THERMAL IMAGING AS A VIABLE TOOL FOR MONITORING PLANT STRESS

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Abstract

Aims: The objective of this paper was to describe an approach to the use of thermal data for shaded leaves rather than areas fully exposed to the sun. Secondly to make use of infrared thermography as a powerful tool to measure effects of solar radiation on berry temperature.

Methods and results: Thermal images were obtained with a long-wave thermal imager. There is often less variability within an image for a shaded portion of the canopy than for a sunlit canopy. The temperature frequency distributions of sunlit leaves displayed a far wider range of temperature variation compared to shaded leaves.

Conclusion: With thermal imagers it is feasible to select precisely the leaves for investigation. The remote sensing approach using infrared thermography combined with techniques available for image analysis open up a number of opportunities for comparative studies such as screening activities.

Significance and impact of study: Infrared thermography can be implemented as a first line of detection to determine the onset of plant stress due to changes in stomatal aperture. This approach can give reliable and sensitive indications of leaf temperature and hence to calculate stomatal conductance.

Key words: infrared thermography, leaf temperature, stomatal conductance, crop water stress index, berry temperature, bunch exposure

Résumé

Objectif : L'objectif de l'étude était de décrire une approche d'analyse de données thermiques visant à considérer les feuilles du côté ombragé plutôt que les zones exposées directement au soleil. Un deuxième objectif était d'utiliser la thermographie infrarouge comme outil fiable pour mesurer les effets des radiations solaires sur la température des baies.

Méthodes and résultats : Les images thermiques ont été obtenues en utilisant un imageur thermique dans les hautes longueurs d'onde. On observe en général moins de variabilité pour une image effectuée sur une partie ombragée de la canopée plutôt que sur une partie exposée. La distribution des fréquences de température d'une feuille exposée est répartie sur un intervalle beaucoup plus grand comparé aux feuilles à l'ombre.

Conclusion : Grâce aux images thermiques, il est possible de sélectionner précisément les feuilles destinées à l'étude. L'approche de télédétection utilisant la thermographie à infrarouge combinée aux techniques existantes d'analyse d'image ouvrent un grand nombre de possibilités pour des études comparatives telles que des activités d'observation pré-symptomatiques.

Signification et impact de l'étude : La thermographie à infrarouge peut être utilisée en première ligne pour déterminer un stress hydrique en comparant les changements d'ouverture des stomates. Cette approche permet de donner des indications fiables et précises de la température des feuilles et ainsi de calculer la conductance stomatique.

Mots clés: Thermographie à infrarouge, température de feuille, conductance stomatique, indice de stress hydrique (CWSI), température de la baie, exposition des grappes

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INTRODUCTION

Due to improved operability and sinking prices, infrared thermography has developed into an important tool and has found application in many fields. More than 40 years have passed since thermography was first applied for plant examination (Monteith and Szeicz (1962); Tanner (1963)). The measurement of canopy temperature as an indicator of stress was put on a sound footing by Idso (1982) who defined a 'Crop Water Stress Index' (CWSI). 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 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 distributions of temperature over the area and, if necessary, to include only areas which are known to be the surface of interest.

The advantage of the approach based on reference surfaces is that it allows appropriate scaling of the leaf or canopy temperature measurements for current environmental conditions. Nevertheless the use of reference surfaces to obtain the data, or other analogous Water Stress Indices (Jones, 1999) 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, 1999) 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 thermal frequency distributions to estimate plant water loss was proposed by Fuchs (1990) who pointed out that the variation in temperature within a canopy as conductance changed was greatest for sunlit leaves. 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.

The objective of this paper was to describe an approach to the use of thermal data when images are available, which involves the analysis of data for shaded leaves rather than areas fully exposed to the sun as are more commonly studied. A second objective was to make use of infrared thermography as a powerful tool to measure effects of solar radiation on berry temperature. Smart and Sinclair (1976), who pursued an energy balance approach, indicated that short-wave solar radiation and convection caused by wind speed were the two most important determinants of fruit temperature. Fruit size, albedo, wind direction, and long-wave radiation were less important. The relationship between sun exposure and temperature is of importance to berry composition and metabolism.

MATERIAL ANS METHODS

Field experiments

The field measurements were made at the Portuguese Ministry of Agriculture Research Station at Pegões, Portugal (80°40´W; 38 °38´30´´N) in July 2001. Measurements were made on mature grapevines (*Vitis vinifera*, cvs. Moscatel and Periquita (=Castelão)) growing on a deep sandy soil with north-south row orientation at a vine spacing of 1 m within the row and 2.5 m between rows. 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 ET supplied through two trickle lines placed 20 cm each side of the row).

Thermal imaging

Thermal images were obtained with an Infrared Solutions SnapShot 525 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 x 120 pixels at 16 bit 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 2*D*tan(8.6), where D is the camera-object distance, so the *iFOV* or pixel size at closest focus (0.25 m) is 0.64 mm, increasing to 25.6 cm 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. These effects were minimised by subtracting an appropriate correction image of a constant temperature background obtained after running the camera continuously for an appropriate period from each observed image (Jones *et al.*, 2002).

Reference surface

Various types of reference surface were compared. Natural references were actual vine leaves (either detached 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_{dry}). 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. In addition, white filter paper stained green in an attempt to match the spectral properties of the leaves was also tested.

Energy balance modelling

The equations describing the energy balance of plant leaves have been discussed extensively (see Jones 1992, etc.). We have used a standard rearrangement of the Penman-Monteith equation for evaporation (Jones, 1992; Eq. 9.6):

$$T_l - T_a = \frac{r_{HR}(r_{aW} + r_{lW})\gamma R_{ni}}{\rho c_p [\gamma(r_{aW} + r_{lW}) + s r_{HR}]} - \frac{r_{HR}\delta e}{\gamma(r_{aW} + r_{lW}) + s r_{HR}} \quad (1)$$

where T_l - 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, Rni is the net isothermal radiation (the net radiation that would be received by an equivalent surface at air temperature), δe air water vapour pressure deficit, r_{HR} is the parallel resistance to heat and radiative transfer (see Jones 1992; p. 108), γ is the psychrometric constant, ρ 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, 1999) were calculated from the measured mean canopy temperature (T_{canopy}), a modified crop stress index (CWSI, (2)), and an index, I_G (3), that is proportional to stomatal conductance (g_{lW}).

$$CWSI = \frac{(T_{canopy} - T_{wet})}{(T_{dry} - T_{wet})} \quad (2)$$

$$I_G = \frac{(T_{dry} - T_{canopy})}{(T_{canopy} - T_{wet})} = g_{lw}(r_{aW} + \left(\frac{s}{\gamma}\right) r_{HR}) \quad (3)$$

Other measurement

Stomatal conductances were obtained using a Li-Cor 1600 steady-state porometer (Li-Cor, Lincoln, Nebraska, USA). Measurements of stomatal conductance (g_s) were made on sun-exposed and fully expanded leaves, with a minimum of four replicates per irrigation treatment.

Statistical Analysis

The effects of treatments were analysed by analysis of variance (ANOVA). Significant differences between means were determined with Tukey-Test.

RESULTS AND DISCUSSION

Canopy temperature variation

When using infrared thermometers, the temperature of soil, trunk or sky may inadvertently be included in the sensed area with consequent error in estimated the true canopy temperature. Furthermore, the average canopy comprises both sunlit and shaded leaves, with the sunlit temperature being much higher than comparable shaded leaves. With thermal imagers, however, it is feasible to exclude areas outside the canopy and furthermore to select precisely the leaves for investigation. The use of reference surfaces within the image can be used as a further possibility to eliminate extraneous surfaces and to define the variance of temperature. However, the choice of reference may affect the temperature. For this purpose the use of wet and dry filter paper (stained green) and "wet" and "dry" leaves was compared. Real leaves, either covered in petroleum jelly to stop transpiration ("dry leaves") or sprayed with water ("wet leaves"), provided the best references because they more closely mimicked the radiometric and aerodynamic properties of the canopy than did the artificial wet and dry stained filter paper. By using "dry" and "wet" references, threshold temperature become available to define the range outside which temperature values can be rejected. Compared to threshold temperature obtained from filter paper references, the temperature range from leaf references agreed closely to temperature ranges of a small canopy area. Hence, this approach allows the semi-automated analysis of a large area of a canopy that includes, for example, some sky and/or some soil in the image. The average temperature or the frequency distribution of temperatures over selected areas can then be calculated. Such analysis for images has been taken both face on to the canopy (Fig. 1a,b), and

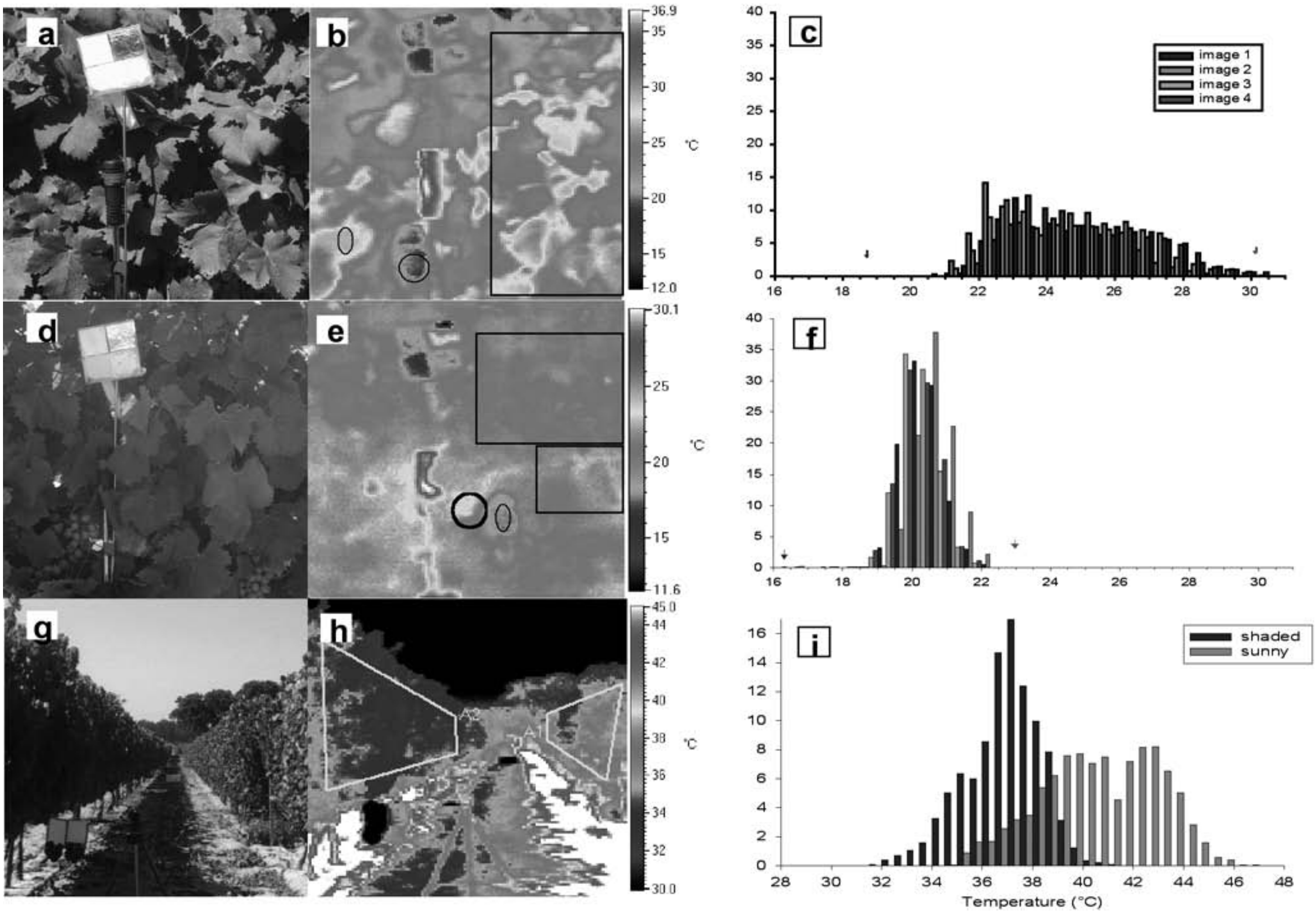

Figure 1 - Digital images of grapevine (Moscatel) canopies in the sun (a) and shade (d) and the corresponding thermal images (b, e) taken during the morning of 20 July 2001.
The section of the images that were analysed are outlined (rectangles). Note that these exclude sky, soil and grapes. The thermal images shown are the first in a series of four taken at c. 1 min intervals. The temperature frequency distributions of the selected area of all four images is shown (c,f). The red and blue arrows correspond to the temperatures of the dry and wet leaves, respectively, which are marked with black outlines on the thermal images. Corresponding images taken looking down the rows (g,h) showing the shaded and sunlit sides, while the temperature frequency distributions for the outlines areas of the canopy are shown in (i) (reproduced with permission from Jones *et al.*, 2002).

Table 1 - Temperature of Moscatel and Castelão (20 July 2001)

Values are means (°C) together with standard deviations between images (σ_B calculated from the error variance of ANOVA), and within images (σ_w). These images were taken along rows over a period of app. 30 min for each cultivar, with four replicates per treatment.

Cultivar	Treatment	Sun			Shade		
		Mean	σ_B	σ_W	Mean	σ_B	σ_W
Moscatel	FI	35,1	0,70	1,27	32,6	0,71	1,02
	NI	36,5			33,5		
Castelão	FI	36,3	1,76	1,28	33,8	1,10	0,90
	NI	40,1			36,2		

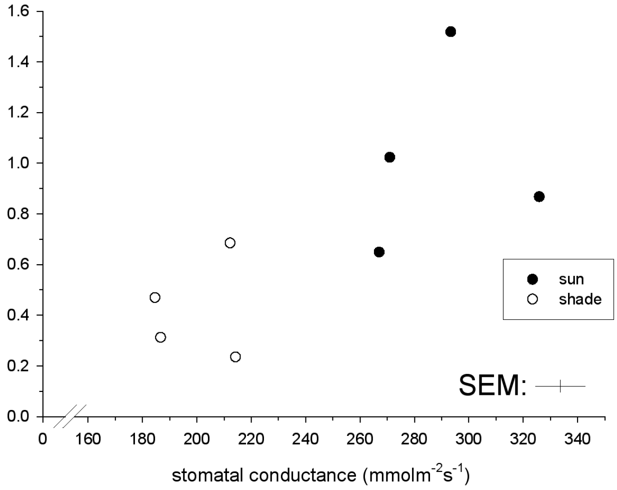

Figure 2 - The relationship between stomatal conductance and IG of leaves from sunny (filled circles) and shaded (open circles) Moscatel grapevine canopies during the morning of the 20 July 2001.
Each symbol represents data from different plots. SEM: standard error of the mean for each group.

along the row of vines. This figure illustrates the different temperature distribution between sunlit and shaded canopies.

The frequency distributions were clearly different, with sunlit canopies displaying a far wider range of temperature variation, whether viewed face-on or down the rows. There was significant overlap in the temperature ranges of sunlit and shaded sides down the rows; this is at least partly related to the fact that there are always sunlit leaves on the shaded side and some shaded leaves visible on the sunlit side. To estimate stomatal conductance the index I_G (3) can be used, if an estimate of boundary layer conductance is available. There are several ways in which boundary layer conductance could be estimated. For example a comparison of heated and unheated leaves can be used, or it can be derived from measurements of wind-speed, from the leaf energy balance of non-transpiring leaves, or else from the dynamics of surface temperature after perturbation (see Jones, 1992). Even without such an estimate, however, the positive relation between porometer measurements of stomatal conductance and I_G (Figure 2) in this experiment supports other evidence for a close relationship between a stomatal index and stomatal conductance in other species (Inoue *et al.*, 1994 ; Jones, 1999).

Furthermore, temperature difference can be related to the water status of the vine with non irrigated vines showing both higher absolute temperatures compared to the fully irrigated vines and greater differences between sunlit and shaded sides (Table 1). The average leaf

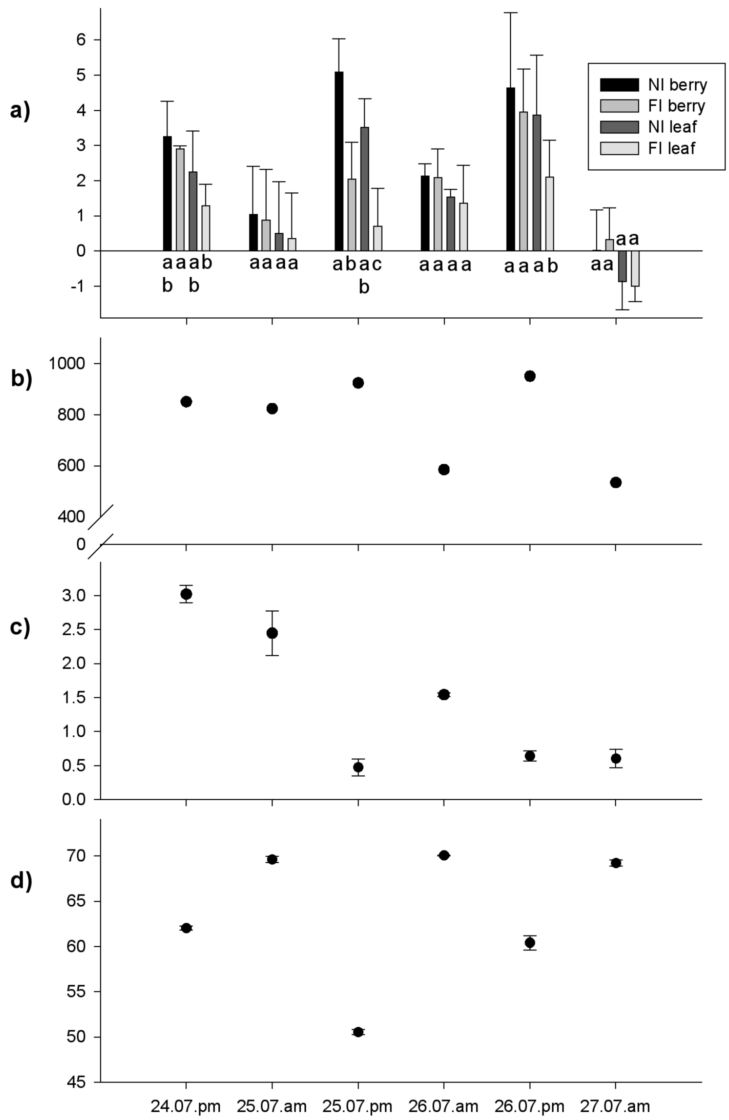

Figure 3 - Variation in thermal data from fully irrigated (FI) and non-irrigated (NI) grapevine canopies and bunches between 24th July and 27th July 2001, with corresponding variation in Temperature (expressed as difference between grape berry and leaf temperature to air temperature (a)), solar radiation (b), wind speed (c) and air humidity (d) during the measuring period in the morning (=am: 10.30 am to 12.30 pm) and afternoon (=pm: 3 pm to 4.30 pm).
The vertical bars in temperature variation (a) represent air temperature during measurements. n=4. Data are means ±SE. Different letters indicate significantly different means in Tukey test following ANOVA (P<0.05).

Table 2 - Temperature of individual berries (AXy) and bunches (BXy) as a fruit response to cluster location within the canopy (*Vitis vinifera,* cvs. Moscatel and Castelão).

The images were taken on 28 July 2001. Values are means (°C) together with standard deviations (σ) (3 pm; air temperature 28 °C; wind speed 0.7 ms^{-1}; total solar radiation 1007 W m^{-2}).

Castelão	mean	σ	Max	Min	**Moscatel**	mean	σ	Max	Min
AC1	33,4	0,23	33,7	33,1	AM1	32,3	0,35	33,2	32,1
AC2	37,7	0,32	38,5	37,3	AM2	42,8	0,35	43,4	42,2
AC3	43,0	0,34	43,3	42,0	AM3	40,8	0,37	41,4	40,2
BC1	39,3	1,44	43,5	36,2	AM4	36,3	0,25	36,8	36,1
BC2	34,5	1,3	37,0	32,1	BM1	40,3	1,19	43,4	35,5
BC3	35,0	0,87	37,0	32,7	BM2	37,1	1,51	41,5	31,1

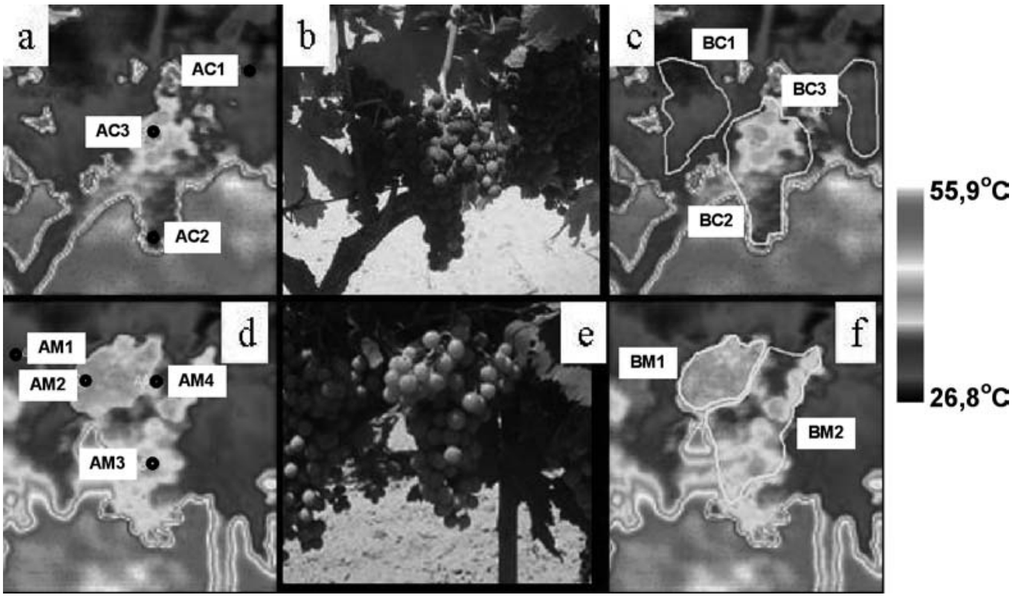

Figure 4 - Digital images of grapevine bunches: Castelão (b) and Moscatel (e) and representatives of the corresponding thermal images for Castelão (a,c) and for Moscatel (d,f) taken at 1 pm (28 July 2001).

The sections of the images that were analysed are outlined for berries (“circles”) and bunches (“lasso”). Note that these exclude sky, soil and grapes.

temperature of sunlit leaves was approximately 3 °C higher than leaves on shaded side with this difference being somewhat greater for non irrigated vines than for fully irrigated vines. There is often less variability within an image for a shaded portion of the canopy than for a sunlit canopy. This difference arises because leaf orientation has little effect on the energy balance of a shaded leaf, but a larger effect on sun-exposed leaves, as pointed out by Fuchs (1990). A further advantage of using shaded leaves is that differences in radiation absorbed by the leaves will be small.

Treatment effects on absolute canopy temperature of shaded leaves closed-by bunches were found (Figure 3). For the afternoon of the 25th and 26th of July the stressed plants were significantly hotter compared to non-stressed vines. No significant effect of absolute temperature of shaded leaves between treatments was observed at the afternoon of 24th of July and during morning measurements.

Temperature differences found between stressed and non stressed plants are encouraging for the application of thermal imaging as a useful tool to distinguish between different water stress treatments or to take advantage by applying this methodology for irrigation scheduling.

Fruit temperature variation

Thermal imaging allows detailed information about the distribution of surface temperature. There was no significant difference in berry temperature between the two treatments except for the 25th of July when solar radiation was high and wind speed was low (figure 3). Measurements of shaded - but exposed - bunches indicate that the berry temperature of NI was on average 0.2oC higher than that of FI berries. Alternatively, it is possible that the irrigation treatment might affect canopy structure and, consequently, either increases the radiation penetration into the canopy or the radiation penetration to the soil and hence the fruit temperature.

Depending on canopy architecture, grape bunches can develop in various conditions varying from heavily shaded (inner canopy) through to fully exposed bunches. The effect on temperature variation of fully sun exposed bunches (approximately 0.5 m above ground) has been illustrated for Castelão and Moscatel (Figure 4). The areas selected have been outlined in the thermal image. With an air temperature of 28 °C, bunch temperature was up to 11 °C and 12 °C above ambient for Castelão and Moscatel respectively. Furthermore, temperature of single berries was up to 15 °C and 14.8 °C higher than air temperature for Castelão and Moscatel respectively (Table 2).

The interactive effects of light and soil radiation reflection on fruit temperature, causing excessive absolute temperatures, can have a detrimental effect on fruit quality. For red varieties there is extensive evidence that canopy microclimate alters anthocyanin concentrations (Haselgrove *et al*., 2000; Spayd *et al.*, 2002). Thermal imaging appears to be a suitable tool for studies of either single berry or whole bunch temperature with much more effective replication and precision that can be achieved with other methods such as the use of thermocouples. The choice of which measure to use depends on the canopy architecture and the availability of exposed fruit. One of the main advantages of infrared thermography is that it covers a variety of different berry positions within a bunch which can hardly be achieved using thermocouples. Furthermore, infrared thermography remains best suited for comparative studies, such as analysing different berry position within a canopy and potentially provides high precision.

More information is needed on critical temperature ranges under field conditions for biosynthesis and degradation of fruit composition in berry skins, recognizing that it may vary with bunch exposure and cultivars. For such comparative studies infrared thermography can be used as a viable tool.

CONCLUSION

The remote sensing approach using infrared thermography combined with techniques available for image analysis open up a number of opportunities that are not currently feasible with conventional infrared thermometers or by leaf gas-exchange instrumentation. The technique remains best suited for comparative studies such as screening activities.

Infrared thermography can be implemented as a first line of detection to determine the onset of plant stress due to changes in stomatal aperture. This approach can give reliable and sensitive indications of leaf temperature and hence to calculate stomatal conductance.

Bunch exposure and bunch temperature are closely related. It is most likely that the synthesis of flavour compounds depend on complex interactions between light and temperature effects. Thermal imaging can be used to detect heat stress in various positions of the canopy and has the potential to help to improve canopy managing practices.

The technique has much more effective replication and precision than can be achieved with other methods. Such early and remote-sensing may have important implications for the adoption of irrigation scheduling strategies and can become an asset in the area of decision support systems or precision viticulture.

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