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REVIEW PAPER

Thermography to explore plant–environment interactions

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Abstract

Stomatal regulation is a key determinant of plant photosynthesis and water relations, influencing plant survival, adaptation, and growth. Stomata sense the surrounding environment and respond rapidly to abiotic and biotic stresses. Stomatal conductance to water vapour (g_s) and/or transpiration (E) are therefore valuable physiological parameters to be monitored in plant and agricultural sciences. However, leaf gas exchange measurements involve contact with leaves and often interfere with leaf functioning. Besides, they are time consuming and are limited by the sampling characteristics (e.g. sample size and/or the high number of samples required). Remote and rapid means to assess g_s or E are thus particularly valuable for physiologists, agronomists, and ecologists. Transpiration influences the leaf energy balance and, consequently, leaf temperature (T_{leaf}). As a result, thermal imaging makes it possible to estimate or quantify g_s and E. Thermal imaging has been successfully used in a wide range of conditions and with diverse plant species. The technique can be applied at different scales (e.g. from single seedlings/leaves through whole trees or field crops to regions), providing great potential to study plant–environment interactions and specific phenomena such as abnormal stomatal closure, genotypic variation in stress tolerance, and the impact of different management strategies on crop water status. Nevertheless, environmental variability (e.g. in light intensity, temperature, relative humidity, wind speed) affects the accuracy of thermal imaging measurements. This review presents and discusses the advantages of thermal imaging applications to plant science, agriculture, and ecology, as well as its limitations and possible approaches to minimize them, by highlighting examples from previous and ongoing research.

Key words: Crop stress, genetic improvement, remote sensing, screening and phenotyping, stomata, thermal infrared.

Introduction

Plant–environment interactions and stomata

The physical environment is often adverse to plant growth and survival as well as to crop yield and quality. Factors such as insufficient water or nutrients, high or low temperature, salinity, diseases, and insect damage are likely to restrict plant growth at some stage. The predicted global climate change will increase the incidence of extreme climate events (drought spells and heat waves) and related stress, leading to changes in plant biodiversity and reduced crop yields (Fedoroff *et al.*, 2010). Increased sensitivity of crops to pests and diseases and the spread of novel pests and diseases are also likely to occur (Gregory *et al.*, 2009).

Plants interact with the surrounding environment namely through carbon, water- and energy-exchange processes, maintaining an equilibrium that permits them to grow and adapt to variable growing conditions. Stomatal regulation of leaf gas exchange (CO_2 and H_2O fluxes) in response to the

environment plays a key role in this adaptation, allowing a compromise between photosynthetic gains and water loss as well as allowing regulation of canopy temperature (T_{canopy}) (Jones, 1992; Chaves *et al*., 2003).

Heat loss by plants occurs mainly via evaporative cooling, resulting from leaf transpiration (E) (Jones, 1992). Reduced transpiration under water deficits and high irradiance raises the risk of leaf temperature (T_{leaf}) increasing above the optimum for metabolic activity or above the threshold that leads to irreversible leaf tissue oxidative stress. The control of stomatal aperture results from coordinated alterations of guard cell turgor dependent on ionic fluxes, cytoskeleton changes, membrane transport, and gene expression. This regulation involves the concurrence of different signals in a complex and coordinated network, resulting in a tight and fast modulation of stomatal aperture in response to a fluctuating environment (Hetherington and Woodward, 2003).

If we consider the linear relationship between stomatal conductance to water vapour (g_s) and E under a constant air vapour pressure deficit (VPD), and the non-linear relationship between g_s and photosynthetic rate, decreased stomatal aperture under the first stages of stress development may improve intrinsic water use efficiency (WUE_i) with a positive impact on plant growth and adaptation to the environment. However, when stress becomes severe, a strong decline in carbon assimilation leads to decreased WUE_i, with photosynthesis being restricted not only by stomatal closure but also by biochemical and photochemical limitations (Chaves *et al*., 2003).

Regulation of water loss by an efficient control of the opening/closing of stomata also minimizes the risks of xylem embolism by reducing xylem cavitation, and by regulating water fluxes in the plant, indirectly influences water and nutrient uptake. Stomata also influence plant response to biotic stresses. In general, plants respond to surface-inoculated pathogens by reducing stomatal aperture as part of the innate immune response to restrict bacterial invasion (Melotto *et al*., 2008).

Improved understanding of stomatal regulation of leaf gas exchange is needed to better predict and model key factors influencing crop growth and yield as well as ecosystem sustainability under increasing environmental stress. However, leaf gas exchange measurements involve contact with leaves and often interfere with leaf functioning. Besides, they are time consuming and can be limited by the reduced dimension of the leaf samples and/or the large number of measurements to be done. Therefore, faster, remote and non-invasive high-throughput analysis based on imaging are mandatory.

Remote sensing and functional imaging for plant science

Remote sensing of vegetation is a non-invasive methodology to monitor physical and physiological characteristics of plants and to evaluate the effects of environmental stresses on plant performance (Jones and Vaughan, 2010). Functional imaging permits observations at different scales (from single leaves/seedlings to entire branches/plants or trees or fields) and the assessment of dynamic and spatial variability of processes. The basis of nearly all remote sensing is electromagnetic radiation. Remote sensing involves the measurement of the amount of reflected and emitted radiation at different spectral wavelengths (e.g. ultraviolet, visible, infrared (IR), and microwave). It includes several imaging techniques such as visible imaging, near-IR and thermal IR imaging, chlorophyll a fluorescence imaging, and multispectral imaging and luminescence imaging (Chaerle and Van der Straeten, 2001; Baker and Rosenqvist, 2004; Havaux *et al*., 2006; Lee *et al*., 2010; Jiménez-Bello *et al*., 2011; Zarco-Tejada *et al*., 2012). Among those, thermal imaging (thermography) is one of the most used in agronomic and environmental sciences and also in the agri-food industry (Jones and Vaughan, 2010; Maes and Steppe, 2012).

Remote sensing is the basis of precision agri-horticulture, which aims to use more efficiently the inputs (e.g. water, biocides, fertilizers), optimize yield, and minimize environmental impact (Lee *et al*., 2010). This is in line with the need to increase food production under increasingly unfavourable climate, scarcer natural resources (water, arable land) (Wilkinson and Hartung, 2009; Fedoroff *et al*., 2010), stricter environmental legislation, and increased consumer demands (Lubin and Esty, 2010).

Remote sensing is also applied in phenomics, an innovative approach towards high-throughput plant phenotyping (Furbank and Tester, 2011), to aid breeding of more productive and stress-resistant cultivars.

IR thermal imaging and stomatal conductance

IR radiation and imaging

Heat transfer by radiation occurs in the IR region of the electromagnetic spectrum, between 0.75 and 1000 μm (Kaplan, 2007). According to Planck's radiation law, every object at a temperature above absolute zero (0 Kelvin) emits electromagnetic radiation in the IR region of the spectrum. The amount of IR radiation emitted by an object depends on its emissivity (ε) and absolute temperature, in accordance with the Stefan–Boltzmann law (Equation 1):

$$W = \varepsilon \, \sigma \, T_s^4 \qquad (1)$$

where W is spectral radiant excitance (total radiation emitted) (W m^{-2}), ε is emissivity (dimensionless), σ is the Stefan–Boltzmann constant (5.67×10^{-8} W m^{-2} K^{-4}), and T_s is the surface temperature (K).

The emissivity, at a particular wavelength, represents the amount of radiation emitted by a theoretical object as a fraction of the radiation emitted by a perfect emitter, named blackbody, with ε = 1. 'Real-world' objects (grey bodies) absorb a certain fraction of the incident radiation and reflect and transmit the remaining part, resulting in ε < 1. Plant material has high ε, varying between 0.91 and 0.97. Soils have slightly lower ε (0.94–0.95) and sand can have ε as low as 0.89 (Jones and Vaughan, 2010).

When determining the temperature of an object with thermal imaging, we must consider that the total radiation (W) detected by camera's sensor is the sum of three major IR radiation streams: (1) the radiation leaving the object's surface; (2) the radiation emitted by the object's surroundings and further reflected by the object's surface, commonly named background radiation ($W_{background}$) (both stream 1 and 2 are modified by transmission through the atmosphere); and (3) any radiation emitted by the atmosphere (W_{atm}) (Equation 2). Therefore, the total radiation is given by:

$$W = \tau\,[\varepsilon\sigma\,(T_s)^4 + (1-\varepsilon)\,W_{background})] + W_{atm} \quad (2)$$

where τ is atmospheric transmissivity (dimensionless), $W_{background}$ is background radiation (W m^{-2}), and W_{atm} is the radiance emitted by the atmosphere (W m^{-2}).

The contribution of the atmosphere is relevant for airborne and satellite measurements (Jones and Vaughan, 2010) but it can be neglected for ground-based measurements and laboratory experiments and when IR radiation is sensed at appropriate wavelengths (e.g. 3–5 and 7–14 μm), in which the atmospheric transmission for IR radiation is close to a maximum (Kaplan, 2007). In this case the total radiation leaving the surface of the object is calculated as follows:

$$W = \varepsilon\sigma\,(T_s)^4 + (1-\varepsilon)\,W_{background} \quad (3)$$

To discard the effect of the $W_{background}$, it can be estimated by measuring the temperature of a crumpled sheet of aluminium foil (high reflectivity and $\varepsilon = 0.03$), placed in the same location as the target object and setting the camera with $\varepsilon = 1$ (Jones *et al*., 2002). Most thermal cameras will automatically provide T_s, once the ε and $W_{background}$ have been input.

Relationship between g_s and T_{leaf}

As thoroughly reviewed by Maes and Steppe (2012), leaf and plant temperature depends on the radiation and atmospheric conditions (time of day, clear or cloudy sky, air temperature, wind speed), soil conditions (soil type, soil water content, etc.), and canopy properties (morphology, density, height) that all together influence the size and ratios of the radiant, sensible and latent heat fluxes. The relationship between g_s, the inverse of stomatal resistance (r_s, leaf resistance to water vapour loss, assumed to be dominated by the stomatal resistance component), and T_{leaf} is summarized by the leaf energy balance equation (Jones, 1992, 1999) given by:

$$T_{leaf} - T_{air} = [r_{HR}\,(r_{aw} + r_s)\,\gamma R_{ni} - \rho\,c_p\,r_{HR}\,VPD] \div [\rho\,c_p\,\{\gamma\,(r_{aw} + r_s) + s r_{HR}\}] \quad (4)$$

where T_{leaf} is leaf temperature (K), T_{air} is air temperature (K), r_{HR} is parallel resistance to heat and radiative transfer (s m^{-1}), r_{aw} is boundary layer resistance to water vapour (s m^{-1}), γ is the psychrometric constant (Pa K^{-1}), R_{ni} is net isothermal radiation (the net radiation for a leaf at air temperature) (W m^{-2}), ρ is density of the air (kg m^{-3}), c_p is specific heat capacity of air (J kg^{-1} K^{-1}), s is the slope of curve relating saturating water vapour pressure to temperature (Pa K^{-1}), and VPD is air vapour pressure deficit (Pa).

Fluctuating environmental conditions and thermal indices

It is clear from Equation 4 that T_{leaf} depends not only on g_s, but also on T_{air}, R_{ni}, VPD, and wind speed (Jones, 1999). If comparing, for example, the impact of different management practices on crop physiology while the weather is stable, T_{leaf} alone can provide the required information about relative stress in the different treatments. If the aim is to determine the development of stress in a crop over time, on the other hand, it is necessary to normalize T_{leaf} in relation to references to account for changing meteorology (Jones *et al*., 2009). A wide range of different types of stress index have been used to address this issue. Here an overview is provided to allow the reader to determine what technique might suit a particular experiment. For more detailed assessment of the pros and cons of each, including analysis of the impact of such factors as leaf size and albedo, T_{air} and VPD, and R_{ni}, see Maes and Steppe (2012).

That T_{leaf} increases as a result of stress was first exploited by Jackson *et al*. (1977), who developed the stress degree day to detect stress by using thermometers in field conditions. The stress degree day is the accumulated difference in temperature between the leaf (or crop canopy) and the air along a certain period. According to this index, if T_{canopy} is lower than T_{air}, then plants are assumed to be well watered. If T_{canopy} is greater than the T_{air}, then plants are assumed to be drought stressed. While this index represents an improvement over the use of T_{canopy} alone, since it allows for fluctuating T_{air}, it does not take into account changes in VPD, solar radiation, or wind speed.

Based on energy balance considerations, Jackson *et al*. (1981) appreciated that the canopy to air temperature difference ($T_{canopy} - T_{air}$) depends on VPD: under non-limiting soil water conditions, a crop transpires at the potential rate (i.e. evapotranspiration is the maximum it can be, but maximum evapotranspiration increases with increasing VPD). Thus for several crops, when water availability is not limiting and when measured under clear sky conditions, there is a linear relationship between $T_{canopy} - T_{air}$ and VPD. Jackson *et al*. (1981) called this linear relationship the theoretical non-water-stressed baseline. For a given crop, at a given VPD, this theoretical baseline provides the minimum possible value of $T_{canopy} - T_{air}$. The $T_{canopy} - T_{air}$ for a non-transpiring crop is insensitive to VPD, and can be estimated if wind speed and net solar radiation are known. This sets the 'upper limit' to $T_{canopy} - T_{air}$. Jackson *et al*. (1981) used the idea of 'upper and lower' baselines, to create a crop water stress index (CWSI):

$$CWSI = \frac{(T_{canopy} - T_{air}) - (T_{canopy} - T_{air})_{nwsb}}{(T_{canopy} - T_{air})_{ul} - (T_{canopy} - T_{air})_{nwsb}} \quad (5)$$

where $T_{canopy} - T_{air}$ is the measured difference in temperature, $(T_{canopy} - T_{air})_{nwsb}$ is the estimated difference at the same

VPD under non-limiting soil water conditions (non-water-stressed baseline), and $(T_{canopy} - T_{air})_{ul}$ is the non-transpiring upper limit. This CWSI allows to relate crop's temperature to the maximum and minimum values possible under similar environmental conditions. The higher the CWSI, the greater the crop stress is assumed to be. Yuan *et al.* (2004) and Testi *et al.* (2008) for example, found CWSI to be inversely correlated with leaf water potential.

A disadvantage of the above form of CWSI is the need to determine the non-water-stressed baseline by plotting $T_{canopy} - T_{air}$ against VPD. This requires substantial time to be spent determining the baseline for a well-watered crop, and the VPD needs to be known when measuring T_{canopy} of the crop of interest. Also, this index does not account for changes in T_{canopy} due to irradiance and wind speed, and the non-water-stressed baseline is not necessarily the same under different radiation conditions. Finally, the non-transpiring upper limit also varies, with a wide range of values being reported (Ben-Gal *et al.*, 2009).

Use of artificial references

An alternative approach is to replace the non-water-stressed baseline and the non-transpiring upper limit, respectively, with the T_{leaf} or T_{canopy} from which there is maximum transpiration and the T_{leaf} and T_{canopy} from which there is no transpiration, measured in the same environment and at the same time as the crop of interest. The fact that these 'references' are in the same environment as T_{canopy} means that there is no need for theoretical estimations of baselines, as they will be exposed to the same VPD, R_{ni}, and wind speed as the canopy of interest. The temperatures of the references are referred to as T_{wet} and T_{dry}, respectively. Jones (1999) adapted the crop water stress index to include these reference temperatures, giving the following form:

$$CWSI = \frac{(T_{canopy} - T_{wet})}{(T_{dry} - T_{wet})} \quad (6)$$

This version of CWSI (referred to as $CWSI_d$ in the review by Maes and Steppe, 2012) has been shown to inversely correlate with leaf water potential (e.g. Cohen *et al.*, 2005; Grant *et al.*, 2007). To ensure that there are suitable references in each thermal image, leaves can be sprayed with water (T_{wet}) and covered in Vaseline to artificially close stomata (T_{dry}) (Fig. 1A–C). Other approaches include the use of wet and dry filter paper (Jones *et al.*, 2002; Loveys *et al.*, 2008), wet and dry cotton (Fig. 1D, E), and wet and dry tensiometers (Fig. 1F, G). Metal artificial leaves can be used as well (Fig. 1H, I). To prevent the wet reference from drying out, sometimes a reservoir of water is provided, with material acting as a wick (Alchanatis *et al.*, 2010).

An alternative index, based on a rearrangement of the energy balance equation, is thermal index of relative stomatal conductance (I_G) (Jones, 1999):

$$I_G = \frac{(T_{dry} - T_{canopy})}{(T_{canopy} - T_{wet})} \quad (7)$$

For most values of g_s, I_G is linearly proportional to g_s, as has now been demonstrated under a wide range of conditions (as reviewed by Maes and Steppe, 2012). This index uses the same references as the second form of CWSI (Equation 6), but gives low values in stressed crops and higher values with increasing g_s.

As the inclusion of wet and dry reference surfaces in every image can sometimes be logistically difficult, an alternative to the above indices is to use an actual non-water-stressed plant/crop and a stressed plant/crop as extremes and relate the temperature of the crop of interest to these (Grant *et al.*, 2007). This is appropriate for example where the crop of interest is deficit irrigated and hence expected to have a T_{canopy} intermediate between those extremes. Since the reference crops, however, cannot usually be included in every image, there is the problem that meteorological conditions can change between imaging the reference crop and the crop of interest. Grant *et al.* (2007) therefore interpolated the temperatures of the reference crops between a series of images to estimate their temperature at the precise time at which the crops of interest were imaged.

Separating canopy from soil temperature

The above indices are appropriate where only leaves are being analysed (i.e. either the crop completely covers the soil, or only leaves are selected to acquire T_{canopy}). An alternative index called the water deficit index (WDI) was developed for applications where soil and crop temperatures could not be separated (Moran, 1994). This index uses the difference between the temperature of the surface (which includes vegetation and bare soil) and the temperature of the air – $(T_s - T_{air})$ – along with an index of vegetation cover. At 100% vegetation cover, the values of WDI will fall within the same limits of the CWSI. For more details on the estimation and the pros and cons of the WDI, see Maes and Steppe (2012).

Alternatives to the stress indices

An alternative to the use of stress indices is to estimate g_s (Leinonen *et al.*, 2006; Guilioni *et al.*, 2008) from T_{leaf}. This requires that T_{air} and VPD, net radiation, and wind speed are measured at the same time as T_{leaf}. Berni *et al.* (2009) used this approach to estimate canopy conductance of olive trees under different irrigation regimes, with T_{leaf} being derived from an image obtained from an unmanned aerial vehicle (UAV). They also estimated CWSI using meteorological data rather than references to obtain T_{wet} and T_{dry}, and found that the estimated CWSI was strongly inversely correlated with leaf water potential. Ben-Gal *et al.* (2009) compared estimation of CWSI using meteorological data with estimation using $T_{dry} = T_{air} + 5°C$ (which is rather arbitrary) and T_{wet} being the temperature of a wet cloth. The two methods gave similar results, and the authors suggest using meteorological data is preferable, to avoid the need of a wet reference in every image. This approach, however, does mean that the reference temperatures are not obtained at the same environmental conditions as T_{leaf}, since a full set of meteorological data cannot be collected at each plant of interest.

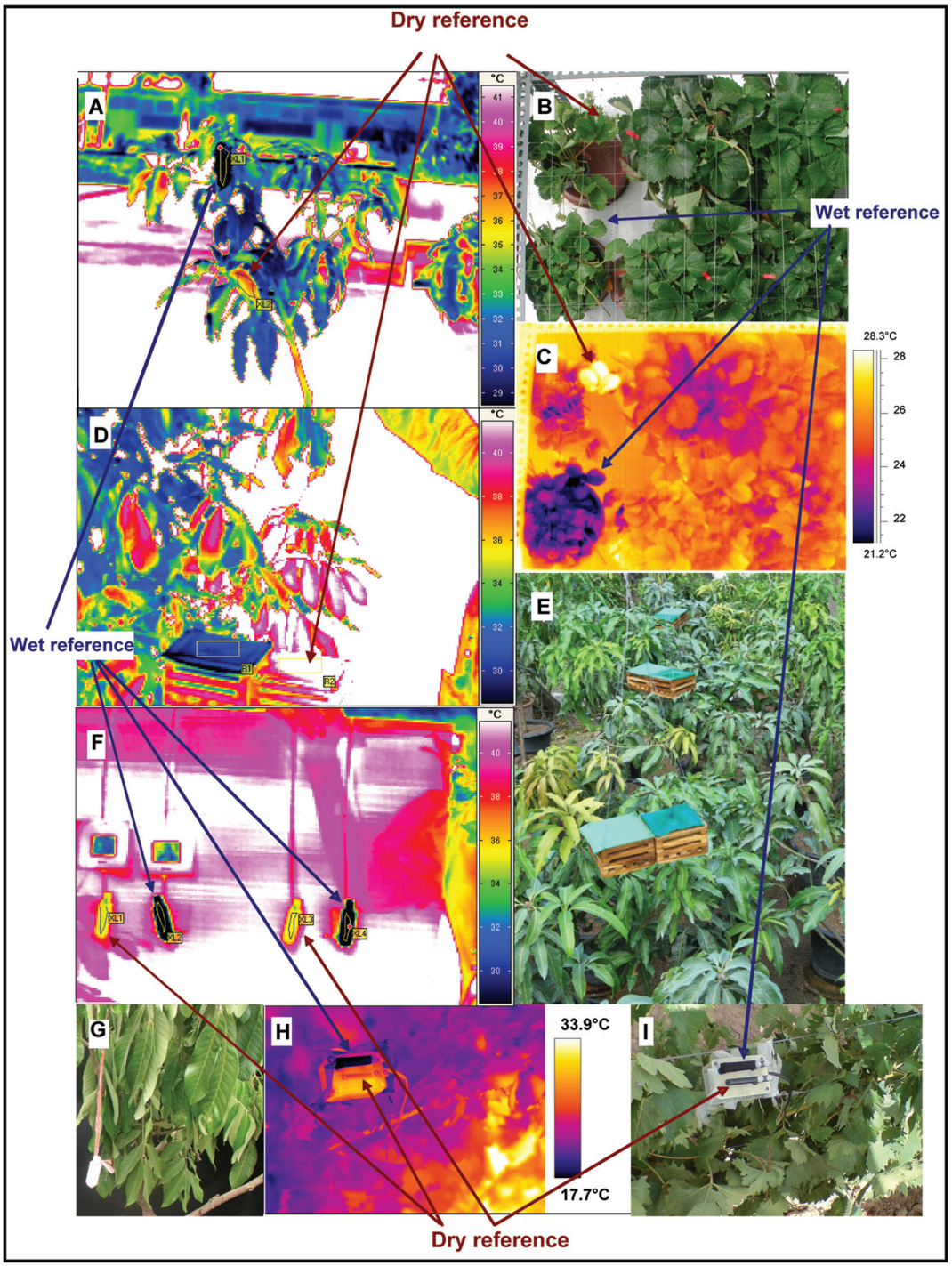

Fig. 1. Different approaches to providing wet and dry reference surfaces to estimate T_{wet} and T_{dry}. (A–C) Wet and dry leaves on the plant of interest. (D, E) Wet and dry cotton material covering pairs of reservoirs (D), with the reservoir filled with water in the case of the wet reference and left empty in the case of the dry reference – such a system can be hung from trees (E). (F, G) Wet and dry tensiometers (F), which can also be hung from trees (G). (H, I) Wet and dry artificial leaves of a sensor designed to monitor wet leaf depression (Evaposensor, Skye Instruments, Powys, UK): the artificial leaves are metal, but the wet artificial leaf is kept wet by means of a wick in a reservoir of water. Images A, D, F, and H were taken with IR cameras; all others are RGB digital images; images relate to various projects, with participation of U Srikasetsarakul, W Spreer, S Zia (A, D–G), and H Ochagavía (H, I), and funding from sources including Deutsche Forschungsgemeinschaft (A, D–G) and the UK Department of the Environment, Food and Rural Affairs (B, C).

A compromise is to use one reference: if T_{dry} is measured, then the need to measure net radiation is removed – an approach that gave consistent results in both field (Leinonen *et al.*, 2006) and greenhouse (Grant *et al.*, 2012) conditions. If T_{wet} is also measured, T_{air} and the boundary layer resistance are the only additional information required to estimate g_s.

Some authors have empirically determined the relationship between CWSI and leaf water potential, or between T_{canopy} and stem water potential or g_s, and then used this relationship to map variation in water potential or g_s (Cohen *et al.*, 2005; Alchanatis *et al.*, 2010; Baluja *et al.*, 2012). The step of determining the empirical relationship for a subsample is not necessary because g_s can be mapped from T_{leaf} based simply on Equation 4.

A different approach is to focus on variability of temperature, rather than absolute temperature. Given the large influence of g_s on the energy budget of a canopy, a greater range of temperatures will be found in a canopy as stomata close, due to the relatively greater influence of variation in leaf exposure as compared to a non-stressed, fully transpiring canopy (Fuchs, 1990). This, however, will not apply to canopies with a non-random distribution of leaf exposure (Grant *et al.*, 2007). There has been little experimental assessment of this approach with canopies that do show a random distribution of leaf angle and orientation, with the exception of a study by Bryant and Moran (1999), which showed that crop temperatures deviate from a normal distribution as plant water stress increases.

González-Dugo *et al.* (2006) considered that variation in temperature in a crop will increase with stress not because of increased variation in leaf exposure when transpiration is low, but because variability in rooting depth or soil structure, for example, will increase as soil dries. They therefore compared the standard deviation of T_{canopy} to that of CWSI and found that the standard deviation of T_{canopy} correlates with CWSI for moderate but not for severe water stress.

Thermal imaging: applications in plant and agricultural sciences

Forward genetics screens and characterization of mutants and transgenics

Forward genetics and selection of stomatal mutants has been providing new biological tools to study and better understand the genetic, physical, and physiological basis of stomatal responses to environment (Papdi *et al.*, 2009; Dodd, 2013). Forward genetics involves induction of artificial genetic variation by chemical, physical, or biological mutagenesis and the screening of mutants for a certain phenotype of interest (Papdi *et al.*, 2009). Mutants identified in novel screens are useful to identify signalling pathway components and potential genes that can be used to improve plant performance under stress (e.g. drought) (Plessis *et al.*, 2011). The use of thermal imaging in forward genetics and related large-scale screens and mutant characterization is rather recent (Merlot *et al.*, 2002) and accompanied rapid developments in plant molecular physiology and functional genomics. In the context of studies on stomatal regulation, the aim of forward genetics is to dissect different signalling pathways (e.g. abscisic acid) at the genetic level involved in regulation of guard cell response to internal and environmental signals.

With thermal imaging it is possible to screen several thousands of young seedlings and isolate those presenting a temperature difference relative to the respective wild type, as a result of different stomatal regulation (example in Fig. 2A). The model species *Arabidopsis thaliana* has been used to identify and characterize molecular regulators of transpiration using forward genetics (Nilson and Assmann, 2007). Mutants with abnormal stomatal response to drought (Merlot *et al.*, 2002; Plessis *et al.*, 2011), light (Merlot *et al.*, 2007), air CO_2 concentration (Hashimoto *et al.*, 2006), air VPD (Xie *et al.*, 2006) and ozone (Saji *et al.*, 2008) have been isolated to date. Genetic characterization of these mutants led to the identification of new genes encoding proteins such as an abscisic acid-activated protein kinase (Merlot *et al.*, 2002) and a plasma membrane H^+-ATPase (Merlot *et al.*, 2007), as well as other key mediators involved in signalling networks regulating stomatal aperture/closure in response to the environment (Papdi *et al.*, 2009; Sirichandra *et al.*, 2009). For example, Merlot *et al.* (2002) isolated mutations at two novel loci designated as *OST1* (OPEN STOMATA 1, At4g33950) and *OST2*. *ost1* and *ost2* were the first mutations altering abscisic acid responsiveness in stomata and not in seeds (Merlot *et al.*, 2002). More recently, Negi *et al.* (2008) isolated another *Arabidopsis* gene, *SLAC1* (SLOW ANION CHANNEL-ASSOCIATED 1, At1g12480), encoding the S-type anion channel, which was shown to mediate sensitivity of stomata to CO_2. *SLAC1* was found essential for stomatal closure in response to CO_2, and *SLAC1*-deficient mutants showed constitutively higher g_s as result of larger stomatal aperture.

In rice, thermography helped to isolate and characterize *SLAC1*-deficient mutants with a constitutive low T_{leaf} phenotype and consequently higher g_s (Kusumi *et al.*, 2012). These mutants had higher photosynthetic rates than the wild type, which could improve growth and yield. Transgenic lines of rice overexpressing a transcription factor (bHLH family) were characterized for their response to drought stress. The overexpressing line showed lower T_{leaf} (and consequently higher g_s) than the silencing line (Chander *et al.*, unpublished) (Fig. 2B).

Thermal imaging is also useful to support preliminary physiological characterization of novel mutants with abnormal leaf growth (small and curled leaves), which limit leaf gas exchange measurements. Characterization of *ESK1*, which results in reduced growth and deficient vascular water transport, was based on measurements of T_{leaf} in detached leaves (Lefebvre *et al.*, 2011).

Stress detection and management

Water stress monitoring and irrigation management

Given the relationship between T_{leaf} and crop stress, the potential of thermal imaging to monitor crop water status is clear. In fact, thermal imaging can show differences between irrigated and non-irrigated plants and between different intensities of irrigation (Cohen *et al.*, 2005; Grant *et al.*, 2007; Möller *et al.*, 2007; Berni *et al.*, 2009; Padhi *et al.*, 2012; Zarco-Tejada *et al.*, 2012). In some cases, a single thermal image is sufficient to reveal spatial variation in plant water status. On a larger scale, aerial thermal imaging is used to detect variation in crop water status at a single point in time. In this case, resolution is critical: Berni *et al.* (2009) were able to determine the temperature

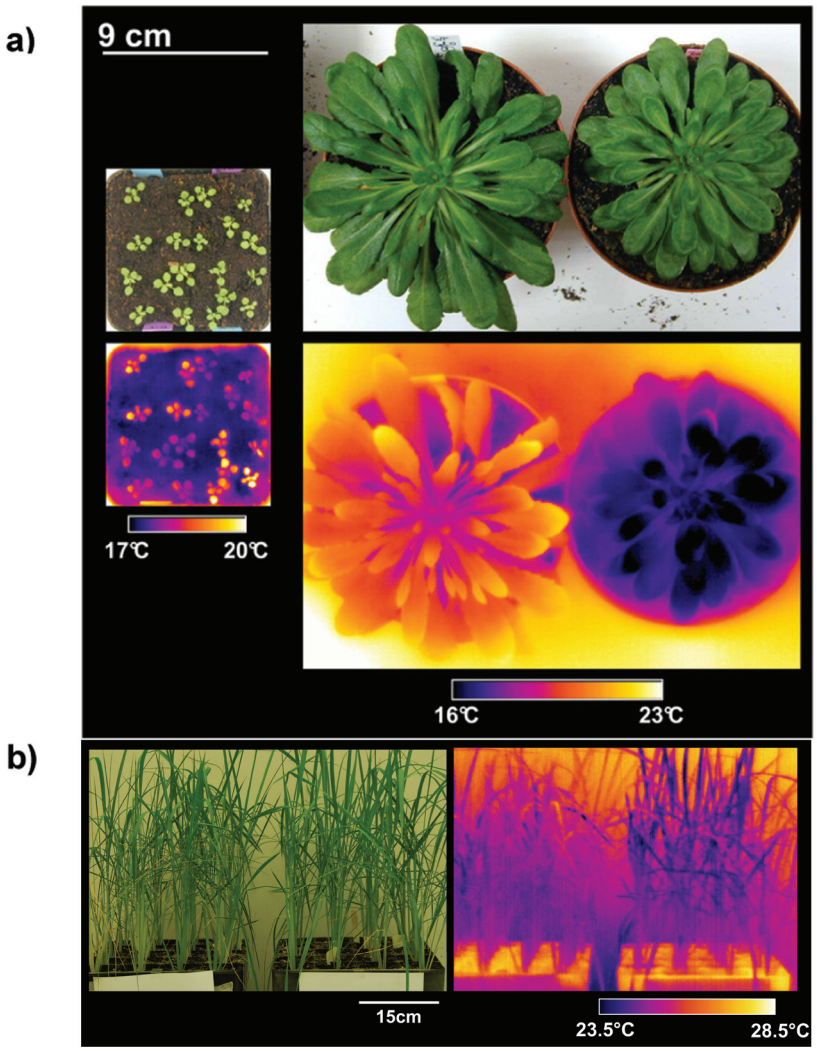

Fig. 2. Examples of thermal imaging in forward genetics and plant phenotyping. A: Visible (upper) and false-coloured thermal (lower) images at two growth stages (young seedlings and mature stage), for wild type (WT, ecotype Columbia) (left) and mutant *ost2* (Merlot *et al.*, 2007) (right) *Arabidopsis thaliana* after plants were dark adapted. The WT close their stomata in the dark whereas the *ost2* plants do not. Therefore, the WT show higher leaf temperatures than mutants. (B) Visible and false coloured IR images of 4-week-old rice plants subjected to drought and overexpressing (right) and silencing (left) a bHLH transcription factor (Chander S, Almeida D, Serra T, Barros P, Costa JM, Santos T, Oliveira MM, Saibo N, unpublished, with permission).

of individual trees from a thermal image taken from an UAV, providing a resolution of 40 cm; this was not possible when thermal images were taken from a higher-flying aeroplane that provided a resolution of only 2 m.

The greatest temperature differences between stressed and non-stressed canopies are found in hot, dry environments. There are limitations concerning measurements in more humid areas with low leaf-to-air vapour pressure differences (Thomson *et al.*, 2012). Nevertheless, detectable differences were measured under relatively humid, cool, and low radiation conditions, as noted by Maes and Steppe (2012).

The capacity of thermal imaging to detect variation in crop water status depends on the plant's stomatal response. In species with more marked anisohydric behaviour (e.g. sunflower, wheat, soybean, almond tree), leaf water potential falls with increasing evaporative demand (Tardieu and Simonneau, 1998), due to poor stomatal control over tissue water loss. For this reason, in these species leaf water potential is a better indicator of soil moisture than g_s. Isohydric species (e.g. maize, lupin, pea, poplar), on the other hand, close their stomata in response to a decrease in soil water and/or an increase in VPD, controlling plant water potential. Under such conditions, g_s is a better indicator of soil moisture than water potential. Therefore, drought detection using thermal imaging is more suited to species or varieties exhibiting isohydric behaviour (Jones *et al.*, 2009).

A logical step forward for the use of this methodology is to use thermal imaging to decide where, when, and how much to irrigate. This, however, requires that a threshold for crop temperature, a stress index, or a value of g_s is established beforehand. Irrigation would be applied when this threshold is reached. Threshold values may vary according to the environment and species or variety/cultivar (Cohen *et al.*, 2005; Möller *et al.*, 2007; Costa *et al.*, 2012; Fuentes *et al.*, 2012).

Further progress is still required before thermal imaging is routinely used for irrigation scheduling. There is the need for monitoring crops over the whole season and ultimately compare thermography with other scheduling methods. Where the costs involved in frequent thermal imaging are prohibitive, infrequent thermal imaging (e.g. aerial or satellite) that will provide information on spatial variation may be combined with frequent (or continuous) alternative means of monitoring water stress. The latter would provide poor spatial resolution, but high temporal resolution. For example, a single aerial or satellite image may be sufficient to pinpoint areas of a crop with different water demands, and representative plants within each zone could then be continuously monitored (e.g. by measuring stem diameter fluctuations or sap flow) in order to schedule irrigation over a season. Garciá-Tejero *et al.* (2011) suggested that the combined monitoring of maximum daily trunk shrinkage (to obtain information regarding individual plants) and $T_{canopy} - T_{air}$ (to obtain information regarding plots) would help to determine irrigation requirements in orchards with spatial variation in plant water status. Aerial and ground thermal imaging can also support detection of malfunctioning (e.g. leaks) of irrigation canals and delivery systems, which result in large water losses (Thomson *et al.*, 2012).

Crop protection

Pests and diseases limit the genetic potential of crops regarding their growth and yield. Pests and diseases can change the amount and direction of radiation reflected and emitted by plants (Jackson, 1986) or can modify plant temperature due to stomatal deregulation and/or changes in plant water relations (Nilsson, 1995; Allègre *et al.*, 2007). Thermal imaging can thus be used to monitor infection patterns of diseases or infestation by pests, which assume typical patchy distributions (Mahlein *et al.*, 2012), or even to detect the stress before symptoms are visible (Jackson, 1986; Nilsson, 1995; Oerke *et al.*, 2006; Stoll *et al.*, 2008; Chaerle *et al.*, 2009).

Increase in plant temperature can co-occur with senescence due to biotic stress, resulting from modified plant–water relationships due to the interruption of normal function of the root system or/and blockage of water and nutrient transport in the stem or leaves (Nilsson, 1995). Foliar pathogens can disrupt cuticular and/or stomatal regulation of transpiration and influence plant water relations and WUE_i (Grimmer *et al.*, 2012). Thermal imaging also supports studies of the action of herbivorous insects: for example, transpirational water loss in soybean (*Glycine max*) leaflets occurred mostly from injuries on cuticle and cut edges of the attacked leaves (Aldea *et al.*, 2005).

Combined thermal imaging and gas exchange studies under laboratory conditions showed that the accumulation of salicylic acid (a hormone produced in plant defence against infections) in response to tobacco mosaic virus infection in tobacco leaves was paralleled by stomatal closure: T_{leaf} increased after virus inoculation prior to cell death (Chaerle *et al.*, 1999). However, if the infection has only a minor effect on transpiration, detection using thermography may not be possible, particularly in field conditions.

Moreover, combination of stresses can also pose limitations when monitoring infection with thermal imaging. For example, in grapevine, the maximum temperature difference between infected and non-infected areas reached 0.9 °C in irrigated plots, but only 0.3 °C for non-irrigated plots (Stoll *et al.*, 2008). Although there are limitations in the identification of the causal agent of biotic stress (disease, pest) (Jackson, 1986), thermal imaging can be used in field conditions to localize spots where the crop is more affected and demanding more urgent intervention (Sankaran *et al.*, 2010).

Other stresses

Shimshi (1967) observed that, under conditions favouring stomatal opening (non-limiting soil moisture content) nitrogen and iron deficiency would induce stomatal closure in different crop species (e.g. beans, wheat, sugar beets, maize, groundnuts). Under controlled conditions, Chaerle *et al.* (2007) using thermal imaging showed that bean plants growing on magnesium deficient solution had a higher temperature (by about 0.5 °C) compared to control plants. Preliminary thermal imaging of spring barley also indicated higher canopy temperature in crops that did not receive any nitrogen fertilizer compared to those that were well fertilized (165 kg N ha^{-1}; Fig. 3). This suggests stomatal closure in the nutrient-starved crops. On the contrary, wheat plants growing under higher nitrogen conditions for two consecutive years exhibited lower T_{canopy} (Tilling *et al.* 2007). In field measurements, combining thermal and spectral properties of canopies may help to identify more precisely nutrient deficiency and distinguish between water and nutrient stress (Christensen *et al.*, 2005).

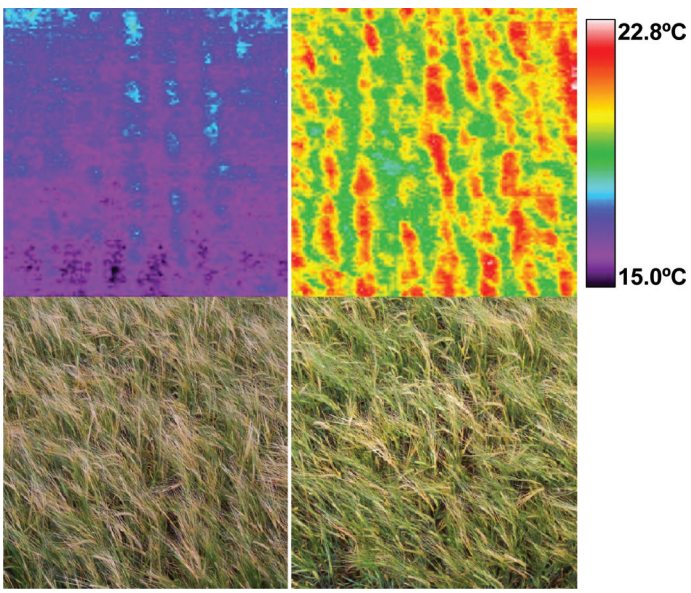

Fig. 3. Spring barley crops subjected to a high nitrogen fertilizer input (165 kg N ha^{-1}; left) and no nitrogen fertilizer (right), and imaged with an IR Snapshot 525 (Infrared Solutions, Minneapolis, USA) 120 × 120 pixel line scan imager, 8–12 μm (top) and a digital camera (bottom) from a ladder, showing higher crop temperature in the nitrogen-deprived crop.

In *Trifolium subterraneum*, ozone exposure resulted in higher T_{leaf} in long-day plants as compared to short-day plants, suggesting that ozone reduces E in long-day plants (Vollsnes *et al*., 2009). Energy released during freezing results in an increase of temperature, which can be recorded by thermal imaging (Wisniewski *et al*., 2008), with the advantage over contact thermometry of detecting the actual site of ice initiation and the number of ice nucleation events. The technique has been applied to study frost resistance in woody plants (Pramsohler *et al*., 2012).

Crop phenotyping and breeding

The ultimate goal of phenotyping in plant breeding is to quantify and rank the success of a range of genotypes in certain environments (Walter *et al*., 2012). It involves comparison of large numbers of genotypes, which requires fast and robust measurement procedures, with a high degree of automation (Roy *et al*., 2011; Walter *et al*., 2012).

Novel crop genotypes are often characterized on the basis of leaf gas exchange traits (e.g. photosynthetic assimilation, g_s, WUE_i). Thermal imaging emerged as a faster method to carry out high-throughput field phenotyping compared to point measurements of T_{leaf}, porometry, or leaf gas exchange (Furbank and Tester, 2011; Walter *et al*., 2012). Besides, it allows phenotyping of different sized plants (small seedlings to entire plants/canopies) in controlled or field conditions (Jones *et al*., 2009; Walter *et al*., 2012). Genotypes of different crops have been characterized in their response to different environmental stresses using thermal imaging (Munns *et al*., 2010; Walter *et al*., 2012).

High-yielding rice cultivars have been selected on the basis of their lower T_{leaf} and higher canopy diffusive conductance to water vapour (Jones *et al*., 2009; Berger *et al*., 2010), and several quantitative trait loci for T_{leaf} differences have been mapped in relation to stomatal behaviour traits (Pelleschi *et al*., 2006). In maize, lower T_{leaf} was positively correlated with biomass accumulation under water stress, supporting the use of thermal imaging in breeding and selection of drought tolerant maize genotypes (Liu *et al*., 2011). The low T_{canopy} of resistant lines observed under drought stress is an indicator of plant water status and of drought-avoidance mechanisms and may be related to deep root growth that allow plants to continue water uptake and transpiration that cools the leaves (Jones *et al*., 2009; Henry *et al*., 2011).

In strawberry, thermal imaging indicated that some cultivars display low g_s whereas others show high g_s under similar well-watered conditions (Grant *et al*., 2012). In grapevine, thermal imaging can also be used in breeding programmes for WUE_i since both g_s and photosynthetic assimilation are genetically dependent traits in this species (Flexas *et al*., 2010). Recently, Costa *et al*. (2012) observed different T_{leaf} and g_s phenotypes between grapevine varieties with similar water status, suggesting different types of stomatal regulation.

High-resolution thermal imaging can also help to characterize morphology and plant architecture traits that are important for selecting superior varieties for different environmental conditions (Chéné *et al*., 2012).

Ecological studies and environmental monitoring

Remote sensing has been used by ecologists and conservation biologists in larger scale studies to better understand environmental changes and their consequences in plant ecosystems (Jones and Vaughan, 2010). Thermal remote sensing allows monitoring of physiological activity of vegetation (Jacob *et al*., 2008), and satellite thermal sensing has been used to support modelling of regional fluxes of water and mapping of evapotranspiration and moisture availability (Anderson *et al*., 2008; Chávez *et al*., 2008; Sobrino *et al*., 2009), as well as in monitoring drought and water use, administering irrigation projects, predicting local and regional water demand, and supporting hydrological and weather forecast models (Anderson and Kustas, 2008).

Scherrer and Körner (2010) have used thermal imaging to monitor alpine landscapes over time with very high spatial resolution. They quantified variation between microhabitats by the deviation of vegetation surface temperature from T_{air}. This is an advance compared to previous approaches based on conventional climate station data that only enabled monitoring of individual plants or soil plots. More recently, Scherrer and Körner (2011) used high-resolution IR thermal imaging to assess the spatial and temporal variation of plant surface and ground temperatures for hundreds of plots distributed across three alpine slopes of contrasting exposure. The results emphasize the need to take microhabitat temperature variation into account when predicting climate change impacts on vegetation: meter-scale thermal contrasts were far greater than the average increase in temperature predicted for the next 100 years.

Scherrer *et al*. (2011) established a drought-sensitivity ranking of deciduous tree species based on thermal imaging and found that in drier sites and at higher temperatures some species could be less competitive than others. Pronounced drought might change the competitive abilities of tree species in favour of those that are able to maintain transpirational fluxes and cooler canopies, such as *Fraxinus excelsior* and *Quercus petraea*. Complementary ground thermal imaging at key locations could enhance the usefulness of satellite sensing (Qiu and Zhao, 2010), namely in ecology (Kerr and Ostrovsky, 2003), by providing the fine local resolution that satellite imaging cannot.

Other applications in agro-food industries

Thermal imaging can be used to monitor quality of horticultural products, namely seed viability (Kranner *et al*., 2010), health of transplants (Kim and Lee, 2004), and graft union quality (Torii *et al*., 1992). In orchards and groves, thermal imaging can help to assess fruit number (Bulanon *et al*., 2008). Additionally, thermal imaging of fruits under field conditions could assist in understanding the impact of extreme temperatures on fruit quality, namely on the incidence/resistance to sunscald (yellowing/browning of a fruit's skin and softened flesh from exposure to high temperatures) (Prohens *et al*., 2004).

Thermal imaging can also support optimized climate control in greenhouse horticulture. Greenhouse climate control could be based on continuous measurements of T_{leaf} instead

of T_{air} because T_{leaf} is more closely linked to plant performance (Ehret *et al.*, 2001).

Future prospects

As advances in detector technology and progress in image processing increase the diagnostic power of thermal imaging, the main challenge may shift from such technical aspects to the optimization of data collection. Physiologically, there is still a need to establish leaf/canopy thresholds (thresholds of relevant thermal indexes) for different biotic or abiotic stress effects and for different species and varieties. Studies that deal with a longer time scale are required as well, rather than mere correlations between temperature variables and standard indicators of stress based on measurements on single dates.

Technologically, future developments will include the combination of thermal imaging with imaging in other spectral wavelengths (visible and red/infrared reflectance, chlorophyll fluorescence) (Bulanon *et al.*, 2008; Jiménez-Bello *et al.*, 2011). The use of UAVs for civil applications opened up a new era of remote sensing, aiding assessment of plant–environment interactions at a larger scale (e.g. crop fields and forest plots) (Berni *et al.*, 2009), although there exists legal restrictions in its use in many countries. Coupling of ground and airborne measurements must be improved, in order to increase the accuracy of retrieved data. Thermal imaging in parallel with wireless sensor networks and geographical information systems will allow a more precise mapping and monitoring of, for example, irrigation and fertilizer requirements (Lee *et al.*, 2010).

Coupling of thermal imaging with modelling approaches is expected (Maes and Steppe, 2012), which should be supported by improved software tools to optimize automation and speed up robust image analysis (Fuentes *et al.*, 2012).

With respect to routine application in the land-based industry sector, as opposed to scientific research, enhanced benefits will arise from reduction in costs of thermal imaging devices that will permit extending the use of the technique in agronomy to a wider range of crops and situations than only the most advanced and intensive agricultural/horticultural systems.

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