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Opportunities and pitfalls in the use of thermal sensing for monitoring water stress and transpiration

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Abstract:

This paper reviews recent progress in the development of thermal sensing (both in-field and remotely from Unmanned aerial vehicles (UAVs), aircraft or satellites) as a tool for irrigation control, for plant phenotyping, for the study of plant water relations and for estimating stomatal conductance and transpiration from crops. Approaches to the use of thermal sensing in horticulture using both single point sensors (especially when incorporated into wireless sensor networks) and imagers are compared with discussion of the information that can be obtained. Particular challenges limiting the wider uptake of thermal sensing including the treatment of mixed pixels, the selection of appropriate reference surfaces for absolute estimates of evaporation or stomatal conductance and the critical need for improved software for extraction of information from images are discussed.

Keywords: imaging, infra-red thermography, stomatal conductance, transpiration, UAVs

INTRODUCTION

Since the physical basis of the control of plant temperature was clarified in the 1950s (Raschke, 1956) and 1960s (Raschke, 1960; Tanner, 1963) it became clear leaf temperature could be a sensitive indicator of evaporation rate from leaves (Monteith, 1965; Tanner and Pelton, 1960) because the high latent heat of evaporation of water caused evaporative cooling to be a major factor in the leaf energy balance. Although there had been various attempts to make use of this phenomenon over the succeeding years, it was only with the ready availability of relatively cheap infrared thermal sensors that the use of canopy temperature sensing as a routine tool for the detection of water deficit stress in crops (as indicated by a reduction in canopy transpiration) was popularised by Idso and colleagues in the United States (Idso, 1982; Jackson et al., 1981; Jackson et al., 1977).

Leaf or canopy temperature at any time is determined not only by the evaporation rate, but also by rapidly changing environmental factors such as ambient temperature, humidity, incident radiation and windspeed, as well as by stomatal conductance, according to the leaf energy balance which can be formulated (Jones, 2014a; Leinonen et al., 2006) as

$$T_{\mathrm{leaf}} - T_{\mathrm{a}} = (r_{\mathrm{HR}}(r_{\mathrm{aW}} + r_{\mathrm{s}})\gamma R_{\mathrm{ni}} - \rho c_p r_{\mathrm{HR}} D)/(\rho c_p(\gamma(r_{\mathrm{aW}} + r_{\mathrm{s}}) + s r_{\mathrm{HR}})) \qquad (1)$$

where T_{leaf} and T_{a} are the leaf and air temperatures, r_{HR}, r_{aW} and r_{s} are, respectively, the parallel resistance to sensible and radiative heat loss, the boundary layer resistance to water loss and the stomatal resistance (=1/g_s, the stomatal conductance), R_{ni} is the net isothermal radiation absorbed by the leaf, D is the air vapour pressure deficit, ρ is the density of air, c_p is the specific heat capacity of air, γ is the psychrometric constant and s is the slope of the curve relating saturation vapour pressure of water to air temperature. Rearrangement of this equation allows one to estimate stomatal resistance or conductance from canopy temperature if the other variables are known. Another rearrangement of the energy balance (the Penman-Monteith equation; Monteith, 1965) allows one to estimate evaporation from the same environmental variables.

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The most convenient applications of thermal imagery are where all treatments of interest can be on one image, or in a series of overlapping images, so that relative temperatures can easily be obtained (Jones et al., 2009; Prashar et al., 2013). This can often be the case for plant phenotyping by plant breeders who may not require absolute estimates of stomatal conductance or evapotranspiration (ET). Where absolute information is required, however, such as for irrigation scheduling, simple temperature differences are less helpful and methods for normalisation to account for varying environmental conditions become necessary.

A wide range of alternative approaches have therefore been used to normalise the data from thermal sensing studies (Jones and Vaughan, 2010; Maes and Steppe, 2012). The simplest is the temperature difference (ΔT) from air temperature (T_a) (Jackson et al., 1977), but interestingly this is often only loosely related to conductance (García-Tejero et al., 2016) and certainly cannot be used to estimate conductance with any precision (Jones, 2014b). Much better normalisation is possible when comparison is made to appropriate references that mimic dry (non-transpiring) and/or wet canopies.

One approach to the development of such references was that of Idso and colleagues who normalised the observed temperature against what would be expected for a well-watered control crop by defining what was termed a crop water stress index (CWSI) as

$$\mathrm{CWSI} = (T_{\mathrm{crop}} - T_{\mathrm{nws}})/(T_{\mathrm{dry}} - T_{\mathrm{nws}}) \qquad (2)$$

where the canopy temperature (T_{crop}) is expressed relative to what would be expected under similar atmospheric humidity for a well-irrigated crop (a non-water-stressed baseline, T_{nws}), or a non-transpiring crop (T_{dry}). This approach allowed for varying atmospheric humidity by setting up calibration plots for the two extremes (non-transpiring and well watered) to cover the range of water status expected, but it takes no account of variation in the incoming solar radiation (as it was optimised for the midday conditions in a semi-arid climate) though it does allow for varying humidity.

The next major step in normalization of canopy temperature data was the avoidance of a need for having actual reference crops, by using either physical references (Jones, 1994; Jones, 1999a; b; Meron et al., 2003; Möller et al., 2007; Qiu et al., 1996) that mimic these surfaces or by calculation of the theoretical values using meteorological data (Berni et al., 2009; Leinonen and Jones, 2004). A particular advantage of the use of physical reference surfaces over the use of an energy balance calculation from meteorological data is that any errors due to incorrect calibration of the thermal sensor (even quite expensive sensors only have a quoted accuracy of ±2ºC but much better resolution) largely cancel out when using reference surfaces. Leinonen *et al.* (2006) showed that the use of a dry reference alone was almost as good as using both wet and dry references.

TYPES OF SENSOR

Historically temperature sensing in plant physiological studies has been achieved by the use of thermocouples or micro-thermistors embedded in the tissues, but these can affect the measured temperatures and usually require complex wiring and data-loggers that tend to be very inflexible in use and difficult to move so are inconvenient to use in the field. The advent of infrared thermometry allowed the remote measurement of tissue temperatures. The simplest remote thermal sensors were basic long-wave infrared radiometers that measured the radiation emitted by an area of surface (within a narrow field-of-view) in the long-wave infrared region (especially 9-14 µm). Such instruments are available as simple hand-held radiometers or as simple detectors suitable for continuous recording in permanent installations. Earlier thermal imagers tended to be large, expensive and required sensor cooling by Stirling engines or liquid coolant. In the past 20 years prices have come down rapidly with the development of smaller microbolometer sensors that bring thermal imagery within the capacity of many laboratories. Indeed sensors with 240x360 pixels and a thermal resolution of better than 0.1 K (required for useful studies of plant transpiration) can now be purchased for <\$4,000. Imagers are also now light and small enough (e.g. the Tau, FLIR® Systems Inc., www.flir.com, which weighs <70 g) to be mounted on the small unmanned aerial vehicles (UAVs or drones) currently being developed for rapid field surveys. There are even cheap thermal imagers (c.\$250) that can be attached to smartphones and give images with an adequate thermal resolution (0.1K) for studies of plant water relations (FLIR ONE, FLIR® Systems Inc., www.flir.com), though with limited numbers of pixels.

Thermistor and thermocouple temperature sensors (as well as infrared thermometers), can now be engineered to be stand-alone and downloaded via the mobile telephone networks at intervals, connected to passing data loggers by RFID sensors, or enabled as wireless sensor networks. This capacity removes the previous limitation provided by the need for wires throughout the field. This has opened up the opportunities for large numbers of relatively inexpensive thermal point sensors to be mounted in the field for purposes such as field phenotyping for plant water relations (O'Shaughnessy et al., 2011; Rebetzke et al., 2016). Similarly, imagers can now be linked wirelessly through 3G systems to provide remote monitoring of crops as required.

Another type of instrument that makes use of thermal infrared wavelengths is the FTIR spectrometer; rather than direct measurements of temperature, this measures the spectral variation in reflectance (or emissivity) within the medium to long-wave (thermal) infrared region. These spectra can provide information on plant features such as tissue water content, leaf microstructure, leaf biochemistry (including specific O-H and C-O bonds as well as compounds such as cellulose, lignin and oleanolic acids, which can be important components of cuticular waxes). The increased availability of such FTIR spectrometers, including those that can be used in the field, has also opened up possibilities for using the variation in thermal infrared (TIR) spectra (Buitrago et al., 2016; Ribeiro da Luz and Crowley, 2007) as a tool for gaining information about leaf structure and biochemistry.

MODES OF OPERATION

The remainder of this article will concentrate on the application of thermal infrared sensing in studies of plant water relations and water balance. The alternative modes of operation that depend on the specific objectives of any study are outlined below.

Qualitative uses

A potentially very useful application that requires no accurate calibration is to use thermal imagers for simple monitoring of larger areas of crop or hardy nursery stock nurseries to identify failures of the irrigation system, or areas of overwatering or disease. In larger area images of crop, areas where overwatering is occurring or areas of under-watering can readily be detected (Figures 1 and 2).

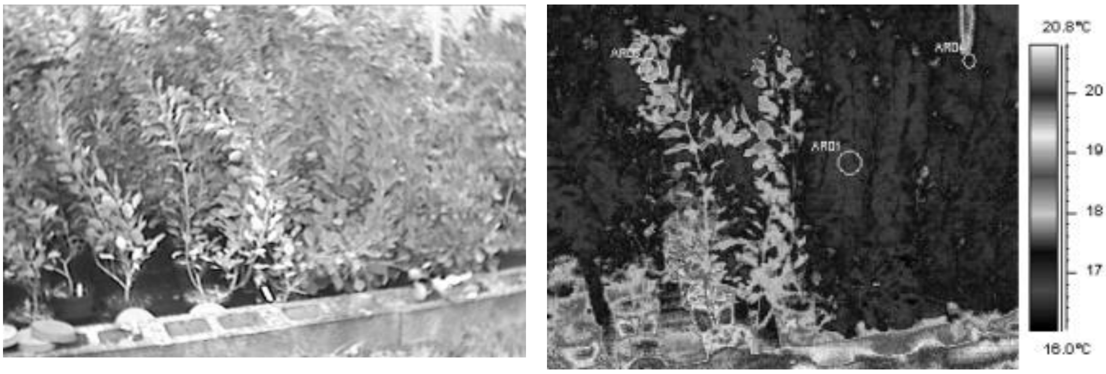

Figure 1. The left-hand panel shows a visible (RGB) image of some potted *Cotinus* plants, while the right-hand panel shows the corresponding thermal image obtained using a hand-held thermal camera (FLIR SC2000). This example illustrates the utility of thermal sensing as

a tool for monitoring for failures in irrigation in a hardy plant nursery with deficient watering identified from higher canopy temperatures.

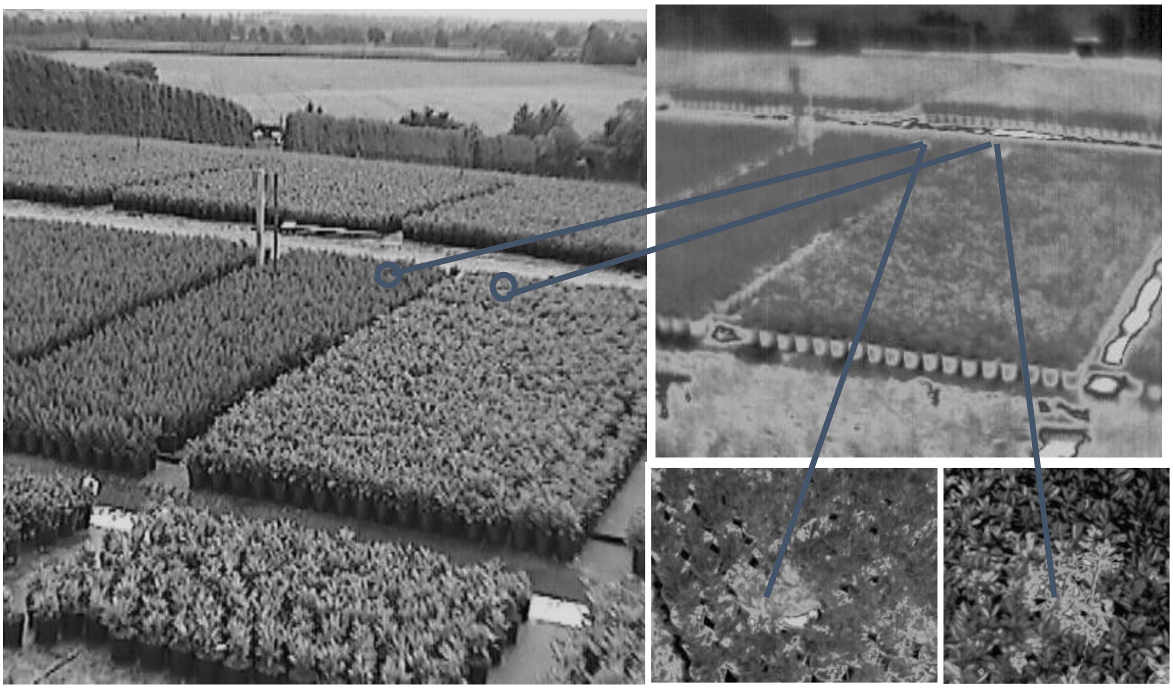

Figure 2. Visible (left-hand panel) and corresponding thermal images (on right) of a larger area of nursery (courtesy of Johnson's of Whixley) showing the monitoring for areas of poor watering using thermal images.

Relative measurements

A slightly more rigorous approach to the use of thermal imagery is to make use of relative measurements or the absolute temperature differences between plants or treatments within an image. Such relative measurements are particularly appropriate for applications such as phenotyping for water stress responses or stomatal conductance where it is common to be able to include many plants of different genotypes in each image (Figure 3). In this case one can eliminate problems caused by the naturally rapid variation of environmental conditions in most field situations and the consequent fluctuating temperatures, as shown in the right panel of Figure 3. Here the expression of the canopy temperature in any plot relative to the average temperature of all plots in the image allows one to rank plots for canopy temperature. A similar approach can also be used where temperatures are measured on only single plots at any time (e.g. when using hand-held infrared thermometers) by expressing any single value relative to a running mean over a series of measurements.

Absolute measurements

The most powerful application of thermal sensing is to make use of absolute temperatures for the calculation of evapotranspiration (*ET*) or stomatal conductance from leaf energy balance (Jones, 2014a). These calculations require a substantial amount of supplementary information on environmental conditions. As indicated in the Introduction this information can either be provided by full micrometeorological data (Ta, windspeed or boundary layer conductance, humidity, net radiation), or else the requirement for many of these variables can be eliminated or reduced by the use of dry and/or wet reference surfaces that mimic canopy properties (Jones, 1994; Jones, 1999b; Qiu et al., 1996).

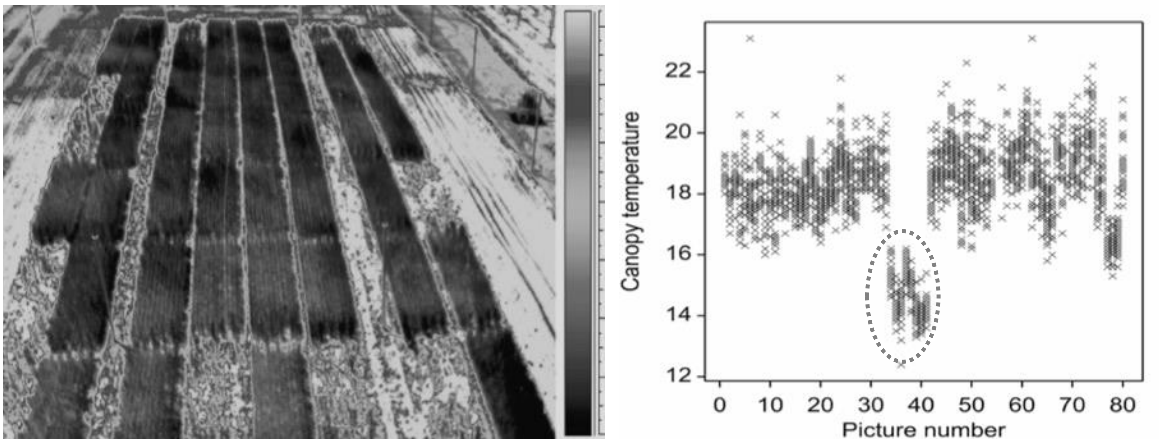

Figure 3. Typical thermal image of a breeder's screening plot for wheat (left-hand panel) and a graph (right-hand panel) showing the individual temperatures of all the plots in each of a series of images, showing the short-term variation in mean image temperature as a cloud passes over (the circled area). If one subtracts the image mean from each plot temperature, this then removes the temporal environmental variation allowing one to rank genotypes for their effect on canopy temperature (Prashar and Jones, 2014).

1. Use of reference surfaces.

In our recent work we have been investigating methods for incorporation of reference surface temperatures for obtaining absolute measures of ET or stomatal conductance. Leinonen et al. (2006) showed that almost as good estimates of conductance can be obtained by using just a dry reference, rather than a combination of a wet and a dry reference, so we have been investigating the use of dry references alone. The challenges involved in developing appropriate reference surfaces are discussed in more detail in the section on Pitfalls and Challenges.

2. Estimation of ET.

Thermal imaging is a powerful tool for estimation of crop transpiration, whether from airborne or satellite platforms or from the ground. There are a range of potential remote sensing energy balance (RSEB) models that can be adopted to estimate crop transpiration (Allen et al., 2007; Bastiaanssen et al., 1998; Bastiaanssen et al., 2005; Jones and Vaughan, 2010; Ortega-Farias et al., 2016) where ET is most usually determined as the residual of the canopy energy balance. Satellite imagery tends to be either too infrequent, or else of too low a spatial resolution to be effective for precision agriculture or phenotyping applications that need to account for the heterogeneity frequently found in spaced tree- or row-crop canopies as is common in horticultural crops. Therefore unmanned aerial vehicles (UAVs) or drones are becoming increasingly valuable for such studies (Berni et al., 2009; Matese et al., 2015). Supplementation of thermal imagery with overlaid multispectral or red-green-blue (RGB) imagery is particularly useful for identifying parts of the image that correspond to vegetation and separating that from the background soil (Jones and Vaughan, 2010).

A critical result that can be readily derived from energy balance theory (Jones, 2014a) and allows canopy temperature (T_c) to be used for estimation of ET is that

$$ET = \rho c_p \,.\, (T_c - T_{dry}).\, g_a/\lambda \qquad (3)$$

where T_{dry} is the temperature of a dry reference surface that mimics the optical and aerodynamic properties of the canopy, g_a is the boundary layer conductance of the canopy, λ is the latent heat of evaporation of water, ρ is the density of air and c_p is the heat capacity of the air. This equation implies that evaporation is linearly related to the temperature of the evaporating surface, assuming that all other conditions remain the same. The appropriate boundary layer conductance (g_a) for canopies or single leaves can be estimated using standard meteorological theory from wind speed (see e.g. Monteith and Unsworth, 2008), or for leaves a comparison of heated and unheated leaf replicas (Brenner and Jarvis, 1995) can be used to estimate g_a from the temperature difference between the two and the amount of power used to heat the heated leaf replica.

The parameter remaining in this equation is the temperature of a similar but non-transpiring canopy (T_{dry}). This temperature can be calculated from energy-balance theory if one knows the net radiation absorbed (R_{n}), the air humidity (e) the air temperature (T_{a}) and g_{a} (Berni et al., 2009; Jones, 2014a; Leinonen and Jones, 2004). Unfortunately, R_{n} especially is generally difficult to estimate accurately, certainly for single leaves and is continuously changing in the field. The idea proposed by Jones (1994; 1999a) is that use of a dry reference surface (whether blotting paper or a non-transpiring leaf) with similar aerodynamic and radiative properties as the leaf being studied will eliminate the need to determine this variable. As outlined in more detail below, it is necessary, however, that the dry reference is fully spectrally and thermally representative of the canopy if errors are not to be introduced.

The accuracy of ET estimates from canopy temperature measurements can be substantially improved by the use of two-source energy balance models where the temperatures of the soil and the vegetation are treated separately, but these require accurate partitioning of the observed mean temperature into the fraction relating to soil and that relating to the green vegetation (Norman et al., 1995). Such models are especially useful for clearly heterogeneous canopies such as orchards (Maes and Steppe, 2012).

3. Estimation of stomatal conductance.

For plant physiologists perhaps the most important application of thermal sensing is its use for estimation of stomatal conductance. The great advantage over any other method is its ability (especially when using imaging) to study large areas of canopy or large numbers of plants and leaves rapidly and non-destructively. A number of approaches have been proposed for estimation of canopy stomatal conductance from temperature measurements (Berni et al., 2009; Blonquist et al., 2009; Leinonen et al., 2006). Rearrangement of equation 1 leads to

$$r_s = 1/g_s = \rho c_p r_{HR}\,(s(T_{\mathrm{leaf}} - T_{\mathrm{a}}) + D)/(\gamma((T_{\mathrm{leaf}} - T_{\mathrm{a}})\rho c_p - R_{\mathrm{ni}})) - r_{\mathrm{aW}} \qquad (4)$$

When one has both wet and dry reference surfaces this simplifies to the following

$$r_s = 1/g_s = (r_{\mathrm{aW}} + (s/\gamma)\; r_{\mathrm{HR}})\;((T_{\mathrm{leaf}} - T_{\mathrm{wet}})/(T_{\mathrm{dry}} - T_{\mathrm{leaf}})) \qquad (5)$$

in which the term $((T_{\mathrm{dry}} - T_{\mathrm{leaf}})/(T_{\mathrm{leaf}} - T_{\mathrm{wet}}))$ has been termed a 'conductance index' (Ig) by Jones (1999a). This can be almost as useful as a true absolute conductance estimate, as it is proportional to stomatal conductance and eliminates any variation due to humidity or net radiation, though it is still affected on boundary layer conductance. Full versions of this equation for leaves with stomata on either one or both sides of the leaf have been provided by Guilioni *et al.* (Guilioni et al., 2008).

SENSOR NETWORKS FOR CONTINUOUS DISTRIBUTED MONITORING

Thermal sensing is ideally suited for installation as a component of a Wireless Sensor Network (WSN) in a field or greenhouse, with sensors distributed throughout the crop and linked to the cloud from a base-station through a wireless network that “daisy-chains” (Figure 4) all the sensors (which may individually have a rather short range) in a robust network that can accept failures of individual components (O'Shaughnessy et al., 2011; Rebetzke et al., 2016). A wide range of wireless sensor networks are available (among the many systems available include LoRaWan from Decentlab, Switzerland – www.decentlab.com; Monnit systems – www.monnit.com; MadgeTech systems – www.madgetech.com; etc.).

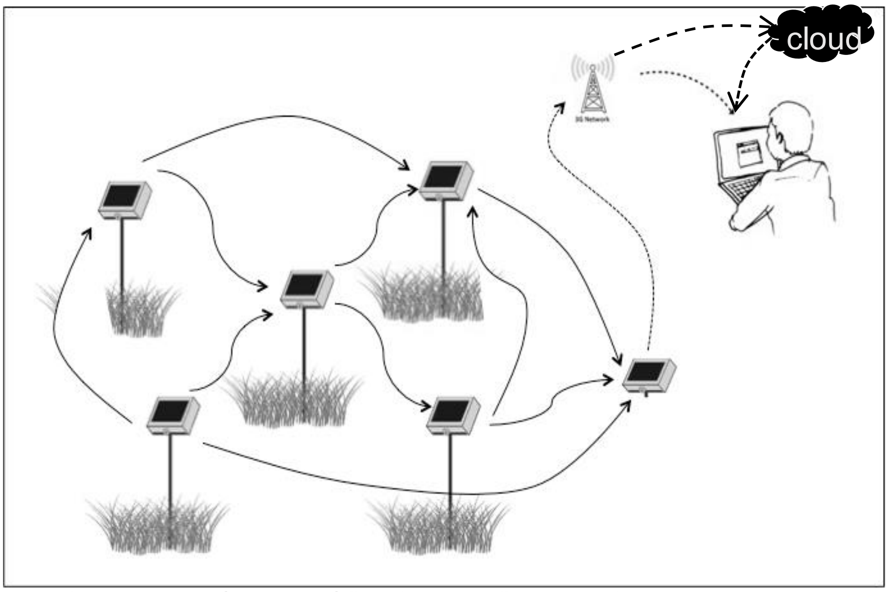

Figure 4. Illustration of a set of "ArduCrop®" thermal sensors in a daisy-chain configuration showing robust alternative pathways of communication to the transmitter, linkage to the "cloud" through a 3G link and the ability to download data direct to computers or smartphones.

The CSIRO High Resolution Plant Phenomics Centre, Black Mountain Laboratories, Canberra, Australia (http://www.plantphenomics.org.au/services/hrppc/) have incorporated WSN capability into their ArduCrop® 3G thermal infrared sensors (see Figure 4). These have the capacity to continuously record canopy temperatures and upload data via daisy-chained sensors to the cloud for nearly real time availability on any internet-enabled computer, tablet or smart-phone (https://www.youtube.com/watch?v=XBhtvnUevLc). The sensors are normally mounted on posts in the canopy, viewing the canopy at an angle of 45° from the nadir, usually angled viewing N (in the Southern hemisphere) so that the solar panel (Facing N) can keep the equipment charged and avoiding any shading of the field of view. A consequence, however, is that the canopy viewed includes a substantial fraction of shaded leaves. The angle of view has substantial effects on the temperature observed, with much higher apparent temperatures observed when viewing a canopy with the sun behind the observer than when viewing against the sun (Duffour et al., 2016; Jones and Vaughan, 2010), though neither of these values strictly estimates the true aerodynamic temperature as required for energy balance calculations of evaporation (Kustas et al., 2003; Norman et al., 1995).

When deployed as a suite of sensors in a field these and similar sensors can follow the temperature of large numbers of plots, as in agricultural or horticultural irrigation experiments, or for phenotyping and variety selection. An example that demonstrates the potential value of networks of thermal sensors for plant phenotyping studies for wheat is illustrated in Figure 5. This figure shows the average diurnal changes in relative temperature for groups of wheat genotypes that fall into different canopy architecture groups, showing that some characters (such as non-rolling leaves or waxless leaves) tend to lead to higher canopy temperatures (Rebetzke et al., 2016).

In this case the normalisation of temperature readings to take account of environmental variation is not necessary, as the experiments are designed solely to identify differences between genotypes and data for all genotypes are obtained simultaneously. Not only do these systems provide continuous trends in temperature throughout the full diurnal cycle, but they also have a great advantage over cases where temperature data are obtained from sequential images from field buggies (Deery et al., 2014) or airborne platforms (Perry et al., 2012; Rebetzke et al., 2016) so that data are not all obtained under identical environmental conditions and therefore require further normalisation.

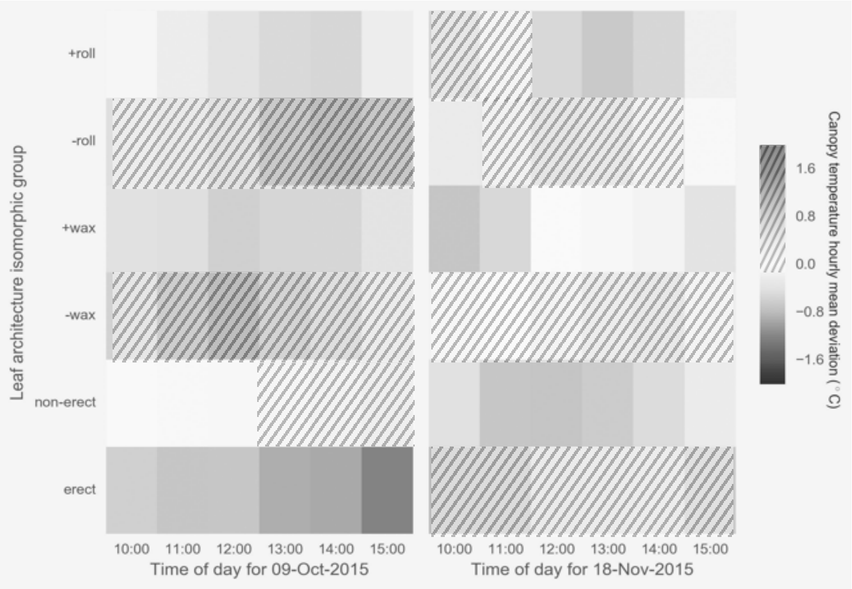

Figure 5. Deviations in mean canopy temperature for leaf architecture isomorphic groups of wheat lines ('roll', 10 rolling vs non-rolling; 'wax', eight waxy vs non-waxy; 'erect', eight with leaves erect vs planophile) throughout the day early (left-hand panel) and late (right-hand panel) in grain-filling. Each hourly value represents averages of ArduCrop® readings across five-minute time intervals for each group of lines (data from Rebetzke et al., 2016).

In the absence of reference surface temperature data, comparisons between sensor outputs are still valid, so the commonest approach is to use the sensors in a relative sense by comparing the temperatures of different plots (as in Figure 5) rather than as estimators of absolute transpiration rates or stomatal conductances. Although sensor outputs could be normalised against air temperature, this is not an ideal basis for normalisation of the thermal readings for absolute calculations of ET or g_s. We are therefore currently investigating the possibilities of incorporating reference surfaces into the sensor networks to allow calculation of ET or g_s.

PITFALLS AND CHALLENGES

Choice of effective references

As discussed in the Introduction, the continuous variation of environment in the field is the main factor that limits the application of thermal sensing. Effective reference surfaces are required for the estimation of ET or stomatal conductance, unless the full energy balance calculation is to be used based on full meteorological data. Where reference surfaces are used it is essential that they truly represent both the aerodynamic and the radiative properties of the leaf or canopy under investigation; ways that this can be achieved are discussed in the following subsections. As an alternative to the use of a physical dry reference surface or a full energy balance calculation, it has been suggested that the dry reference temperature can be estimated as T_a+5ºC (Meron et al., 2003), based on the earlier observations by Irmak et al. (Irmak et al., 2000). This simple assumption, however, is not generally very reliable, especially in more humid climates, as T_{dry} is very dependent on incident radiation, and even in the original paper it is clear that the choice of 5ºC was only approximate. Indeed, others have confirmed that better results can be obtained using real dry references than by using the Ta+ 5 (Reinert et al., 2012). Where wet reference surfaces are used it is essential that the surface can be maintained truly wet; this is difficult in very hot arid climates. Nevertheless a number of self-wetting sensors have been developed for practical application in the field (Maes et al., 2016; Meron et al., 2003; Meron et al., 2010).

It is also important to note that the reference surfaces and leaves should have similar thermal properties (thermal capacity or thermal inertia) so that their time responses to changing radiation are similar, otherwise instantaneous values may not be well synchronised between leaf and reference giving rise to large errors in calculated ET or conductance. Such errors will be greatest where imaging approaches are used, but they will be minimised where continuous measurements are made and the data averaged over periods of at least 5 min.

1. Colour (spectral absorptance).

As pointed out above, it is usual to use a sheet of filter paper, commonly coloured green or grey with a density to mimic the solar absorptance of leaves, as a dry reference, though other materials have been used. It is critical that any physical reference surface has the same solar absorptance as leaves of the canopy of interest, otherwise the dry surface in particular will not achieve the correct temperature. For example a dry black reference surface normal to the solar beam has been shown to be as much as 10ºC warmer than a similar-sized non-transpiring (Vaseline®-covered) grape leaf while a white reference was about 12ºC cooler under the same bright sunlight conditions of South Australia (Jones et al., 2009). Such differences would lead to very large errors in estimation of *ET* or stomatal conductance. Note that the errors are much smaller under cloudy conditions or for wet leaf replicas (with only a range of about 6-7ºC under the same environmental conditions as for the dry leaves), because in this latter case latent heat loss is a much greater fraction of the total leaf energy balance, or under shady or low light conditions.

Because of the need to ensure an appropriate solar absorptance, we have commonly used actual leaves covered with petroleum jelly (to inhibit water loss) as our dry references (Grant et al., 2006; Grant et al., 2007; Jones, 1999a; Jones et al., 2002). Unfortunately petroleum-jelly-covered leaves die quite rapidly in hot environments, and this approach is particularly inconvenient and labour intensive when applied to large areas of canopy as are needed for many remote imaging applications. Therefore it is necessary to optimise the choice of material for true physical reference surfaces. Although it would be ideal for any reference to have the same spectral absorptance as leaves, the errors introduced by using paper (with its rather smaller reflectance in the near infrared) rather than leaves is unlikely to introduce much error and there is no strong reason for using green paper as has often been used. Indeed it is probably more repeatable to use a grey-scale whose density is clearly defined and chosen empirically to mimic the temperature of a non-transpiring leaf; we have therefore been testing grids printed with differing grey densities and using the resulting standards in our field studies.

It seems possible in principle to correct for different illumination of individual leaves as a result of shading by making use of the visible radiance reflected. I am not aware, however, of effective use having yet been made of this opportunity.

2. Angle.

A second problem with developing an appropriate physical reference is getting the reference surface to have the same exposure to the sun as do the canopy leaves, which are usually oriented in a wide range of directions and some of which may be shaded by others. For a canopy, different leaves will have a wide range of irradiances (and hence temperatures) depending on the proportion of shaded leaves in the field of view and on leaf angle relative to the incident solar beam; these will vary depending both on the view angle of the thermal sensor and on the canopy structure (Jones et al., 2009). As a first attempt at generating an appropriate average temperature for the differently illuminated leaves in a canopy we first tested an inverted cone of filter-paper where the temperatures of different sectors were detected using thermocouples (Loveys et al., 2008) and the temperatures averaged (Figure 6) to give a 'radiation weighted' dry reference temperature.

Since a typical plant canopy has approximately randomly oriented leaves which can be represented by arranging the leaves on the surface of a sphere (see Jones, 2014a; Monteith and Unsworth, 2008), it seems reasonable to suggest that one way of obtaining a representative temperature for a dry reference would be to construct it as a hemisphere. Following suggestions by Brian R. Loveys (personal communication) we have now tested a hemispherical dry reference sensor constructed from a half table tennis ball whose temperature is sensed using an infrared thermometer (Figure 6). As expected temperature on different sides of the hemisphere depends on its exposure to the sun.

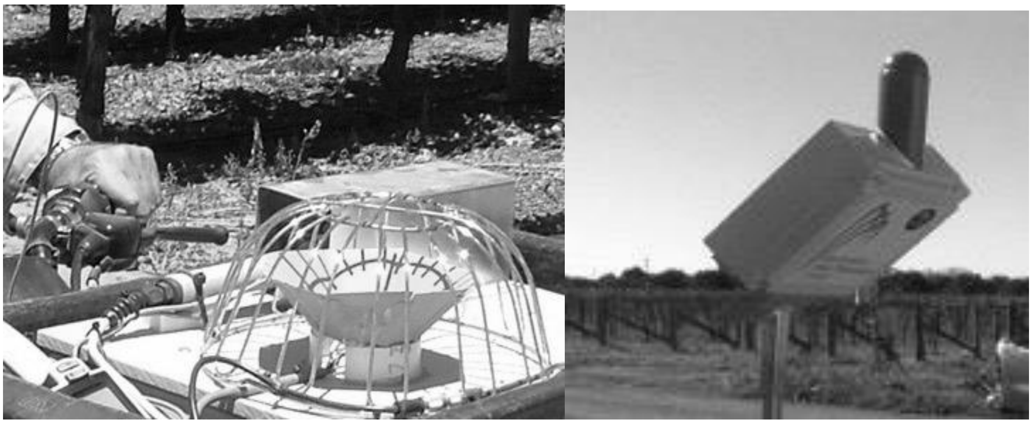

Figure 6. Possible formats for dry reference sensors to mimic radiation distribution in real canopies. The left-hand panel shows the inverted paper cone design of B R Loveys (personal communication), while the right-hand panel shows a hemisphere whose temperature is sensed using an infrared thermometer (P. Hutchinson, personal communication).

3. Scaling and mixed pixels.

Problems of scaling and mixed pixels, caused by the inclusion of background such as soil in thermal images of canopies have been extensively reviewed by Jones and Sirault (Jones and Sirault, 2014a; b). In brief, as the pixel size increases the probability that what is viewed is entirely leaf decreases, with an increasing proportion of pixels contaminated in some way by background material such as soil - which may have much higher temperatures than the leaves. Methods for extracting the true canopy temperature have been reviewed (Jones and Sirault, 2014a; b) and can be separated into methods for identifying pixels that are wholly leaf to methods that allow one to 'unmix' each pixel to obtain the separate temperatures of leaf and background. The most powerful methods to eliminate, or correct for, background contamination are based on combining thermal with visible or even hyper- or multi-spectral imagery to separate leaf from soil by the use of indices such as the normalised difference vegetation index (NDVI) (Leinonen and Jones, 2004; McCabe et al., 2008; Shi, 2011).

4. Some other considerations.

For automated irrigation control it is critical to distinguish drought-related stomatal closure from the responses to other stresses such as water-logging or disease which can also lead to stomatal closure. Unless the algorithms used correctly distinguish these different stresses, it is possible to end up substantially overwatering crops.

It is also necessary to distinguish changes in leaf temperature from any changes that result from differences in the proportion of non-leaf in the image, whether it arises from ground cover or from factors such as the proportion of flowers or fruits (or in cereals flowering/fruiting spikes) or stems/wood in the field of view. If these organs are non-transpiring it is necessary to develop procedures to correct for resulting errors in apparent temperature. Other factors such as pubescence of the leaves or waxiness, leaf rolling or leaf angle distribution all affect the spectral absorptance of the canopy and hence its temperature (Rebetzke et al., 2016).

Automated image extraction, especially separation of sunlit and shaded leaves (Jones et al., 2009; Maes and Steppe, 2012), as well as for separating leaves from background soil (Leinonen and Jones, 2004) will be crucial for wider application of thermal sensing for applications such as irrigation control and for phenotyping (Raza et al., 2014; Wang et al., 2010a).

DISCUSSION AND CONCLUSIONS

In addition to the challenges posed by problems such as choice of reference surface and its orientation or colour, and difficulties relating to mixed pixels and distinguishing water deficit stress from other stressors, there are several other considerations that need to be considered. Firstly, where thermal sensing is to be used for irrigation control it will be critical to determine the appropriate 'thresholds' for initiating irrigation: these will depend both on the crop and on the environment within which it is grown. It will be necessary to include robust algorithms to ensure that irrigation is not triggered by other stresses such as waterlogging or disease that might also close stomata and raise canopy temperature. When canopy temperature is used for phenotyping by breeders it is also necessary to recognise that canopy temperature can vary as a function of crop height (generally being higher for shorter crops with equal stomatal conductance; Giunta et al., 2008; Rebetzke et al., 2013). This latter effect is generally attributed to an aerodynamic effect associated with the typical atmospheric temperature profile. It is also worth remembering that stomatal conductance and hence leaf temperature tends to be more sensitive to changes in water availability in so-called 'isohydric' plants, where stomata act to maintain leaf water status relatively stable, than in more 'anisohydric' plants (Hochberg et al., 2013; Jones, 2014a).

Perhaps the greatest limitation to the wider use of thermal imaging at the present time is the need for better software for automation of image extraction and analysis. Although substantial advances have been made in image handling and the analysis of thermal imagery (Rebetzke et al., 2016; Wang et al., 2010b; Yang et al., 2012) there is still some way to go before systems are adapted to general use in a 'user-friendly' manner.

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