Chapter 17

Assessing Drought Responses Using Thermal Infrared Imaging

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Abstract

Canopy temperature, a surrogate for stomatal conductance, is shown to be a good indicator of plant water status and a potential tool for phenotyping and irrigation scheduling. Measurement of stomatal conductance and leaf temperature has traditionally been done by using porometers or gas exchange analyzers and fine-wire thermocouples attached to the leaves, which are labor intensive and point measurements. The advent of remote or proximal thermal sensing technologies has provided the potential for scaling up to leaves, plants, and canopies. Thermal cameras with a temperature resolution of <0.1 K now allow one to study the temperature variation within and between plants. This chapter discusses some applications of infrared thermography for assessing drought and other abiotic and biotic stress and outlines some of the main factors that need to be considered when applying this to the study of leaf or canopy temperature whether in controlled environments or in the field.

Key words Thermal imaging, Stomatal conductance, Plant stress, Drought, Water use efficiency

1 Introduction

Water deficit is one of the major constraints for agriculture and future climatic predictions suggest an increase in the frequency of extreme weather conditions. Thus, breeding of crops for drought avoidance, escape, and tolerance is likely to be needed for sustainable agriculture. Under water-deficit or stress conditions, root-sourced abscisic acid (ABA) is conveyed through xylem resulting in stomatal closure, which, especially in isohydric plants, often occurs before plant water status declines [1, 2]. Thus, stomatal closure can be used as an indication for response to water stress and in regulating crop irrigation [3]. Most of the traditional ways of measuring stomatal conductance use porometer or infrared gas analyzers which give point measurements and are time consuming and labor intensive. With recent technological advances, infrared thermography (IRT) has become viable as an alternative for the indirect estimation of stomatal conductance, because the temperature of

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leaves, plants, or canopies is an indicator of leaf transpiration rate (and hence of stomatal opening and closing). The fact that stomata tend to close with water-deficit stress means that stomatal closure, indicated by the use of IRT, has become increasingly used as a tool for irrigation scheduling and in phenotyping for drought tolerance [3–7]. IRT has also been used to understand the variation in leaf/canopy temperature measurements in response to other abiotic and biotic stresses [8–10], for energy balance and aerodynamic studies, and for studies of biochemical activity (especially relating to thermogenic respiration) in plants [11, 12].

1.1 Thermal Imaging Theory

Temperature sensing in the thermal infrared is based on the fact that all objects emit thermal radiation (R; W m^{-2}) as a function of surface temperature according to the Stefan–Boltzmann equation:

$$R = \epsilon \sigma T^4 \tag{1}$$

where ε is the emissivity, σ is the Stefan-Boltzmann constant (5.6697×10^{-8} W m^{-2} K^{-4}), and T is the temperature (K). The emissivity is introduced to take account of the fact that not all surfaces are perfect emitters of radiation (i.e., black bodies) and relates the actual radiance for a body to the amount that would be emitted by a perfect emitter, and varies between 0 and 1 (for a perfect emitter or "black body"). In order to allow measurements to be made in sunlight, thermal imagers for use outdoors need to be restricted to the long-wave infrared radiation window (c. 9.5 μm to 14 μm) and to exclude shorter wavelengths. Other cameras (so-called short-wave thermal cameras sensitive to radiation between 3 and 5 μm) are available that are optimized for engineering applications where surface temperatures may be of the order of 500–1000 K: these are unsuitable for vegetation studies as they can detect reflected solar radiation, thus giving incorrect results when used in the daytime outdoors. Long-wave cameras are little affected by solar radiation and are therefore useful in the field.

1.2 Plant Environment Interaction: Leaf Temperature Regulation

Plants interact with environment through interface "stomata," maintaining carbon-water and energy exchange balance, and adapt to ever-changing conditions. Thus stomata play an important role in plant adaptation and growth by balancing the need to minimize water loss while maintaining photosynthetic gains [13]. Evaporative cooling through transpiration is a major component of the leaf energy balance, and the leaf temperature (T_l) at any time is given by Eq. 2:

$$T_l - T_a = [r_{HR}(r_{aW} + r_s)\gamma R_{ni} - \rho c_p r_{HR} D] / [\rho c_p[\gamma(r_{aW} + r_s) + s r_{HR}]] \tag{2}$$

where T_a is the air temperature (K), R_{ni} is the net isothermal radiation absorbed by the leaf (W m^{-2}), D is the atmospheric air humidity deficit (Pa), ρ is the density of air (kg m^{-3}), c_p is the specific heat capacity of air (J kg^{-1} K^{-1}), s is the slope of the curve relating saturating water vapor pressure to temperature (Pa K^{-1}), γ is the psychrometric constant (Pa K^{-1}), r_{HR} is the parallel resistance to heat and radiation transfer, r_{aW} is the boundary layer resistance to water vapor transfer, and r_s is the stomatal resistance to water vapor transfer (refer to Ref. 13 for details).

The use of IRT for remotely sensing stomatal closure and transpiration offers a great potential for irrigation scheduling and as a high-throughput phenotyping tool. Indeed IRT can be used as an effective tool not only in evaluating crop water status but also for other abiotic and biotic stresses in several agricultural crops.

In this chapter we highlight different steps involved in experimental setup, image acquisition, processing using different normalization techniques, and data analysis involved typically in a field trial, with examples on controlled conditions also addressed.

2 Materials

2.1 Equipment

Thermal cameras that are sensitive to radiation in the 8–14 μm band and having a thermal resolution of 100 mK or better are available from a range of companies. This high temperature sensitivity is essential for most plant stress-sensing applications (*see* **Note 1**) with their absolute accuracy being less important.

2.2 Plant Trials

In general a replicated case–control designed trial consisting of a control and corresponding water stress treatments either in glasshouse conditions (controlled) or field conditions (natural environment) is required. This can also include different genotypes if genotypic variability needs to be assessed for case–control studies and future breeding.

2.3 Reference Surfaces

Reference markers in the form of marked banners or labels are placed in the field as position indicators; these can also be used as artificial wet or dry references [4, 14]. Environment data is also often needed for data normalization. In addition to environment data, different referencing methods can be used (*see* **Note 2** and Ref. 14 for details).

2.4 Software

Thermal cameras come with their own proprietary software for extracting temperatures from images; in adddition statistical or other image analysis software for image processing (e.g., Excel, Genstat, R) are often required.

3 Methods

3.1 Thermal Imaging

A flow diagram indicating the various steps involved in thermal imaging is shown in Fig. 1. Software built into the camera transforms the detected radiation into temperature, taking account as

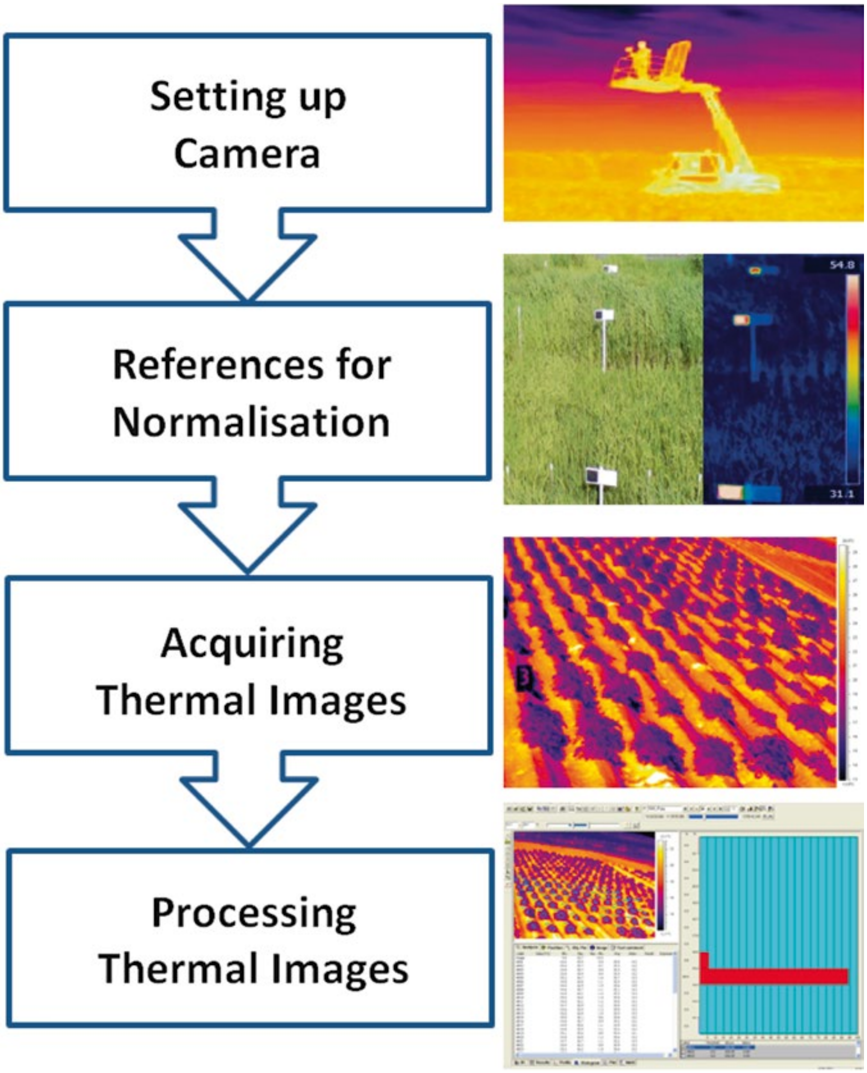

Fig. 1 Steps involved in using the imaging as a tool for phenotyping or crop monitoring

necessary of atmospheric humidity, object-to-camera distance, and surface emissivity. These temperatures are then commonly displayed as false color images or can be analyzed using raw digital numbers.

3.1.1 Setting Up Thermal Camera

1. Imaging can be done using either handheld or mounted (*see* **Note 3**) cameras at an appropriate distance depending on the spatial resolution required and the specific research question being addressed. It is useful to distinguish the use on single leaves or small plants like *Arabidopsis* from use on whole canopies in the field. Experiments in controlled conditions have allowed identification of individual *Arabidopsis thaliana*

mutants with altered stomatal responses [15], while field studies of breeding lines have also identified genetic differences [4, 5, 16].

2. Set the camera parameters. For acquisition of a default value of emissivity (ε), enter 0.965 into camera settings (*see* **Note 4**). Similarly, the distance of the camera to the subject/target object, air temperature, and humidity need to be measured and entered into the camera settings.
3. The camera parameters such as reflected, atmospheric, and optics temperatures along with the environment conditions (*see* **Note 5** and Subheading 3.3) affect the calculation of the target temperature. The radiation received, and hence the temperature recorded, by the thermal camera depends on (a) the temperature of the object being viewed, (b) the emissivity (ε) of the object's surface, (c) the incoming thermal radiation from the environment (background radiation) reflected by the object (*see* **Note 6**), and (d) any absorption or emission of thermal radiation by the atmosphere between the object and the camera. Most thermal cameras come with software that allows for correction of (c) and (d) given a knowledge of ε and the atmospheric humidity. Luckily, for most close-range applications in plant science the error caused by (d) is small and can usually be neglected.

3.1.2 Acquisition of Images

1. Thermal images should be acquired at times when both stomatal conductance and transpiration tend to be relatively constant and depend upon whether the experiment is under controlled or field conditions.
2. Thermal images generally include both foreground (target of interest) and background regions. It is usually necessary to separate these and only perform further analysis on the pixels representing leaves (*see* **step 2** of Subheading 3.2.2 for details).
3. When imaging in the field, images taken at an oblique angle to the horizontal (as compared with the nadir view) help in maximizing the canopy area in any image, but this can introduce complexities in analysis relating to perspective and varying camera-object distances (*see* **Note 7**).
4. Use of reference surfaces: The identification of a particular canopy or leaf in a group of canopies is easier if a set of markers which make the image processing easier when extracting canopy temperature from a group of similar canopies/areas is used. These markers can be reference surfaces (see Subheading 3.3) or labels to identify or distinguish different plants or pots. It can be useful to ensure overlap of neighboring images to allow better normalization to account for changing environmental conditions and provide increased replication with reduced standard error as discussed in normalization section [5].

3.2 Analysis

3.2.1 Image Processing

The next step after image acquisition is the image analysis. A wide range of softwares are available for image handling and analysis, many of which are specific to the camera used, but the greatest flexibility is available if the original images can be exported into nonproprietary formats for analysis in programs such as Excel (Microsoft Office, 2010), Matlab (The Mathworks, Inc., UK), or Fiji [17]. Nevertheless much useful work, such as identification of object outlines and extraction of object temperature, can usually be achieved in the camera-specific software (e.g., ThermaCAM Researcher (FLIR systems), and Fluke SmartView (Fluke Corporation)).

3.2.2 Separating Leaf and Canopy Temperature from Background

An advantage of using thermal imagers as compared with simple infrared sensors is that the images allow the user to distinguish leaves from background soil. Many thermal cameras come with a built-in visible camera and most of the thermal cameras available in the market come with a data fusion option, where thermal and visible images are available on the camera's LCD for identification and distinguishing foreground and background components while screening (Fig. 2). In subsequent image processing, there are a couple of ways in which the information on leaf temperature may be isolated from a complex thermal image including soil and other objects (*see* **Note 8**).

1. *Overlay with visible image*: The simplest approach is to overlay the thermal image with a visible image of the same scene. A number of methods are available for this that include (1) automated image recognition and alignment algorithms (e.g., [18]); (2) freeware implementations of alignment programs such as the Fiji image analysis platform, which uses ImageJ's interface and plug-ins relevant to biological research [17]; and (3) utilization of the high reflectance of leaves in the near IR in the form of some vegetation index approach (e.g., Ref. 19).
2. *Use of temperature histograms*: An alternative is to make use of the expectation that leaves will generally be substantially cooler than the background soil, and use histogram thresholding to determine which pixels to use (e.g., Ref. 20), possibly with an automated histogram separation method such as the Otsu method [21]. A similar approach has also been used for manual extraction of canopy/leaf temperature eliminating background noise due to soil [5]. In a related approach, Giuliani and Flore [22] used a high temperature background screen to facilitate the use of thresholding to extract canopy temperature.

3.3 Normalization

The surface temperature of plant canopy or leaf depends on the biological factors but is also influenced by the environmental factors including irradiance and wind speed, which are continually changing (*see* **Note 5**). Therefore, it is necessary to isolate treatment dif-

Fig. 2 Example of data fusion from FLIR camera E50 with Picture in Picture option available on the camera's LCD for identification and distinguishing foreground and background component elimination while screening/phenotyping

ferences in temperature from this background variation using a normalization technique. Different normalization techniques can be used as mentioned below, depending on the experimental design and the conditions (whether controlled or field trials).

1. An early normalization was the derivation of the Crop Water Stress Index (CWSI) [23, 24] as shown in Eq. 3:

$$CWSI = (T_{\text{canopy}} - T_{\text{nwsb}}) / (T_{\max} - T_{\text{nwsb}}) \tag{3}$$

   where T_{canopy} is the canopy temperature, T_{nwsb} is the temperature of a non-water stressed reference crop under similar conditions, and $T_{\max}$ is an upper temperature for a non-transpiring crop.

2. However, the use of CWSI as a normalization is limited by the fact that T_{nwsb} is site dependent and does not account for variation in net radiation and aerodynamic resistance [25]. Also, it is sometimes hard to have access to a non-transpiring crop reference. Therefore, it has been suggested that an analogous stress index (SI_{cwsi}) could be defined where T_{wet} and T_{dry} are the temperatures of wet and dry (non-transpiring) physical reference, respectively:

$$SI_{\text{cwsi}} = (T_{\text{canopy}} - T_{\text{wet}}) / (T_{\text{dry}} - T_{\text{wet}}) \tag{4}$$

Alternatively, an index (I_G) that is proportional to stomatal conductance could be defined [3]:

$$I_G = \frac{T_{\text{dry}} - T_{\text{canopy}}}{T_{\text{canopy}} - T_{\text{wet}}} = g_{1W}(r_{aW} + (s/\Upsilon)r_{HR}) \tag{5}$$

where r_{aw} is the boundary layer resistance to water vapor, s is the slope of the curve relating saturation vapor pressure to temperature, Υ is psychrometric constant, and r_{HR} is parallel resistance to heat and radiative transfer as defined by Jones and colleagues [3, 20, 25]. An advantage of this index over the *CWSI* or SI_{CWSI} is that I_G is nearly linearly related to stomatal conductance.

3. The physical reference surfaces used must have similar radiative properties to the plant leaves of interest, and ideally should also have similar aerodynamic properties [26, 27]. The appropriate choice of reference surface depends on the scale of observation but studies suggest that real leaves, either sprayed with water or covered in petroleum jelly to stop transpiration, provided the best references because of similar radiometric and aerodynamic properties for single leaf or small plot studies (reviewed in Ref. 14). This can be extended to large areas of well-irrigated reference crop for satellite-scale observations (e.g., Refs. 11, 13, 20, 26).
4. The actual canopy temperature can also lag behind the current equilibrium canopy temperature due to thermal lag in the system. When screening large numbers of genotypes under field conditions, a normalization technique based on using the temperature difference from the image mean has been shown to give highly reproducible results [4, 5]. Figure 3 gives an overview of the process using overlapping images and the calculation of genotype temperature. This overlap strategy and the associated normalization technique have been shown to provide enough power to identify quantitative trait loci (QTLs) [5].

4 Notes

1. It is worth noting that the absolute accuracy of most readily available cameras is only ±1 or ±2 °C, though in most applications the accuracy is not a major limitation as one is concerned with the measurement of temperature differences. This depends on the questions being answered through the experiment. If one is interested in difference between temperature for different genotypes and breeding scenarios, absolute value is not critical. But in case the experiment is designed to understand the energy balance and the morphophysiological relations, absolute temperature may be required as temperatures need to be related to air temperature (not measured by the camera).
2. Different reference surfaces or methods used previously include wet and dry leaf canopies (WDLC), paper references, comparison with air temperature, and calculations made by using

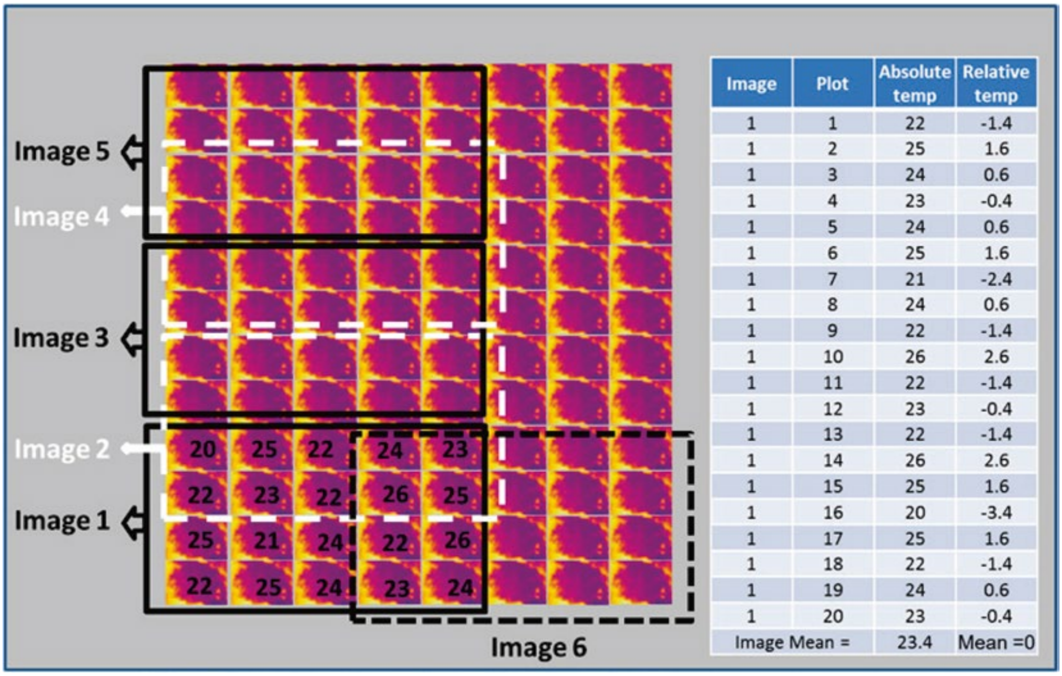

Image	Plot	Absolute temp	Relative temp
1	1	22	-1.4
1	2	25	1.6
1	3	24	0.6
1	4	23	-0.4
1	5	24	0.6
1	6	25	1.6
1	7	21	-2.4
1	8	24	0.6
1	9	22	-1.4
1	10	26	2.6
1	11	22	-1.4
1	12	23	-0.4
1	13	22	-1.4
1	14	26	2.6
1	15	25	1.6
1	16	20	-3.4
1	17	25	1.6
1	18	22	-1.4
1	19	24	0.6
1	20	23	-0.4
Image Mean =		23.4	Mean =0

Fig. 3 Field experimental design showing the image overlap sequence for repeated measurements and table showing an example of normalization method used by Jones and colleagues [4, 5]. The plot number in the table corresponds to the row and column in the image (e.g., row 1, column 1 = plot 1, row 2, column 2 = plot 2)

meteorological data in the form of "wet and dry temperatures." Each of these has its own advantages and disadvantages; for example comparison with air temperature does not take account of other environmental variables, while wet reference surfaces are difficult to maintain under hot conditions.

3. In case the camera is mounted at a height on a tripod where the focus function on camera is inaccessible, firewire cable or USB can be used to connect the camera to computer. The Thermacam Researcher Professional software (FLIR systems) or FLIR IR camera player (FLIR systems) on the computer can be used to manage the live imaging and its acquisition.

4. Choice of value for emissivity (ε): For single leaves, typical emissivities are between 0.93 and 0.98 [28]; in the absence of further precise information a value of 0.965 is recommended. Note that emissivities appropriate to soils tend to be only slightly lower (say 0.94–0.95) though sands may average as low as 0.89. When one views a canopy of leaves from a distance, however, the effective emissivity is higher than that of the component leaves, averaging approximately 0.99 [28].

5. Rate of canopy/leaf transpiration depends on the difference in air-to-leaf vapor pressure. Therefore under high wind, humid, and low irradiance conditions, the temperature difference

between a leaf with open stomata and one with closed stomata is much less as compared to low-wind, low-humidity, and high-irradiance conditions.

6. Correction for the background radiation: It is difficult to estimate the background (incoming to the object) thermal radiation accurately, but one approach to its estimation is to measure the apparent temperature of some crumpled aluminum foil placed in the position of the object (using an emissivity of 1)—the measured temperature is the effective background temperature (T_b) for use in the camera's software (for a leaf within a canopy T_b should be close to canopy temperature, while at the surface of a canopy T_b may be closer to the sky temperature which could be as low as 40 °C for clear sky).
7. In addition to environment, the effect of solar angle and angle of view should be taken into account. Image timing during the day is an important consideration in screening for canopy temperature as direction and angle between the sun and imager causes variation in apparent reflectance with overall reflectance highest when sun is behind the imager and lowest when opposite to imager. Thus imaging from two different angles will provide different results.
8. Field of view (FOV) is critical for image analysis and when collecting temperature data from canopy and elimination of background. Higher resolution or having more number of pixels in the image allows better temperature prediction and background elimination of the concerned object, instead of using low resolution or small number of pixels for temperature prediction making it harder to eliminate the background noise.

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