the temperatures of a number of different leaf surfaces and therefore give an underestimate of the true variability. Table 2 shows how the standard deviation of temperature varies as a function of the pixel size chosen for a number of different canopies and environmental conditions. For comparison, some data are presented for nearby citrus (grapefruit) canopies as well as for the grapevine. In Table 2 larger ‘virtual’ pixels were created by arithmetically averaging blocks of different numbers of the raw pixels. In order to confirm this more theoretical analysis, relatively close and more distant data from the images taken down the rows were compared (as in Fig. 3c). Areas of canopy between c. 5 and 10 m from the camera were averaged as ‘near’, and areas between c. 12 and 34 m were averaged as ‘far’. On three separate occasions where such comparisons were made with a minimum of eight replicates, there was no consistent significant difference in the average temperatures recorded for ‘near’ and ‘far’ areas, but there was a slight increase in standard deviation of pixels within the image by an average of 10% for the nearer region.

Choice of canopy illumination for optimal discrimination of stomatal conductances

The sensitivity of \( T_{\text{leaf}} \) to changes in stomatal conductance, and hence the utility of thermal imaging for the study of stomatal conductance, depends both on the absorbed radiation and on the boundary layer conductance and atmospheric humidity. Figure 4a shows how the modelled difference between the temperatures of wet and dry leaves varies as a function of absorbed radiation and windspeed. This difference, which is a measure of the maximum potential sensitivity of \( T_{\text{leaf}} \) to stomatal conductance, was calculated by appropriate substitution in equation (2).

Figure 4 shows that this sensitivity increases with radiation absorbed. Interestingly, although the temperature range tends to decrease as the amount of absorbed radiation decreases, at low incident radiation the temperature range (sensitivity) actually increases with increasing windspeed (= increasing boundary layer conductance). The critical value of absorbed radiation at which this change occurs is that at which leaf temperature equals air temperature. By contrast, the various stress indices that can be calculated (Jones, 1999a) are independent of radiation (though errors in their determination are not), but they do change significantly in response to windspeed as shown in Fig. 4b.

The greater sensitivity of leaf temperature to stomatal conductance for sunlit as compared with shaded leaves suggests that it might be best to use sunlit leaves for the estimation of stomatal conductance from thermal data. On the other hand there is often significantly less variability within an image for a shaded portion of canopy than for a sunlit canopy (Fig. 3). This difference arises because leaf orientation has little effect on the energy balance of a shaded leaf, but a large effect on sun-exposed leaves, as pointed out by Fuchs (1990). A further advantage of using shaded canopies is that errors resulting from differences in radiation absorbed by reference and transpiring leaves will be smaller when the incident radiation is less. Indeed, as is apparent from Fig. 2, it is more likely for the light environment of the reference surfaces not to be truly representative of the measured canopy when sunlit.

Effects of atmospheric environment on canopy temperature and calculated indices

Leaf temperatures can vary rapidly for a given section of canopy in response to environmental fluctuations including air turbulence and changing radiation (Figs 5, 6). Any asynchronous variation of the temperatures of leaves and references can lead to errors in the calculation of any stress

<table>
<thead>
<tr>
<th>Canopy</th>
<th>Time/date</th>
<th>Shade/sun</th>
<th>Distance: (size of 1 pixel)</th>
<th>Standard deviation of averages over different numbers of pixels as indicated below (( \sigma; ^\circ\text{C} ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citrus No. 105</td>
<td>9:58 on 14/09/00</td>
<td>Deep shade</td>
<td>1.8 m (4.5 mm)</td>
<td>0.28 0.25 0.24 0.23 0.20</td>
</tr>
<tr>
<td>Citrus No. 106</td>
<td>10:00 on 14/09/00</td>
<td>Sunlit side</td>
<td>1.8 m (4.5 mm)</td>
<td>1.37 1.35 1.34 1.29 1.15</td>
</tr>
<tr>
<td>Citrus No. 452</td>
<td>10:10 on 14/09/00</td>
<td>Sunlit tree</td>
<td>10 m (25 mm)</td>
<td>2.51 2.47 2.41 2.41 2.32</td>
</tr>
<tr>
<td>Grape No. 377</td>
<td>16:15 on 18/07/00</td>
<td>Sunlit side</td>
<td>1.5 m (3.8 mm)</td>
<td>2.52 2.48 2.45 2.36 2.11</td>
</tr>
<tr>
<td>Grape No. 477</td>
<td>12:00 on 20/07/00</td>
<td>Sunlit side</td>
<td>2 m (5.0 mm)</td>
<td>3.47 3.37 3.27 3.07 2.75</td>
</tr>
<tr>
<td>Grape No. 573</td>
<td>15:23 on 20/07/00</td>
<td>Shady side</td>
<td>2 m (5.0 mm)</td>
<td>1.61 1.54 1.50 1.42 1.28</td>
</tr>
<tr>
<td>Grape No. 571</td>
<td>15:22 on 20/07/00</td>
<td>Sunlit side</td>
<td>2 m (5.0 mm)</td>
<td>2.42 2.34 2.27 2.13 1.88</td>
</tr>
</tbody>
</table>

\( \sigma \) relative to single pixels

<table>
<thead>
<tr>
<th></th>
<th>100</th>
<th>50</th>
<th>25</th>
<th>20</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Theory</td>
<td>100</td>
<td>50</td>
<td>25</td>
<td>20</td>
<td>10</td>
</tr>
</tbody>
</table>
Typical magnitudes of the short-term variation is illustrated for sun and shaded canopies in Fig. 3: the four histograms presented in each case were for four images of the same area of canopy taken at approximately 1 min intervals. Table 3 summarizes both the average short-term variation between means of four replicate images taken of a constant area of canopy at 1 min intervals (representing instrumental and measurement errors and short-term environmental fluctuations), and long-term variation (including instrumental, environmental (30 min) and plot variation). These data provide the raw information that could permit an approximate error analysis of indices calculated using thermal data. However, as one might expect, there was a high degree of covariance between $T_{leaf}$, $T_{wet}$ and $T_{dry}$; indeed multiple regression showed that variation in $T_{wet}$ and $T_{dry}$ explained more than 90% of the variation in $T_{leaf}$ for 20 July and more than 94% of variation across the other experimental days. There was also some evidence from Table 3 that there is greater variation in temperatures of the reference surfaces than in the canopy temperature, possibly because of the smaller area of the references and the opportunities for at least the wet surface partially to dry between measurements.

Because of the covariance between temperatures, rather than attempting a rigorous error analysis, the variation in calculated indices in Table 3 is shown. For these data, the average coefficient of variation ($CV=100 \times \text{standard deviation (σ)/mean}$) for $I_{G}$ averaged around 17% for the short-term variation, but up to 50% for the longer term measurements (equivalent to 8.5% and 25%, respectively, for averages of four readings). The corresponding CVs for CWSI were 6.7% and 16%.
The consequence of such large errors for experimental design can be readily calculated. As a rule of thumb one needs four replicates to have an 80% probability of detecting at the 5% level (two-tailed test) a true difference between two treatments ($d$) where $d/s=2.0$. 16 replicates would be required to detect a true difference where $s=d$ (Snedecor and Cochrane, 1967). A potential advantage of thermal imagery is that large numbers of samples averaging large areas of canopy are much more readily obtained than is a similar number of stomatal conductance measurements with a porometer.

Table 3. Average temperatures (°C) and stress indices for the Moscatel canopies for 20 July 2001 and for shaded leaves for 24–28 July 2001, together with the error ($\sigma$) as calculated from the square root of the residual mean square from ANOVAs involving all treatments

For 20 July the error (24 df) relates to mean variation between replicate images of the same area of canopy taken at 1 min intervals using data from all four treatments. For the other dates the measurements were taken over periods of up to 30 min and includes variance due to environmental fluctuations, different image areas and plot–plot variation (3 df).

<table>
<thead>
<tr>
<th></th>
<th>$T_{\text{leaf}}$</th>
<th>$T_{\text{wet}}$</th>
<th>$T_{\text{dry}}$</th>
<th>$I_{\text{C}}$</th>
<th>CWSI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Error</td>
<td>Mean</td>
<td>Error</td>
<td>Mean</td>
</tr>
<tr>
<td>Short-term error</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20 Jul sun</td>
<td>24.4</td>
<td>0.26</td>
<td>19.1</td>
<td>0.58</td>
<td>29.7</td>
</tr>
<tr>
<td>20 Jul shade</td>
<td>21.2</td>
<td>0.26</td>
<td>15.7</td>
<td>0.58</td>
<td>23.5</td>
</tr>
<tr>
<td>Plot/long-term error</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 Jul pm</td>
<td>27.4</td>
<td>0.59</td>
<td>24.0</td>
<td>0.65</td>
<td>29.5</td>
</tr>
<tr>
<td>25 Jul am</td>
<td>22.2</td>
<td>1.15</td>
<td>18.3</td>
<td>1.72</td>
<td>25.4</td>
</tr>
<tr>
<td>25 Jul pm</td>
<td>28.3</td>
<td>0.97</td>
<td>22.7</td>
<td>0.92</td>
<td>32.9</td>
</tr>
<tr>
<td>26 Jul am</td>
<td>25.9</td>
<td>1.18</td>
<td>22.7</td>
<td>1.00</td>
<td>28.5</td>
</tr>
<tr>
<td>26 Jul pm</td>
<td>29.5</td>
<td>1.61</td>
<td>25.5</td>
<td>0.98</td>
<td>31.7</td>
</tr>
<tr>
<td>27 Jul am</td>
<td>21.1</td>
<td>1.79</td>
<td>17.2</td>
<td>1.50</td>
<td>22.8</td>
</tr>
<tr>
<td>28 Jul am</td>
<td>25.4</td>
<td>1.76</td>
<td>22.6</td>
<td>1.30</td>
<td>28.4</td>
</tr>
<tr>
<td>Mean</td>
<td>25.7</td>
<td>1.29</td>
<td>21.9</td>
<td>1.15</td>
<td>28.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The consequence of such large errors for experimental design can be readily calculated. As a rule of thumb one needs four replicates to have an 80% probability of detecting at the 5% level (two-tailed test) a true difference between two treatments ($\delta$) where $\delta/\sigma=2.0$. 16 replicates would be required to detect a true difference where $\sigma=\delta$ (Snedecor and Cochrane, 1967). A potential advantage of thermal imagery is that large numbers of samples averaging large areas of canopy are much more readily obtained than is a similar number of stomatal conductance measurements with a porometer.

Some representative data for an expanded time scale are presented for 27 July 2001 in Fig. 6, showing a close association between environmental conditions and canopy temperature. Part of the discrepancy probably arises because the meteorological instruments were up to 50 m from the section of canopy being imaged.

**Relationships between temperature, water status, stress indices, and stomatal conductance**

Predawn leaf water potentials in cv. Moscatel by the time of the measurements were only significantly different between NI and FI, with the averages (four dates between 17 July and 1 August 2001) being $-0.25\pm0.06$ MPa for NI and $-0.11\pm0.031$ MPa for FI. The values for the other treatments was intermediate. Midday leaf water potentials
Stomatal conductances were measured on most occasions when thermal data were collected and are summarized in Table 4. In Moscatel, only small differences in predawn leaf water potential and none in stomatal conductance as measured with the Li-Cor 6400, were apparent at the time of the field campaign in late July with no significant treatment differences apparent on 20 July 2001. There was, however, a consistent and often significant difference between the sunlit and shaded sides of the canopy with \( g_{leaf} \) for the sunlit leaves averaging about double the value of the shaded leaves. On no occasion was there either a significant main irrigation effect on \( g_{leaf} \), or a significant irrigation \( \times \) exposure interaction. Though stomatal data were not available for Castelão during the measurements in July, significant treatment differences were already apparent by 12 July (data not shown).

The mean values for both the raw temperature data and the calculated indices between 24 and 27 July 2001 for the Moscatel NI and FI treatments are presented in Table 5. Although \( I_G \) was, on average, 10% higher for the FI treatment, this difference was not significant, nor was the small difference in CWSI significant. There was, however, a reasonable association between \( I_G \) and stomatal conductance when comparing the sunlit and shaded leaves (Fig. 7). Table 5 also shows the treatment effects on leaf and reference temperatures. In particular there was a highly significant treatment effect on \( T_{leaf} \) which, for the shaded leaves, was 1 °C warmer for the NI treatment than for the FI treatment. Much more surprising, however, was the observation that there were similar, or even stronger, highly consistent and significant treatment effects on both the reference temperatures (\( T_{dry} \) and \( T_{wet} \)). This result was so surprising that other data available to the authors from the previous year’s thermal measurements on the same experiment were investigated.

Analysis of the data obtained on four measurement dates during August 2000 (detailed data not shown), confirmed the observations of July 2001. For the 2000 measurements, there was again a statistically significant (at the 10% probability level) higher temperature for NI canopy (29.4 °C) and dry reference (33.2 °C) than for the corresponding FI canopy (28.3 °C) or dry reference (32.9 °C), though the temperatures of the wet reference surfaces did not differ significantly. It is notable that the canopy was significantly sparser in 2000 than in 2001. The clear treatment effects on reference temperatures suggests that small changes in the crop water status can have detectable effects on the canopy microclimate. This might occur if the reduced transpirational cooling for NI plants raises the air temperature within the canopy. Alternatively, it is possible that the irrigation treatment might affect canopy structure and, consequently, either the radiation penetration into the canopy from the sunlit side to the shaded side or the radiation penetration to the soil and hence the amount of soil heating. No obvious irrigation effects on canopy development were apparent to the eye during July 2001, as all treatments had been mechanically trimmed to a comparable size the week before the measurements. Nevertheless, in 2000 the leaf areas per vine were 4.1 ± 0.2 m² for NI and 5.4 ± 0.2 m² for FI. The suggestion that the treatment effect may arise partly from

**Fig. 5.** Thermal data (\( T_{leaf} \), \( T_{wet} \) and \( T_{dry} \)) obtained for the shaded side of a Moscatel grapevine canopy on various measurement occasions between 24 and 28 July 2001 (a). Corresponding environmental variation is shown: (a) air temperature, (b) radiation, (c) air humidity, and (d) wind speed. Thermal images were taken face-on at approximately 1.5 m from the canopy, and values outside of the dry-wet leaf threshold range were ignored. Thermal data in (a) were pooled for the two irrigation treatments, full irrigation (FI) and no irrigation (NI).
differences in energy partitioning between the canopy and soil is supported by the observation of a consistent effect on radiometrically-sensed soil temperature for the downrow images collected during 2000 (data not shown). Although the $I_G$ is not designed to compare shaded and sunlit leaves, it is encouraging to note that the consistent differences in stomatal conductance between these surfaces were reflected in appropriate differences in $I_G$ (Fig. 7). The positive correlation between stomatal conductance and $I_G$ in this experiment agrees with previous evidence for reliable estimates of stomatal conductance using thermal sensing in soybean (Inoue et al., 1994), cotton (Inoue et al., 1990) and runner beans (Jones, 1999a).

### Calibration for boundary layer conductance (windspeed)

The use of wet and dry reference surfaces for the calibration of the thermal approach for estimation of leaf conductance or evaporation rate was pioneered by Jones (1999a, b). Unfortunately, the various indices derived, such as equation (1) above still depend on the boundary layer conductance and to a lesser extent on the temperature, through its effects on the ‘constants’ $s$ and $\gamma$. A proportion of the scatter in the relationship between $I_G$ and $g_{leaf}$ may, therefore, result from variation in boundary layer conductance (wind speeds were markedly different on the various days, see Fig. 5). The index $I_G$ can be used to estimate leaf conductance if an estimate of boundary layer conductance is available. There are a number of possible ways in which this boundary layer conductance can be estimated (Dixon and Grace, 1983; Brough et al., 1986; Jones, 1992, appendix 8), though these usually either require information on absorbed radiation and leaf and air temperature (and possibly humidity), or else follow the dynamics of surface temperature after perturbation.

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**Table 4. Average stomatal conductance ($g_{leaf}$ mmol m$^{-2}$ s$^{-1}$) of Moscatel canopies July 2001**

<table>
<thead>
<tr>
<th></th>
<th>NI</th>
<th>FI</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sun</td>
<td>Shade</td>
<td>Sun</td>
</tr>
<tr>
<td>20 Jul am</td>
<td>287</td>
<td>209</td>
<td>260</td>
</tr>
<tr>
<td>24 Jul</td>
<td>174</td>
<td>116</td>
<td>195</td>
</tr>
<tr>
<td>25 Jul am</td>
<td>158</td>
<td>138</td>
<td>150</td>
</tr>
<tr>
<td>25 Jul pm</td>
<td>303</td>
<td>115</td>
<td>358</td>
</tr>
<tr>
<td>26 Jul am</td>
<td>324</td>
<td>109</td>
<td>223</td>
</tr>
<tr>
<td>26 Jul pm</td>
<td>197</td>
<td>115</td>
<td>258</td>
</tr>
<tr>
<td>Mean</td>
<td>215</td>
<td>119</td>
<td>267</td>
</tr>
<tr>
<td>Treatment mean</td>
<td>167</td>
<td>193</td>
<td></td>
</tr>
</tbody>
</table>

*E* refers to the exposure treatment (sun versus shade) and *I* refers to the irrigation treatment (NI versus FI). LSD=least significant difference, ns=not significant, +=$P<0.1$, **=significant at $P<0.01$, ***=significant at $P<0.001$. On 24 July, measurements were only taken on one row for FI, and two for NI; there was only one measurement in each treatment $\times$ exposure on 20 July; otherwise $n=4$. Measurements on 20 July were taken with the Li-Cor 1600 steady-state porometer, all others with the Delta-T porometer.

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**Fig. 6.** Variation in thermal data from shaded areas of fully irrigated (FI) and non-irrigated (NI) grapevine canopies during measurements on the morning of 27 July 2001, with corresponding variation in air temperature (a), radiation (b), air humidity (c), and wind speed (d).
General conclusions and recommendations

It is apparent from the data presented here that useful information on the water relations of grapevine canopies can be obtained from infrared thermography of plant canopies, though it is important, for example, to avoid the inclusion of non-leaf material in the analysis of the images. With imaging, this can be achieved either by selection of appropriate areas or by the use of dry and wet threshold temperatures to define the range outside which temperature values are rejected. Thermography allows the semi-automated analysis of large areas of canopy with much more effective replication that can be achieved with porometry.

Analysis of the sources of variation suggest that variability or errors in the measurement of the reference temperatures has a major contribution to errors in \( I_G \); these may arise from slight differences in exposure to incoming radiation or slight differences in radiative properties. It is concluded that real leaves, either sprayed with water or covered in petroleum jelly to stop transpiration, provided the best references because of their similar radiometric and aerodynamic properties to the canopy being studied. Some specific points include:

(i) Thermal imaging and image analysis allow automated correction of images, with, for example, the elimination of pixels representing sky or soil. Image analysis further opens a range of techniques not previously feasible with infrared thermometry, for example Fuchs (1990) method, although this is only likely to be appropriate for homogeneous crops, not row crops such as the grapevine studied here.

(ii) A new approach to the calculation of stress indices is proposed that is based on the study of shaded portions of canopies; this is likely to be particularly appropriate for row or tree crops. Potential limitations of this approach include the tendency for stomata to be more closed in the shade and the smaller range of temperatures expected for a given range of conductances, but these disadvantages can be offset by improved data consistency.

(iii) Evidence was provided for grapevine that, not only leaf or canopy temperatures but also the temperatures of other surfaces within the canopy (including wet or dry reference surfaces), were dependent on the water relations of the crop.

(iv) Coefficients of variation for calculated stress indices were substantial, thus limiting the potential discriminatory power of the techniques for giving absolute estimates of stomatal conductance. However, nearly comparable errors exist for other more labour-intensive approaches such as the use of porometry.

(v) Thermal imaging remains best suited for comparative studies, such as screening activities, because of the potentially high precision of within-image comparisons.

Acknowledgements

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Table 5. The treatment means, together with the least significant difference (LSD), for the different temperatures and for the calculated indices \( I_G \) and CWSI for Moscatel measurements (shaded leaves) between 24 and 27 July 2000

<table>
<thead>
<tr>
<th>Treat Day</th>
<th>( I_G )</th>
<th>CWSI</th>
</tr>
</thead>
<tbody>
<tr>
<td>FI treatment</td>
<td>0.83</td>
<td>0.58</td>
</tr>
<tr>
<td>LSD</td>
<td>0.24</td>
<td>0.06</td>
</tr>
<tr>
<td>NI treatment</td>
<td>29.1</td>
<td>0.83</td>
</tr>
</tbody>
</table>

\( ** \) = significant at \( P < 0.01 \), \( *** \) = significant at \( P < 0.001 \).

\( a \) ns=not significant, *=significant at \( P < 0.05 \), **=significant at \( P < 0.01 \), ***=significant at \( P < 0.001 \).

![Fig. 7.](https://academic.oup.com/jxb/article-figures/53/378/2249/426550) The relationship between \( I_G \) and stomatal conductance of leaves from sunny (filled circles) and shaded (open circles) Moscatel grapevine canopies during the morning of 20 July 2001. Each symbol represents data from a different treatment.
References


