

# Thermal infrared imaging of crop canopies for the remote diagnosis and quantification of plant responses to water stress in the field

Hamlyn G. Jones<sup>A,G</sup>, Rachid Serraj<sup>B</sup>, Brian R. Loveys<sup>C</sup>, Lizhong Xiong<sup>D</sup>, Ashley Wheaton<sup>E</sup> and Adam H. Price<sup>F</sup>

<sup>A</sup>Division of Plant Sciences, University of Dundee at SCRI, Invergowrie, Dundee DD2 5DA, Scotland.

<sup>B</sup>International Rice Research Institute, DAPO Box 7777, Metro Manila, Philippines.

<sup>C</sup>CSIRO Plant Industry, PO Box 350, Glen Osmond, SA 5064, Australia.

<sup>D</sup>National Key Laboratory of Crop Genetic Improvement, Huazhong Agricultural University, Wuhan 430070, China.

<sup>E</sup>Melbourne School of Land and Environment, Dookie Campus, The University of Melbourne, Dookie College, Vic. 3647, Australia.

<sup>F</sup>Institute of Biological and Environmental Sciences, University of Aberdeen, Aberdeen AB24 3UU, UK.

<sup>G</sup>Corresponding author. Email: h.g.jones@dundee.ac.uk

*This paper originates from a presentation at the 1st International Plant Phenomics Symposium, Canberra, Australia, April 2009.*

**Abstract.** Thermal imaging using infrared (IR) is now an established technology for the study of stomatal responses and for phenotyping plants for differences in stomatal behaviour. This paper outlines the potential applications of IR sensing in drought phenotyping, with particular emphasis on a description of the problems with extrapolation of the technique from the study of single leaves in controlled environments to the study of plant canopies in field plots, with examples taken from studies on grapevine (*Vitis vinifera* L.) and rice (*Oryza sativa* L.). Particular problems include the sensitivity of leaf temperature (and potentially the temperature of reference surfaces) to both temporal and spatial variation in absorbed radiation, with leaf temperature varying by as much as 15°C between full sun and deep shade. Examples of application of the approach to phenotyping in the field and the steps in data analysis are outlined, demonstrating that clear genotypic variation may be detected despite substantial variation in soil moisture status or incident radiation by the use of appropriate normalisation techniques.

**Additional keywords:** BRDF, drought, grapevine, IR thermography, *Oryza sativa*, phenotyping, rice, stress diagnosis, stress sensing, *Vitis vinifera*.

## Introduction: thermal sensing principles

Leaf temperature measurement using thermal infrared (IR) sensing is primarily used to study plant water relations, and specifically stomatal conductance, because a major determinant of leaf temperature is the rate of evaporation or transpiration from the leaf. The cooling effect of transpiration arises because a substantial amount of energy (the latent heat of vaporisation,  $\lambda$ ; J mol<sup>-1</sup>) is required to convert each mole of liquid water to water vapour, and this energy is then taken away from the leaf in the evaporating water and, thus, cools it. In rare cases leaf temperature may be affected by other physiological processes: for example, the heat generated (the exotherm) as water in a leaf freezes can be readily imaged (e.g. Wisniewski *et al.* 1997), and in extreme cases of particularly high respiratory rates (e.g. as found in the *Arum* spadix) raised temperatures can be used as a measure of these

increased respiration rates (Seymour 1999). In most cases, however, the heat generated by respiration is too small to have a detectable effect on leaf temperature (Breidenbach *et al.* 1997).

Thermal imaging is, therefore, particularly well suited for screening plants for differences in stomatal conductance, especially under laboratory conditions (e.g. Raskin and Ladyman 1988; Merlot *et al.* 2002) where the environmental conditions can be closely controlled. These and other uses of thermal imaging in plant science have been reviewed by Jones (2004). Because leaf temperature is dependent on environmental factors such as air temperature, humidity, wind speed and incident radiation, as well as stomatal aperture, many attempts have been made to normalise the data to account for environmental variation. The first normalisation for environmental variation was in terms of air temperature ( $T_a$ ), achieved by accumulating

differences between leaf temperature ( $T_{\text{leaf}}$ ) and  $T_a$  as a measure of plant stress (Jackson *et al.* 1977). Further normalisation was achieved by Idso *et al.* (1981) who developed the 'crop water stress index' (CWSI), which relates the observed temperature to the temperature of non-stressed and non-transpiring crops under the same environmental conditions; by noting the ambient humidity at the same time, effects of humidity variation could also be corrected. Rather than using actual empirical crop temperatures as references for calculation of CWSI, Jones and others have developed the approach of using physical wet and dry reference surfaces for application to field screening (Jones 1999a, 1999b, 2002; Cohen *et al.* 2005). A particularly helpful feature of the use of wet and dry reference temperatures is that they can readily be used for the derivation of indices that do not require detailed environmental information. A useful index for screening purposes, and in other cases where absolute estimates of stomatal conductance may not be required, is the index of stomatal conductance ( $I_g$ ) introduced by Jones (1999a), where the index is proportional to stomatal conductance (for a constant boundary layer conductance) and is calculated as.

$$I_g = (T_{\text{dry}} - T_{\text{leaf}}) / (T_{\text{leaf}} - T_{\text{wet}}), \quad (1)$$

where  $T_{\text{wet}}$  is the temperature of a wet surface and  $T_{\text{dry}}$  is the temperature of a non-transpiring surface. This can be converted to a stomatal conductance ( $g$ ) using

$$g_l = I_g / (r_{aW} + (s/\gamma)r_{\text{HR}}), \quad (2)$$

where  $r_{aW}$  is the boundary layer resistance to water vapour ( $\text{s m}^{-1}$ ), and  $r_{\text{HR}}$  is the parallel resistance to heat and radiation transfer by the leaf (see Jones 1992).

Theoretical analysis has shown that this approach may be extended beyond the calculation of a stress index to the estimation of an actual stomatal conductance (Leinonen *et al.* 2006), and that as good results may be obtained using only dry reference surfaces, or even, where full environmental measurements are available, calculation of the theoretical wet and dry reference temperatures. From an analysis of the full leaf energy balance, Leinonen *et al.* (2006) and Guilioni *et al.* (2008) showed that stomatal resistance ( $r_s$ ; the reciprocal of the conductance) may be estimated, without the use of reference surfaces, from

$$r_s = -\rho c_p r_{\text{HR}} (s(T_{\text{leaf}} - T_{\text{air}}) + D) / (\gamma((T_{\text{leaf}} - T_{\text{air}}) \rho c_p - r_{\text{HR}} R_{\text{ni}})) - r_{aW}, \quad (3)$$

where  $R_{\text{ni}}$  the net isothermal radiation (the net radiation that would be absorbed by a leaf if it were at air temperature,  $\text{W m}^{-2}$ ),  $\rho$  the density of air ( $\text{kg m}^{-3}$ ),  $c_p$  the specific heat capacity of air ( $\text{J kg}^{-1} \text{K}^{-1}$ ),  $s$  the slope of the curve relating saturating water vapour pressure to temperature ( $\text{Pa } ^\circ\text{C}^{-1}$ ),  $\gamma$  the psychrometric constant ( $\text{Pa K}^{-1}$ ) and  $D$  the air vapour pressure deficit (Pa). Where any of these key variables are unavailable, reference surfaces may substitute, with suitable rearrangement of the equations (Leinonen *et al.* 2006).

Thermal imaging appears to have potential advantages over the use of conventional porometry or gas-exchange measurement when screening for stomatal responses in phenotyping studies. This is because the ability to include large areas of crop, or large numbers of individual plants or plots, in single images means that

comparisons can readily be made, even in rapidly changing environments that are common outdoors. In such cases it is not generally necessary to have fully calibrated absolute data, as one is usually interested in the relative differences in phenotypic responses of the different genotypes. An alternative approach to the collection of thermal data for large areas of crop in the field, and one that has potential value for irrigation monitoring and control applications and that may even be of use for phenotyping is the use of a mobile IR thermometer (Loveys *et al.* 2008). In this paper we evaluate the potential and problems of scaling up thermal remote sensing to the field.

## Materials and methods

In all the studies discussed here, thermal images were obtained using a Thermacam P25 (FLIR Systems, Danderyd, Sweden), long-wave thermal imager with a sensitivity of  $0.08^\circ\text{C}$  and accuracy of  $\pm 2^\circ\text{C}$ . Parallel visible images were obtained with either the onboard digital imager or a normal digital camera. For phenotyping purposes, images can be obtained over a range of distances from the canopy of interest. In some studies the camera may be held within a couple of metres of the canopy, but for most phenotyping applications, and for many agronomic applications, it is necessary to view a larger area of canopy so that substantial numbers of genotypes can be compared simultaneously. As shown later, this minimises problems caused by any rapid changes in solar irradiance. Several options are available to expand the area of view; these include mounting the camera on mobile platforms such as a 'cherry picker' (Möller *et al.* 2007) or other mobile platforms. The camera can also be elevated on a hand-held pole to  $\sim 5$  m above the ground, which, when combined with an oblique view, allows viewing of a useful area of crop. For example, for a camera with a field of view of  $22^\circ$ , this will allow viewing of an area of  $\sim 15 \text{ m}^2$  at 20 m; wide angle lenses allow a larger field of view. Wider fields of view may also be obtained by mounting the camera on a balloon or in an aircraft. Of course, as cameras have a limited number of pixels (usually  $240 \times 320$  for the P25 or  $480 \times 640$ ) greater viewing distances come with less spatial resolution on the ground (larger pixels).

Where absolute estimates of stomatal conductance or of a 'stress index' are required, it is usually necessary to have some wet and dry or dry reference surfaces in the image to allow for changes in environmental conditions (Jones 1999a; Meron *et al.* 2003; Leinonen *et al.* 2006), though in stable and consistent climates such as in Arizona, the normalisation of canopy temperature to air temperature and the use of standard crop responses may be adequate (Idso 1982). For crop phenotyping, however, the usual requirement is the comparison of genotypes, with absolute values of stomatal conductance or stress index being of less interest. In such cases, inclusion of a substantial number of genotypes in each image (or the use of repeated reference varieties) allows effective internal normalisation of the data to correct for changes in incident light or local variation in soil. Unfortunately, in order to include the necessary large numbers of genotypes in any image it is necessary to view the canopy from substantial distances. Where this is not possible, the use of references (or precise environmental measurement) becomes essential to allow

correction for any rapid changes in environmental conditions. In such cases it is critical that the thermal time response of the reference surfaces matches that of the canopy. Optimisation of references will be discussed further in succeeding sections.

### Difficulties of scaling up to the field: radiation variation

Of particular interest for the application of thermal imaging to phenotyping and irrigation studies is the sensitivity of  $T_{\text{leaf}}$  (or of  $I_g$  and other indices) to changes in stomatal conductance as a function of the expected variation due to environmental variables. Here, the thermal approach will be of little value where the environmentally caused variation in temperature is greater than the 'sensitivity', that is the range in temperature caused by a specified variation in conductance (Jones 1994). Nevertheless, by viewing an ensemble of leaves in a canopy, it may be possible to obtain a more robust estimate of the mean temperature (Jones *et al.* 2002). Below we will consider some of the possible errors, and some possible solutions. Simple analyses of the potential sensitivity of thermal sensing for the estimation of stomatal conductance have been provided by Jones (1994, 1999a, 1999b) and Jones *et al.* (1997); these concluded that the sensitivity increases with temperature, vapour pressure deficit, radiation and generally decreases with increasing wind speed.

Several the problems involved in applying thermal sensing to field crops have been analysed (Jones *et al.* 2002), including (i) how one deals with the problem of variation in temperature of both references and crop leaves caused by varying incident radiation, (ii) the choice of reference surface – these may include leaves (Jones *et al.* 2002), models (Jones 1999a; Cohen *et al.* 2005; Loveys *et al.* 2008), or whole canopies (Grant *et al.* 2007), and (iii) the need to ensure that the data collected are for crop leaves alone and are not biased by inclusion of woody parts or the background soil (Guiliani and Flore 2000; Leinonen and Jones 2004).

### Leaf temperature variation as function of absorbed radiation

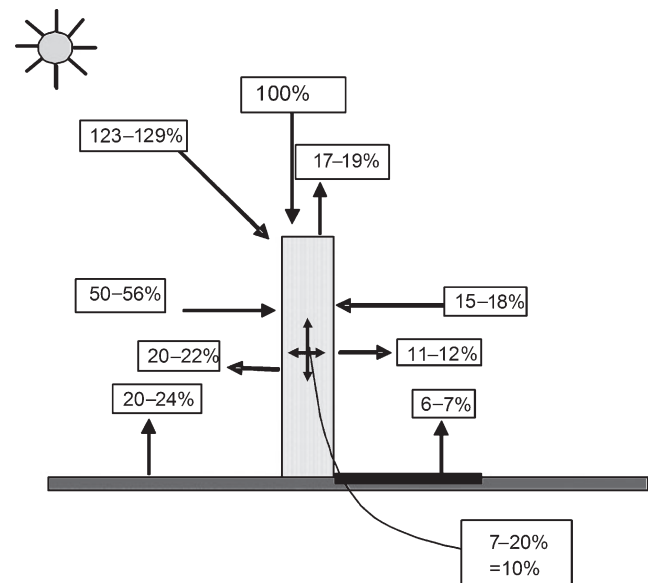
A critical variable in Eqn 3 is the net radiation absorbed by the leaf or canopy. This is because the leaf temperature increases linearly as absorbed radiant energy increases (other factors being constant). Several studies have demonstrated that the range of leaf temperatures for individual leaves in a homogeneous grapevine canopy may easily vary by 10–15 K when comparing leaves directly illuminated normal to the solar beam and those in shaded parts of the canopy (Jones *et al.* 2002; Leinonen and Jones 2004; Grant *et al.* 2007). Data collected on different occasions on a grapevine *Vitis vinifera* (L.) cv. Cabernet Sauvignon vineyard at Waikerie using a moving narrow-beam IR radiometer mounted on a quad-bike (according to the methods described by Loveys *et al.* 2008) gave the mean temperature ranges for any plot when viewed from the sunny side of the row on sunny days (3 January 2008 and 9 January 2008) of  $5.3 \pm 1.03$  K. This range is presumably smaller than the extreme values found by thermography because the field of view of the IR thermometer used included several leaves at the range used (~1.5 m).

For direct solar radiation, the radiation absorbed by a surface will increase proportionately to the solar absorption coefficient

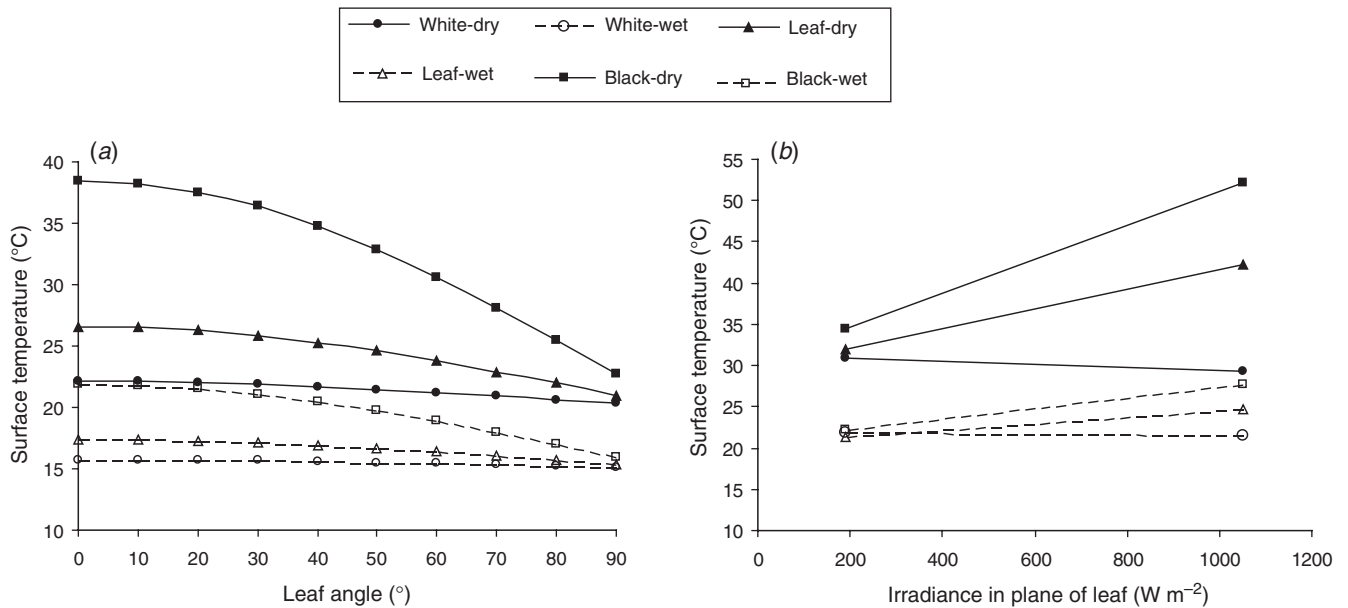
( $\alpha$ ), and proportionately according to the cosine of the angle between the direct beam and the normal to the surface (Jones 1992). For clear sky conditions 80% or more of the irradiance on a horizontal surface may be in the direct beam with only 20% representing diffuse radiation scattered by sky and clouds, so that the incident shortwave radiation on different leaves at one time may easily vary by as much as  $800 \text{ W m}^{-2}$ . Some representative mean values for irradiance on surfaces at different positions and orientations in typical grapevine canopies are illustrated in Fig. 1 for clear-sky conditions. Not only is the mean irradiance greatest for leaves pointing towards the sun at the top of the canopy, but as one considers a leaf further into the canopy the proportion of diffuse radiation increases since it includes an increasing amount of radiation scattered by other leaves in the canopy and by the soil, and the variation between leaves in radiation intercepted decreases.

Eqn 3 can be rearranged to give  $T_{\text{leaf}}$  as a function of all the environmental variables including the absorbed radiation (Jones 1992), so it is possible to calculate the expected variation in surface temperature for wet and dry surfaces of differing absorptivities and leaf angle (Fig. 2a). These are compared with observed temperatures for different surfaces (with differing  $\alpha$ ) at high and low irradiances (Fig. 2b).

We can illustrate the effect of leaf orientation on temperature by using the artificial reference surface described by Loveys *et al.* (2008). This instrument consists of a horizontal frame with four green filter-paper surfaces radiating at  $45^\circ$  from the



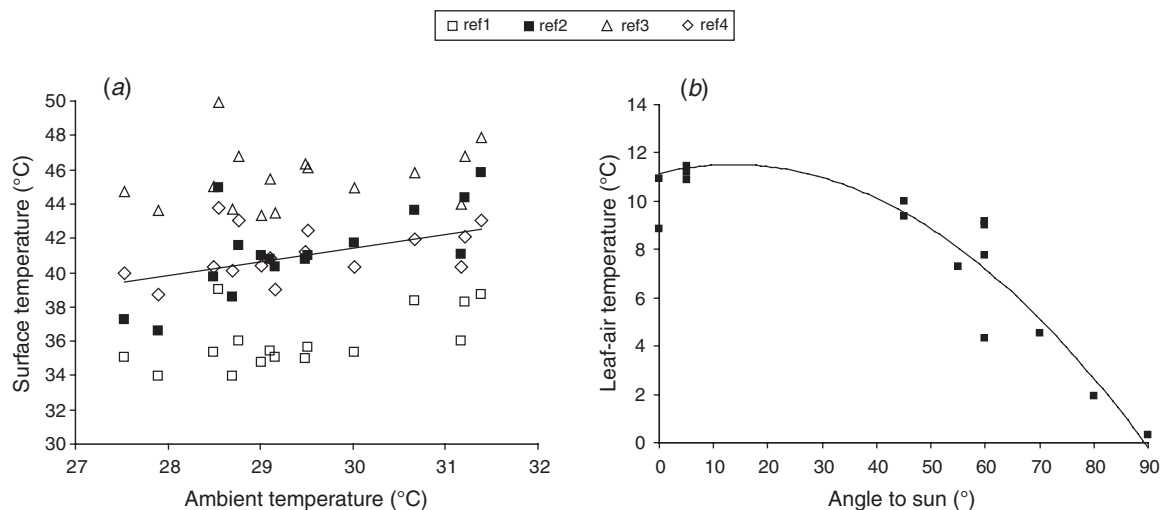
**Fig. 1.** Schematic diagram showing typical mean irradiances in different directions as indicated by the heavy arrows for clear sky conditions for different positions and orientations in a grapevine canopy. Measurements were made by a cosine-corrected Pyranometer sensor (Skye Instruments, Llandidrod Wells, Wales) and expressed relative to the horizontal irradiance at the top of the canopy. Data ranges indicate the means of 20 separate measurements, each on a different vine, for each canopy position in each of two contrasting grapevine canopies, one of Shiraz vines at SARDI, Nuriootpa, Australia, January 2005; and one of Moscatel vines, Estremoz, Portugal, July 2004, with both canopies trained to a solid hedgerow form (H. G. Jones, unpubl. data).



**Fig. 2.** (a) Modelled effect of leaf angle on leaf temperature in full sunlight using the full energy balance equation as the leaf angle is varied from 0° (normal to the solar beam) to 90°, assuming 80% of the irradiance is in the direct beam. Results were calculated according to (Jones 1992) assuming air temperature ( $T_a$ ) = 20°C, wind speed ( $u$ ) = 0.05 m s<sup>-1</sup>, leaf diameter = 0.1 m, relative humidity = 50%, incident irradiance of 1000 W m<sup>-2</sup>, of which 150 W m<sup>-2</sup> is diffuse. Absorptivities of white filter paper, the leaf and black filter paper were assumed equal to 0.08, 0.25 and 0.7, respectively, and calculations were for surfaces where both sides were either wet (open symbols) or dry (closed symbols). (b) Observed relationship between surface temperature and irradiance for the three types of reference surface when wet or dry (Nuriootpa, 24 January 2005).

horizontal and oriented to the four cardinal points; the temperature of each surface was monitored using a copper-constantan thermocouple. This type of reference uses only dry surfaces; Leinonen *et al.* (2006) showed that the use of wet references could be replaced by measurements of atmospheric

humidity with very little loss of precision. Figure 3a shows data collected over a 90 min period indicating that variations in surface orientation may affect temperature by up to  $\pm 7$  K. Simultaneous variation in leaf temperature as a function of angle to the solar beam is shown in Fig. 3b. In this case, leaf orientation alone



**Fig. 3.** (a) Variation of the reference surface temperature for four reference surfaces in the mobile reference sensor (Loveys *et al.* 2008) where the four surfaces are angled at 45° to the horizontal and references 1 to 4, respectively, were sloping to the N (i.e. facing away from the sun in the southern hemisphere), E, S and W. The solid line represents a regression fitted to all the data points shown. Results are plotted against air temperature over a 1.5 h period at Adelaide (13 March 2009), showing the major impact of reference surface orientation on temperature. (b) Simultaneous variation of observed temperatures of non-transpiring leaves (coated with Vaseline (Walton-on-Thames, UK)) (expressed as a difference between leaf and air temperature) plotted as a function of the angle between the solar beam and the leaf plane. Leaf temperatures were measured using a narrow beam infrared thermometer (Everest 1110) (B. R. Loveys, unpubl. data).

affected leaf temperature by up to 11°C. The temperature of any leaf will also depend on the position in the canopy as a result of both the local variation in irradiance, due to canopy structure and due to mutual shading. Note from this figure that the energy absorbed by any leaf will be the sum of that absorbed from the two sides, which equals the sum of the two irradiances multiplied by the absorption coefficient (which for leaf material averages ~0.85). Thus, the radiant energy absorbed by different leaves at any one time may vary by up to an order of magnitude, with consequential substantial impacts on canopy temperature.

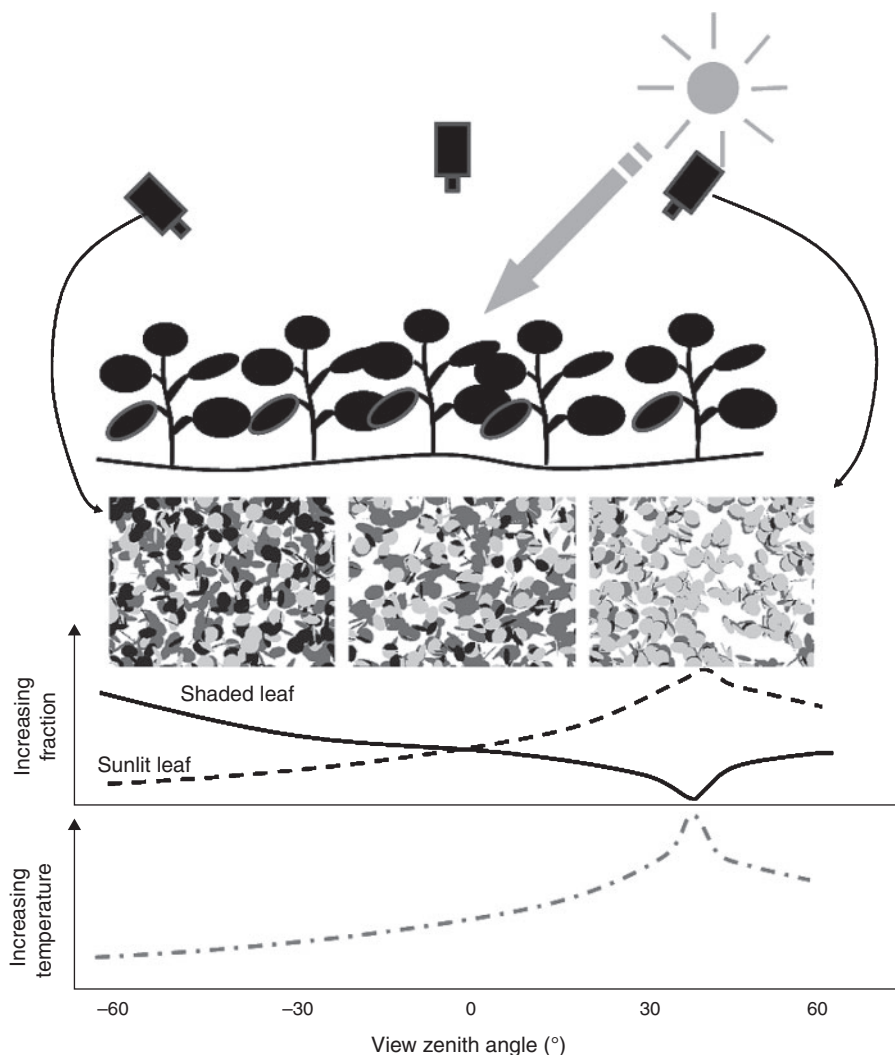
#### *Bi-directional reflectance distribution function*

The canopy temperature observed by a thermal sensor will depend on the direction of view relative to the direction of illumination. The bidirectional reflectance distribution function (BRDF) describes this behaviour (Liang 2004). As is illustrated in

Fig. 4, the fraction of sunlit leaves viewed increases as the view angle approaches the angle of illumination, as does the fraction of viewed soil that is sunlit if the canopy is not too dense. The greater is the sunlit fraction, the greater the absorbed radiation and hence the greater the average temperature of a scene. The individual leaf temperatures in the scene will reflect the amount of radiation absorbed by each of the leaves. It is possible to estimate both the mean canopy and leaf temperature components, or by extrapolation of the approach, the sunlit and shaded leaf component temperatures if one has temperature measurements from more than one view angle (Jia *et al.* 2003).

#### *Extraction of canopy temperature*

In studies such as the grapevine experiments reported above, imaging or thermometer measurements were made from close range, or plot sizes are large compared with the thermal images, so



**Fig. 4.** Schematic illustration of the bidirectional reflectance effect on the proportion of leaves sunlit (dashed line) or shaded (solid line) as the view angle changes relative to the incident solar beam, and the consequent effect on average leaf temperature observed when viewing the canopy from a distance when individual shaded and sunlit leaves cannot be distinguished (dots and dashes). Note that the actual thermodynamic temperature of the canopy is not changing.



it is straightforward to identify the genotype or plot of interest. The only critical requirement is to be confident that one is recording the temperature of the leaves of interest and not the trunks or worse, the background soil (very hot) or sky (very cold). Approaches to the elimination of background temperatures have included the use of a sheet background that heats up above the canopy temperature and so can be excluded from the collected data simply by trimming the thermal histogram (Guiliani and Flore 2000), or mathematically density-slicing of the observed thermal histogram so that it only includes points between the temperatures of the wet and dry reference surfaces (Jones *et al.* 2002). Neither of these approaches requires explicit pixel-related image manipulation. A more sophisticated approach is to identify leaf pixels from visible (RGB) or R and NIR images and only take the temperatures of those pixels, thus, ignoring background pixels (Leinonen and Jones 2004; Möller *et al.* 2007; Guisard *et al.* 2009).

Another approach that is applicable to the extraction of subpixel information where individual pixels contain varying fractions of the different surfaces (e.g. leaves and soil) is to estimate the component surface temperatures by regression of the observed temperature against the fraction of leaf in each pixel of the image, which itself may be estimated from NDVI as suggested by Nemani and Running (1989). The extrapolated extreme temperatures at zero and complete canopy cover represent the true soil and canopy temperatures. This linear unmixing approach has been widely extended in remote sensing applications for the generalisation of crop water stress indices to the situation where there is a variable amount of soil visible in each pixel (or in the thermometer view), and the different pixels may have differing stomatal conductances.

In most cases, however, it is essential to have a corresponding visible image to check for the completeness of canopy cover in any area of canopy. This allows one to select only areas from any plot that are vegetated.

#### Choice of reference

As we have seen, the choice of reference surface and its orientation is crucial for accuracy of the calibration in terms of a CWSI or stomatal conductance. The use of the average temperature of multiple, differently-oriented surfaces as proposed by Loveys *et al.* (2008) (see also Fig. 3) provides a relatively robust approach to providing an average reference temperature for an ensemble of leaves as found in many canopies, though the result may not be so good for individual leaves where it is possible for a non-transpiring leaf to be substantially warmer or cooler than the corresponding dry reference, depending on their relative orientations with respect to the solar beam. The provision of a wetted surface as a reference, or even a surface with known but finite surface conductance (Jones 1999b), is particularly difficult in the field (as it tends to be difficult to maintain a surface wet in hot arid environments), though it is much less sensitive to orientation or absorptivity than is a dry surface because of the greater contribution of latent heat as compared with radiation in its energy balance. For larger-scale sensing, the wetted leaf references used by Jones *et al.* (2002) need to be replaced by larger references such as the 'wet artificial reference surface' proposed by Meron *et al.* (2003), or well-

irrigated crop references. Although the use of both wet and dry reference surfaces is ideal for calculation of stress indices and conductance, Leinonen *et al.* (2006) showed that it is possible to dispense with the wet reference, as incorporation of atmospheric humidity in the calculation can replace this reference with little or no loss of accuracy.

#### Utilisation of variance in canopy temperature

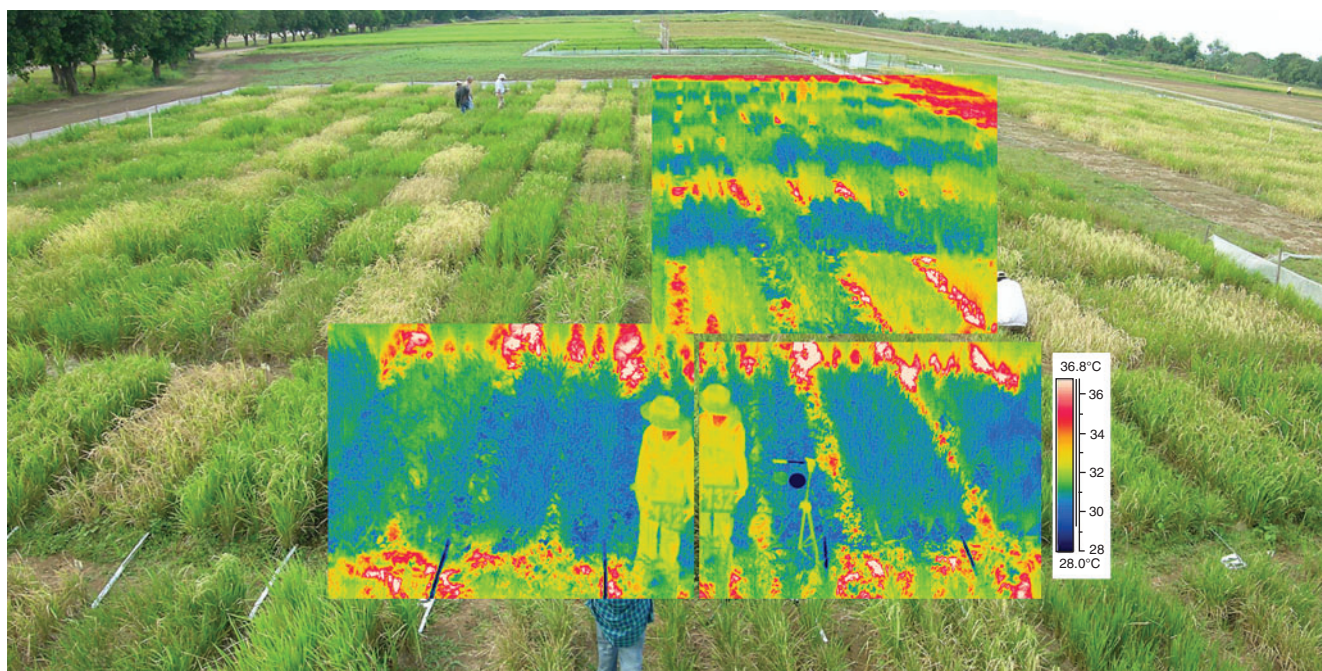
Although the commonest approach to the use of leaf temperature is the calculation of a stress index or the direct estimation of stomatal conductance, there has been some interest in making use of the variability in leaf temperature as a measure of stomatal opening (Fuchs 1990). This approach depends on the increasing contribution of the radiative component to the heat balance as stomata close and evaporation decreases. In addition there may be an increasing proportion of background soil included in the image as drought increases (partly an effect of wilting and partly an effect of reduced leaf expansion or of leaf abscission). In principle, however, the approach depends on a random distribution of leaves. This may explain why Grant *et al.* (2007) found no good correlation in between variance and water status in grapevine canopies in Portugal (probably because the leaves in this case were mostly oriented in the plane of the canopy surface). Others have reported some association of temperature variance with conductance in cotton (González-Dugo *et al.* 2006), and also in grapevine (Jones *et al.* 2002), though it must be said that variability does not appear to be a reliable stress indicator in all cases.

#### Phenotyping in field 'macro-arrays'

An analogue of what can be obtained in conventional micro-arrays for genotyping and phenotyping is the traditional field screening trial or 'macro-array', where a range of genotypes are grown in the field in small replicated plots for phenotypic analysis. Two examples of such experiments are illustrated in Figs 5 and 6. In each case both thermal and corresponding conventional visible (RGB) images are obtained and analysed. The elevated viewpoint may be achieved by mounting the camera on a 'cherry-picker' or similar crane (Möller *et al.* 2007), or by mounting the camera on the top of a 5-m pole with remote control from a computer at ground level using a firewire connection. One advantage of this approach is that individual thermal images cover substantial numbers of individual plots, so it is possible to correct for both spatial and temporal variation in temperature that is unrelated to the genotypic variation by normalising plot temperatures to the mean of each image, and to apply other detrending algorithms.

#### Identification of plots

As the complexity of the experiment increases, or the distance from which samples are viewed increases, it becomes more important to distinguish plots representing different treatments or genotypes. This is particularly true of the two rice 'macro-array' experiments (Figs 5, 6). Initial trials on the IRR experiment (Fig. 5), using sticks placed between rows that heated up in the sunlight and could be seen in thermal images, succeeded in identifying the individual plots, but these markers were visible only in nearby plots, so plot identification proved to



**Fig. 5.** Visible (RGB) digital image, together with some corresponding thermal images, of a rice 'macro array' trial in the dry season of 2006 consisting of 300 plots combining 50 contrasting genotypes, two water treatments (well watered and drought stressed) and three replicates at the International Rice Research Institute (IRRI; R. Serraj and H. G. Jones, unpubl. data). All images were taken from ~4 m above the canopy. To see clearly the temperature differences between plots, view the online version in colour.

be a very tedious and labour-intensive process. It was found in the subsequent Wuhan experiment that plot identification could be greatly facilitated by the use of plot labels with large white labels with black writing, as the black area heated up and could be read in the thermal images (Fig. 6). Möller *et al.* (2007) used aluminium crosses that served the same purpose in their experiments.

Although processing of multiple images, overlaying them and extracting pixels of interest can be greatly accelerated by use of software such as ENVI, Matlab (Mathworks Inc., Natick, MA, USA), Java or Adobe Photoshop, there is usually still a residual need for substantial operator input to the identification and delineation of relevant parts of the image.

#### *Canopy structure*

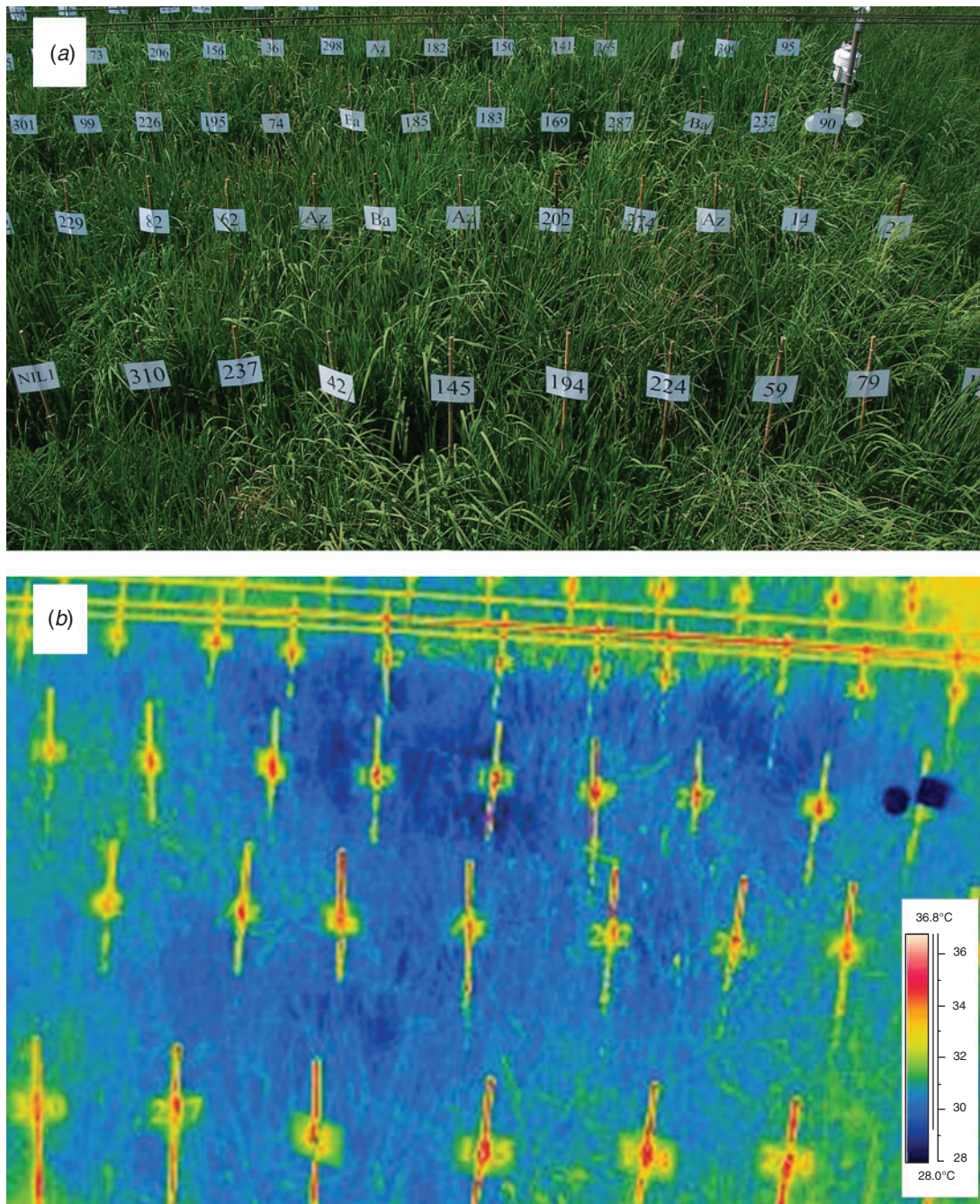
The canopy structure affects not only the proportion of sunlit and shaded leaves and their mean angle in relation to the solar direct beam (and, hence, their temperatures), but also the fraction of leaf and of other plant parts such as flowers or fruits in the image, which will change as plants mature. The canopy structure also affects the observed temperature and how it varies with view angle (Kimes *et al.* 1980; Otterman *et al.* 1999). In cereals, developing and maturing seed heads tend to have stomata, and transpire less than the leaves (e.g. Milthorpe and Moorby 1979; H. G. Jones, unpubl. data) there is an expectation that canopy temperatures will increase. As can be seen from the field macro-array shown in Figs 5 and 6, the stage of development varied between rice genotypes studied. This suggests that the low overall correlation between leaf temperature and stomatal conductance ( $R^2 = 0.16$ ) may have been related to this. It is possible, therefore, that a better explanation of the variation in temperature could have

been obtained by incorporating a developmental stage measure into a multiple regression.

#### *Application to determination of plant water relations responses and quantitative trait loci*

The field experiment conducted at IRRI (Fig. 5) indicated that the average temperature differences due to drought were between 1 and 1.8°C, whereas the temperatures of individual lines varied by as much as 1.9°C for the well-irrigated plots and 3.7°C for the dry plots. These results confirmed the expectation (Fuchs 1990) that temperature variation between leaves will be greatest when crops are drought-stressed. A second experiment analysed in more detail the response of 12 rice genotypes to soil moisture in a line-source irrigation gradient experiment (data not shown). Thermal sensing has been previously used successfully in field screening for monitoring differences in stomatal responses to drought in crops such as rice (Garrity and O'Toole 1995) and wheat (*Triticum aestivum* L.; Reynolds *et al.* 1998). Using an IR thermometer gun on single rice leaves, rice varieties were screened for reproductive-stage drought-avoidance traits (Garrity and O'Toole 1995). They found that both grain yield and spikelet fertility were significantly correlated with midday canopy temperature on the day of flowering, and confirmed the existence of significant differences in canopy temperature among rice cultivars, reporting that lines with high drought-avoidance scores consistently remained coolest under stress (Garrity and O'Toole 1995). However, using an IR gun on single plants is often subject to high field variation and it has limited scope as a high-throughput and reproducible screening method. Hence, IR thermal imagery, if up-scaled to plot or field levels, can offer new





**Fig. 6.** Image of a sample area from a rice 'macro array' consisting of 105 recombinant inbred lines (Bala  $\times$  Azucena population), Price *et al.* (2000) plus 15 others grown in rainout shelters at Huazhong Agricultural University, Wuhan (A. Price, L. Xiong and H. G. Jones, unpubl. data). Plot size is  $1.6 \times 0.6$  m with repeated controls. This image shows the use of plot labels to identify genotypes in the thermal image. At the right-hand side of this image can be seen the wet and dry reference surfaces, which can be used to normalise the data if required. To see clearly the temperature differences between plots, view the online version in colour.

screening tools for drought resistance research in rice (Serraj *et al.* 2009).

The field experiment in Wuhan was based on a mapping population involving 105 recombinant inbred lines and was used to derive quantitative trait loci (QTL) relating to aspects of plant water relations using the thermal data in combination

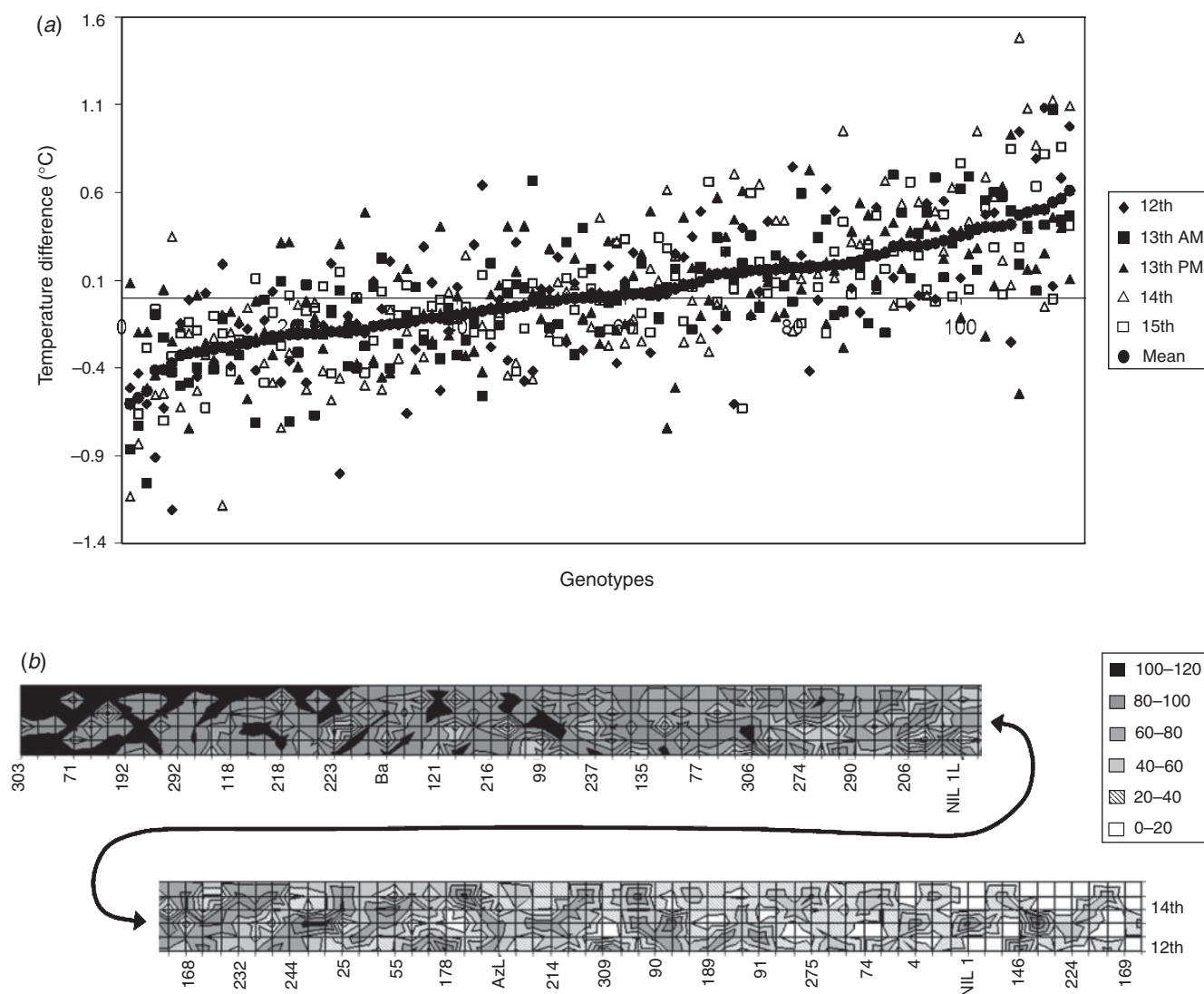
with a range of other physiological and structural data. Here we illustrate the viability of the thermal approach for derivation of genetic variation in stomatal conductance in a field experiment. Although there was substantial variation in underlying soil moisture content over the trial, partly as a result of the need to apply supplementary hand-watering to prevent serious stress,



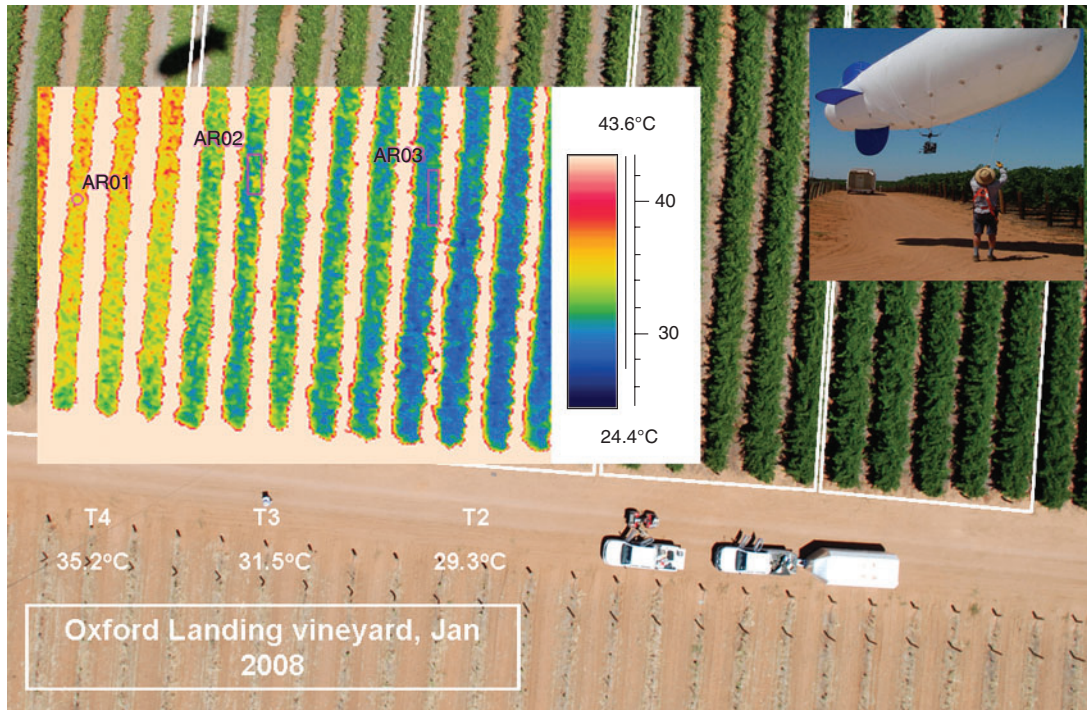
analysis of the thermal data from measurements made over 5 days during the drying cycle demonstrated good heritability and provide clear evidence for thermal QTLs (related to crop transpiration). Two temperature QTLs were identified with LOD scores above 3.2 on chromosomes 2 and 11 (the 5% genome wide threshold) (these will be reported elsewhere in more detail; A. H. Price, unpubl. data).

In order to account for local variation in soil moisture content over the plot (resulting from hand watering) and variation in incident radiation with time, all temperatures were expressed as differences from the mean temperature of the rice canopy in each image. The average temperature difference from the mean was then calculated for each genotype on any measurement date by averaging the observed temperature difference across all images and replicates and plotted in Fig. 7*a*. This figure shows the

temperature differences for each genotype on each of five measurement occasions, with genotypes ranked according to the overall mean temperature difference. This figure shows that for the Wuhan experiment, the mean temperatures of the different lines varied over a range of 1.2°C, and heritability analysis gave a reasonable broad sense heritability of 49% even though the mean temperatures of the two parental lines differed by less than 0.05°C. Despite of the substantial spatial and temporal variation of the data there was an extremely good consistency between the ranking of different genotypes for measurements made on five separate occasions during the drying cycle (Fig. 7*b*). This figure shows that there is a high degree of consistency between the genotypic rankings observed at different stages during the drying cycle. Stepwise regression across the whole experiment indicated that canopy temperature



**Fig. 7.** Illustration of the consistency of the rankings of the different genotypes across five measurement dates for the Wuhan experiment. (a) Mean temperature differences across all replicates for each genotype expressed as a difference from the local mean temperature for each of five sample occasions between 12 and 15 August 2007; genotypes ranked according to their overall mean temperature differences. (b) The same data as (a) expressed as genotype rankings on each occasion (earliest measurements in the top row), with dark areas indicating genotypes ranked in the coolest 20 on any occasion and white areas indicating genotypes ranked in among the hottest 20.

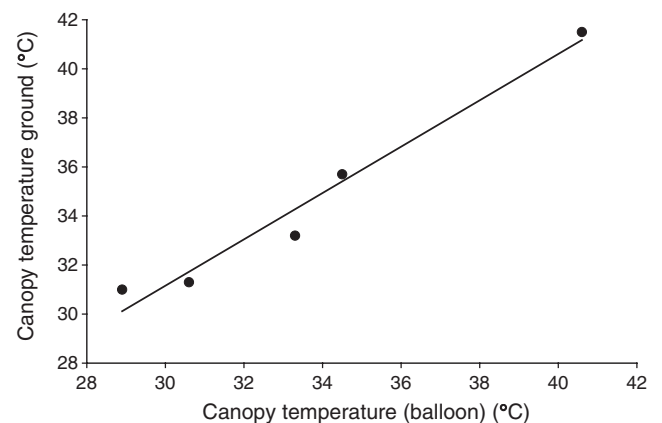


**Fig. 8.** A visible image of a grape vineyard (cv. Cabernet Sauvignon) at Oxford Landing, Waikerie, SA, taken from a balloon at a height of ~80 m at 1240 hours on 9 January 2008, together with a corresponding thermal image also taken from the balloon at the same time. Mean canopy temperatures of three areas of crop corresponding to three irrigation treatments (T2, T3 and T4 with decreasing amounts of irrigation applied) are indicated (A. Wheaton, B. R. Loveys and H. G. Jones, unpubl. data).

was best explained by variation in stomatal conductance with 'leaf firing' score and  $\delta^{13}\text{C}$  being the next most important explanatory variables (A. H. Price, unpubl. data).

#### Extrapolation to larger scales

Figure 8 shows how it is possible to derive canopy temperature from airborne, or in this case, balloon flights. Substantial temperature differences between areas of the canopy corresponding to different irrigation treatments are apparent, with a high correlation between observed canopy temperature and stomatal conductances measured nearly simultaneously (H. G. Jones, B. R. Loveys and A. Wheaton, unpubl. data) but see Loveys *et al.* (2008) for details of the methods. Figure 8 illustrates the problem caused by the bidirectional reflectance effect, with the observed temperature in any row clearly increasing towards the hotspot (where the balloon shadow can be seen), although some of this trend may relate to an 'edge effect' as commonly observed at the end of each row. Figure 9 shows that the treatment temperatures derived from balloon data corresponded closely with near simultaneous data obtained using an IR thermometer driven along the rows mounted on a quad-bike (Loveys *et al.* 2008). The small but consistent difference between the two datasets may relate to differences in the view angle for the two instruments. In addition to the use of balloon-mounted cameras, there has also been an increasing interest in the extension of the approach to airborne (at 1000–2000 m) or even satellite-based thermal sensing, though



**Fig. 9.** The relationship between mean treatment temperatures obtained using an infrared thermometer mounted on a quad-bike and near-simultaneous data obtained from the balloon data at around 1320 hours on 9 January 2007 at Waikerie.

as the pixel size increases, the size of the ground plots needs to increase (e.g. Sepulcre-Cantó *et al.* 2007).

#### Discussion and conclusions

Results presented here show that there is enormous potential for the use of thermal sensing at a field scale for crop phenotyping and for crop and irrigation management purposes, where the interest is in detecting differences in stomatal conductance as a measure of

plant response to water deficit. Although thermal imaging does not directly measure stomatal conductance, in any given environment stomatal variation is the dominant cause of changes in canopy temperature (Jones 2004). Use of remote imagery, when combined with effective image analysis techniques, provides a powerful approach to the comparison of large numbers of genotypes in typical field situations. It has also been widely suggested that thermal imaging can be used as a component of a remote sensing system for diagnosing plant stresses. Unfortunately, any single sensor provides only limited information, indicating changes in only one or two intermediary responses, in the case of thermal imaging providing information on changes in stomatal opening. In its turn, stomatal aperture can be affected by a wide range of primary stresses, including water deficit, flooding, salinity, pollutants as well as biotic stresses such as pests and diseases. It follows that use of thermal sensing for stress diagnosis, as opposed to simple monitoring where the stress is well defined, ideally requires the combined application of one or more other imaging techniques in a multi-sensor approach where thermal sensing is combined with reflectance, fluorescence and other sensing techniques (Chaerle *et al.* 2004, 2007; Leinonen and Jones 2004; Lenk and Buschmann 2006).

## Acknowledgements

We are particularly grateful to various colleagues including Pietà Schofield, Ilkka Leinonen and Laury Chaerle for their contributions to aspects of the work described, and to funding agencies including EU Human Potential Program - HPRN-CT-2002-00254, EU FP6 project 015468 'CEDROME', EU WATERUSE Project (EVKI-2000-22061), UK Defra HortLink project WaterLink2 (project HL0168), the Rockefeller Foundation Project (IRRI), the International Rice Research Institute and the Australian Grape and Wine Research and Development Corporation.

## References

- Breidenbach RW, Saxton MJ, Hansen LD, Criddle RS (1997) Heat generation and dissipation in plants: can the alternative oxidase pathway serve a thermoregulatory role in plant tissues other than specialised organs? *Plant Physiology* **114**, 1137–1140. doi: 10.1104/pp.114.4.1137
- Chaerle L, Hagenbeek D, De Bruyne E, Valcke R, Van Der Straeten D (2004) Thermal and chlorophyll-fluorescence imaging distinguish plant–pathogen interactions at an early stage. *Plant & Cell Physiology* **45**, 887–896. doi: 10.1093/pcp/pch097
- Chaerle L, Hagenbeek D, Vanrobaeys X, Van Der Straeten D (2007) Early detection of nutrient and biotic stress in *Phaseolus vulgaris*. *International Journal of Remote Sensing* **28**, 3479–3492. doi: 10.1080/01431160601024259
- Cohen Y, Alchantis V, Meron M, Saranga Y, Tsipris J (2005) Estimation of leaf water potential by thermal imagery and spatial analysis. *Journal of Experimental Botany* **56**, 1843–1852. doi: 10.1093/jxb/eri174
- Fuchs M (1990) Infrared measurement of canopy temperature and detection of plant water stress. *Theoretical and Applied Climatology* **42**, 253–261. doi: 10.1007/BF00865986
- Garrity DP, O'Toole JC (1995) Selection for reproductive stage drought avoidance in rice, using infrared thermometry. *Agronomy Journal* **87**, 773–779.
- González-Dugo MP, Moran MS, Mateos L, Bryant R (2006) Canopy temperature variability as an indicator of crop water stress severity. *Irrigation Science* **24**(4), 233–240. doi: 10.1007/s00271-005-0022-8
- Grant OM, Tronina L, Jones HG, Chaves MM (2007) Exploring thermal imaging variables for the detection of stress responses in grapevine under different irrigation regimes. *Journal of Experimental Botany* **58**, 815–825. doi: 10.1093/jxb/erl153
- Guiliani R, Flore JA (2000) Potential use of infra-red thermometry for the detection of water stress in apple trees. *Acta Horticulturae* **537**, 383–392.
- Guilioni L, Jones HG, Leinonen I, Lhomme JP (2008) On the relationships between stomatal resistance and leaf temperatures in thermography. *Agricultural and Forest Meteorology* **148**(11), 1908–1912. doi: 10.1016/j.agrformet.2008.07.009
- Guisard Y, Whish JPM, Scollary GR (2009) Canopy temperature as indicator of water stress: image analysis of grapevine canopies. *Acta Horticulturae*, in press.
- Idso SB (1982) Non-water-stressed baselines – a key to measuring and interpreting plant water-stress. *Agricultural Meteorology* **27**, 59–70. doi: 10.1016/0002-1571(82)90020-6
- Idso SB, Jackson RD, Pinter PJ, Reginato RJ, Hatfield JL (1981) Normalizing the stress-degree-day parameter for environmental variability. *Agricultural Meteorology* **24**, 45–55. doi: 10.1016/0002-1571(81)90032-7
- Jackson RD, Reginato RJ, Idso SB (1977) Wheat canopy temperature: a practical tool for evaluating water requirements. *Water Resources Research* **13**, 651–656. doi: 10.1029/WR013i003p00651
- Jia L, Li Z-I, Menenti M, Su Z, Verhoef W (2003) A practical algorithm to infer soil and foliage component temperatures from bi-angular ATSR-2 data. *International Journal of Remote Sensing* **24**, 4739–4760. doi: 10.1080/0143116031000101576
- Jones HG (1992) 'Plants and microclimate.' 2nd edn. (Cambridge University Press: Cambridge)
- Jones HG (1994) Use of infrared thermometry for irrigation scheduling. *Aspects of Applied Biology* **28**, 247–253.
- Jones HG (1999a) Use of infrared thermometry for estimation of stomatal conductance in irrigation scheduling. *Agricultural and Forest Meteorology* **95**, 139–149. doi: 10.1016/S0168-1923(99)00030-1
- Jones HG (1999b) Use of thermography for quantitative studies of spatial and temporal variation of stomatal conductance over leaf surfaces. *Plant, Cell & Environment* **22**(9), 1043–1055. doi: 10.1046/j.1365-3040.1999.00468.x
- Jones HG (2004) Application of thermal imaging and infrared sensing in plant physiology and ecophysiology. *Advances in Botanical Research* **41**, 107–163. doi: 10.1016/S0065-2296(04)41003-9
- Jones HG, Aikman D, McBurney TA (1997) Improvements to infra-red thermometry for irrigation scheduling. *Acta Horticulturae* **449**, 259–266.
- Jones HG, Stoll M, Santos T, de Sousa C, Chaves MM, Grant OM (2002) Use of infrared thermography for monitoring stomatal closure in the field: application to grapevine. *Journal of Experimental Botany* **53**(378), 2249–2260. doi: 10.1093/jxb/erf083
- Kimes DS, Idso SB, Pinter PJ, Reginato RJ, Jackson RD (1980) View angle effects in the radiometric measurement of plant canopy temperatures. *Remote Sensing of Environment* **10**, 273–284. doi: 10.1016/0034-4257(80)90087-5
- Leinonen I, Jones HG (2004) Combining thermal and visible imagery for estimating canopy temperature and identifying plant stress. *Journal of Experimental Botany* **55**, 1423–1431. doi: 10.1093/jxb/erh146
- Leinonen I, Grant OM, Tagliavia CPP, Chaves MM, Jones HG (2006) Estimating stomatal conductance with thermal imagery. *Plant, Cell & Environment* **29**, 1508–1518. doi: 10.1111/j.1365-3040.2006.01528.x
- Lenk S, Buschmann C (2006) Distribution of UV-shielding of the epidermis of sun and shade leaves of the beech (*Fagus sylvatica* L.) as monitored by multi-colour fluorescence imaging. *Journal of Plant Physiology* **163**, 1273–1283. doi: 10.1016/j.jplph.2005.08.018
- Liang S (2004) 'Quantitative remote sensing of land surfaces.' (John Wiley & Sons: Hoboken, NJ)
- Loveys BR, Jones HG, Theobald JC, McCarthy MG (2008) An assessment of plant-based measures of grapevine performance as irrigation-scheduling tools. *Acta Horticulturae* **792**, 391–403.



- Merlot S, Mustilli AC, Genty B, North H, Lefebvre V, Sotta B, Vavasseur A, Giraudat J (2002) Use of infrared thermal imaging to isolate *Arabidopsis* mutants defective in stomatal regulation. *The Plant Journal* **30**(5), 601–609. doi: 10.1046/j.1365-313X.2002.01322.x
- Meron M, Tsipris J, Charitt D (2003) Remote mapping of crop water status to assess spatial variability of crop stress. In '4th European conference on precision agriculture'. (Eds J Stafford, A Wemer) pp. 405–410. (Wageningen Academic Publishers: Wageningen, The Netherlands)
- Milthorpe FL, Moorby J (1979) 'An introduction to crop physiology.' 2nd edn. (Cambridge University Press: Cambridge)
- Möller M, Alcanatis V, Cohen Y, Meron M, Tsipris J, Naor A, Ostrovsky V, Sprintsin M, Cohen S (2007) Use of thermal and visible imagery for estimating crop water status of irrigated grapevine. *Journal of Experimental Botany* **58**, 827–838. doi: 10.1093/jxb/erl115
- Nemani RR, Running SW (1989) Estimation of regional surface resistance to evapotranspiration from NDVI and thermal-IR AVHRR data. *Journal of Applied Meteorology* **28**, 276–284. doi: 10.1175/1520-0450(1989)028<0276:EORSRT>2.0.CO;2
- Otterman J, Brakke TW, Fuchs M, Lakshmi V, Cadeddu M (1999) Long-wave emission from a plant/soil surface as a function of the view direction: dependence on the canopy architecture. *International Journal of Remote Sensing* **20**, 2195–2201. doi: 10.1080/014311699212191
- Price AH, Steele KA, Moore BJ, Barraclough PB, Clark LJ (2000) A combined RFLP and AFLP linkage map of upland rice (*Oryza sativa* L.) used to identify QTLs for root penetration ability. *Theoretical and Applied Genetics* **100**, 49–56. doi: 10.1007/s001220050007
- Raskin I, Ladyman JAR (1988) Isolation and characterisation of a barley mutant with abscisic-acid-insensitive stomata. *Planta* **173**, 73–78. doi: 10.1007/BF00394490
- Reynolds MP, Singh RP, Ibrahim A, Ageeb OA, Larqué-Saavedra A, Quick JS (1998) Evaluating physiological traits to compliment empirical selection for wheat in warm environments. *Euphytica* **100**, 85–94. doi: 10.1023/A:1018355906553
- Sepulcre-Cantó G, Zarco-Tejada PJ, Jiménez-Muñoz JC, Sobrino JA, Spriano MA, Fereres E, Vega V, Pastor M (2007) Monitoring yield and fruit quality parameters in open-canopy tree crops under water stress. Implications for ASTER. *Remote Sensing of Environment* **107**, 455–470. doi: 10.1016/j.rse.2006.09.014
- Serraj R, Kumar A, McNally KL, Slamet-Loedin I, Bruskiewich R, Mauleon R, Cairns J, Hijmans R (2009) Improvement of drought resistance in rice. *Advances in Agronomy* **103**, 41–99. doi: 10.1016/S0065-2113(09)03002-8
- Seymour RS (1999) Pattern of respiration by intact inflorescences of the thermogenic arum lily *Philodendron selloum*. *Journal of Experimental Botany* **50**, 845–852. doi: 10.1093/jexbot/50.335.845
- Wisniewski M, Lindow SE, Ashworth EN (1997) Observations of ice nucleation and propagation in plants using infrared video thermography. *Plant Physiology* **113**, 327–334.

Manuscript received 26 May 2009, accepted 15 September 2009