Agronomy **2014**, *4*, 397-417; doi:10.3390/agronomy4030397

OPEN ACCESS

agronomy

ISSN 2073-4395

www.mdpi.com/journal/agronomy

Review

Infra-Red Thermography as a High-Throughput Tool for Field Phenotyping

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Received: 29 April 2014; in revised form: 6 June 2014 / Accepted: 2 July 2014 / Published: 31 July 2014

Abstract: The improvements in crop production needed to meet the increasing food demand in the 21st Century will rely on improved crop management and better crop varieties. In the last decade our ability to use genetics and genomics in crop science has been revolutionised, but these advances have not been matched by our ability to phenotype crops. As rapid and effective phenotyping is the basis of any large genetic study, there is an urgent need to utilise the recent advances in crop scale imaging to develop robust high-throughput phenotyping. This review discusses the use and adaptation of infra-red thermography (IRT) on crops as a phenotyping resource for both biotic and abiotic stresses. In particular, it addresses the complications caused by external factors such as environmental fluctuations and the difficulties caused by mixed pixels in the interpretation of IRT data and their effects on sensitivity and reproducibility for the detection of different stresses. Further, it highlights the improvements needed in using this technique for quantification of genetic variation and its integration with multiple sensor technology for development as a high-throughput and precise phenotyping approach for future crop breeding.

Keywords: field phenotyping; thermal imaging; high-throughput phenotyping; stress screening

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1. Introduction

Plant stress was defined by Jackson, 1986, as any disturbance that adversely influences growth [1]. Such stresses include both biotic stresses caused by pests or pathogens and abiotic stresses caused by adverse climatic or edaphic conditions. A range of different physiological and anatomical changes occur in response to stresses which can all affect the ability of plants to tolerate the stress, though in general such responses tend to limit plant growth and yield potential [2]. Plant breeders need effective tools to identify those responses and the associated physiological characters that lead to the best tolerance of stresses while maximising commercially important traits like yield and quality. Recent developments in genomics have radically altered the landscape for conducting genetic analysis, and have great prospects for impacting significantly on crop improvement. However the development of high-throughput genotyping methods has not been matched by equivalent advances in plant phenotyping, so that the lack of effective high-throughput phenotyping methods is now becoming a major limiting factor [3,4] in any large genetic study. Recent developments in imaging technology now offer particular opportunities to develop robust high-throughput phenotyping [5,6]. In particular, it is essential that any such techniques are applicable in the field, since the majority of plant traits are currently evaluated in pots under glasshouse conditions where results do not correlate well with field trials [7].

Thus field phenotyping is crucial in an era when breeding for crops under different climatic conditions is essential to meet the population increase. Most of the attention so far has been on the traits which are directly commercially important, but with the changing scenarios of stresses, we also need to understand traits which are indirectly linked to yield and which can give an indication of stress in order to understand how plants adapt and tolerate stress and how pyramiding of these regions can be used in breeding. The increasing availability of different imaging techniques has allowed real time image analysis of physiological changes in plants; such imaging techniques have great potential for high-throughput screening of plant populations [3,5,8], often allowing the pre-symptomatic monitoring of plant stress non-destructively. Although imaging techniques for addressing the "phenotyping bottleneck" have been widely used under controlled environments they are only now starting to be tested under field conditions.

In this review we concentrate on the potential uses of thermal imaging as a tool for stress sensing as a component of high-throughput field phenotyping systems; other papers address other sensing systems (e.g., hyperspectral imaging [9,10]) and the use of different platforms for mounting the sensors [11–13]. The role of thermal imaging as a tool for irrigation scheduling has been widely discussed elsewhere and is therefore only touched on briefly in the present article (see e.g., [14–18]) while wider applications in agriculture have previously been reviewed by several authors [8,19–21]. Thermal imagers estimate surface temperatures from the emission of long-wave infrared radiation which varies as a function of surface temperature according to the Stefan-Bolzmann equation (see [10]). The surface temperature of crop canopies decreases with increasing transpiration as a result of evaporative cooling; the rate of transpiration is itself regulated by, among other factors, stomatal aperture [2]. Therefore changes in leaf or canopy temperature provide a valuable measure of stomatal conductance, which itself is a key indicator of many stress responses. Unfortunately canopy temperature is also dependent on other environmental conditions so methods are needed to separate the effects of varying stomatal conductance from other environmental effects on temperature: some possible approaches are discussed in detail below.

Although canopy temperature sensing using infrared thermometers has been used for many years, especially for irrigation scheduling purposes, the advent of relatively cheap thermal imagers has opened up the possibility of screening large numbers of genotypes. Imaging allows much greater replication as compared with infrared thermometers or the standard research method of porometry (which is highly labour intensive and unsuitable for automation) so imaging is highly suitable for incorporation in high-throughput screening [22–25]. This review outlines the evidence for the sensitivity and reproducibility of infra-red thermography (IRT) as a tool for assessing the variation in stomatal behaviour and describes some of the precautions that need to be adopted in its use for screening purposes.

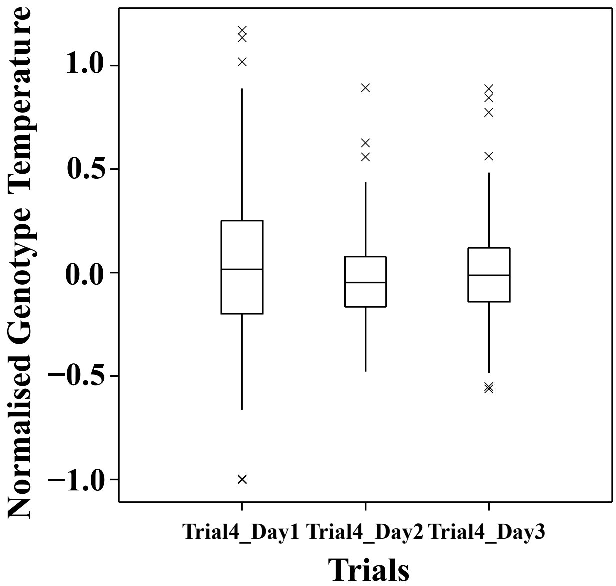
2. Practical Considerations Affecting Thermal Imagery for Phenotyping

Although thermal imaging is well suited for screening genotypes under controlled conditions where all environmental factors are controlled, when applied in the field it is necessary to allow for the fact that temperatures can vary for many reasons other than differences in stomatal conductance. These factors include varying air temperature, wind speed and irradiance, as well as the complications arising from the complexity of canopy structure and the variation of leaf angle in real canopies. For example, varying leaf angles lead to each leaf being at a different temperature as a result of absorbing different amounts of solar radiation [26], while the angle from which one views the canopy also affects the apparent temperature.

2.1. Temporal Variation in Environmental Conditions

All the environmental factors affecting leaf temperature such as air and soil temperature, humidity, cloud cover and wind speed, can change rapidly in natural environments. Furthermore the temperature at any time is not only dependent on the current equilibrium value but also to some extent on previous conditions and the thermal lags in the system [2,27,28]. A particularly useful approach to the partial elimination in phenotyping studies of the rapid or slow changes in equilibrium temperature caused by environmental variation has been described by Jones and colleagues [25,29]; this involves simultaneous collection of temperatures from a number of plots in each image and expressing individual plot temperatures as differences from the mean of all plots in that image. This corrects for temporal changes in temperature as occur, for example, when clouds pass over. The power of this correction method improves as the number of plots in an image increases and as the amount of overlap between images increases, and works well even if the environmental conditions are changing rapidly. Figure 1 illustrates the range of normalised genotype variation for a potato trial imaged on three different days (see [29] for details) showing the temperature variation on each day caused by variation in factors such as air temperature, relative humidity and solar radiation.

Figure 1. Box plot demonstrating the range of temperature variation for trials imaged on different days (see [29] for details). Trial4_Day1 displays a greater variation in temperature during imaging as compared with Trial4_Day 2 and Trial4_Day 3 as a result of greater variation in solar radiation.



The rate of transpiration, and hence cooling, decreases as the leaf-air vapour pressure difference decreases and also tends (at least at high radiative loads) to decrease as the windspeed (or boundary layer conductance) increases [2]; therefore the temperature range corresponding to a given range of stomatal conductances is lower under humid or high wind speed conditions [30–32]. Because the general errors in temperature measurement remain constant, this means that thermal screening of genotypes has less discriminatory power in humid conditions or at high wind speeds.

Rather than normalising to image means, it is possible to use other referencing methods. Approaches available are summarized and compared in Table 1. For example normalisation to air temperature removes some of the variability resulting from environmental variability [33]. More effective at environmental correction, however, is to calculate a stress index based on the difference between observed leaf temperature and the temperatures of artificial wet or dry physical reference surfaces [30,31]; this gives a measure directly related to stomatal conductance that accounts for variation in incoming radiation and other environmental factors. An alternative is to calculate the expected temperatures of notional wet or dry leaves using an energy balance model [34,35]. This use of reference surfaces is closely related to the calculation of a crop water stress index (CWSI) as proposed by Idso and colleagues [36,37] where the canopy temperature was related to empirical relationships describing the temperature of well watered or non-transpiring crops under similar conditions.

Table 1. Comparison of referencing methods for thermal data.

Reference Method	Advantages/Disadvantages	References
Comparison with (T_{air}) air temperature	Removes some variation, but not that due to radiation, or environmental humidity	[33]
Empirical "baselines"	Adequate in fixed environments, but not extrapolatable to other environments/crops	[16,37]
Wet and dry leaves/canopies	Corrects for radiation, windspeed, humidity and removes need for absolute accuracy; but hard to maintain wet surface in hot environments	[31,38]
Paper references	Essential to ensure correct spectral properties or dimensions, otherwise errors introduced; may be hard to maintain wet surfaces	[31]
Larger wet references (WARS)	Good for aerial imaging but inappropriate time constant and spectral properties of polystyrene surface gives erroneous results	[39,40]
Estimation of dry value as T_{air} + 5 °C	Does not take account of varying radiation or windspeed; requires absolute accuracy of thermal data	[40,41]
Calculation of theoretical "wet" and "dry" temperatures	Requires local meteorological data and difficult to estimate radiation absorbed by canopy; requires absolute accuracy of thermal data	[35]

In selecting material for the construction of artificial dry or wet reference surfaces it is essential that the reference surface has a similar short-wave absorptance as the plant leaves of interest, especially when measurements are made under direct solar radiation. A black surface will absorb a greater fraction of incoming radiation and may heat up many degrees above the temperature of a non-transpiring leaf [25]. It is also necessary to consider the thermal mass of any physical reference surfaces used, as massive references will have high heat capacity and hence longer thermal time constants in response to changing environments [2]. Where time constants of reference and leaf are mismatched it is possible for the reference temperature to be substantially out of synchrony with the putative equivalent leaf which can lead to substantial errors. Yet another consideration is that the boundary layer resistance of the reference leaf needs to be similar to that of the real leaf, to ensure that its energy balance is representative. Some artificial reference surfaces that have been proposed do not satisfy fully these conditions: for example the large wet artificial reference (WARS) proposed by Cohen, Alchanatis, Meron and colleagues [40,42,43] for use with airborne imagery has neither the correct short-wave absorptance (it is white and reflects more radiation than real leaves), nor the correct aerodynamic properties (being 40 × 30 cm), nor an appropriate time constant. A further problem is that these authors and following papers [44,45] have assumed that the dry reference is 5 °C warmer than air temperature. Although some evidence for this latter assumption has been provided [39], in most practical situations this assumption can lead to substantial errors [32]. At least the inappropriate spectral properties of the WARS used by these workers has only a small effect on its use as a wet reference (though it would have a large effect on a dry reference) because of the relatively small contribution of radiation balance in the energy balance of a wet surface [2]. As long as ground measurements of air humidity are available, estimates of leaf conductance using just a dry reference are very similar in accuracy to those obtained using both wet and dry references together [35].

Because of the varying orientation of the different leaves in a real canopy (and hence their varying energy balance) it is difficult to provide a reference surface that mimics accurately the mean temperature of an ensemble of leaves. One approach that has some potential is to construct the reference surface from a surface with a range of orientations that simulates the distribution of orientations of real leaves. For a typical canopy with the leaf angle distribution approaching a hemispherical distribution [2] taking the average temperature of a hemispherical sensor should provide a good approximation to the average leaf temperature. Preliminary experiments with such a sensor have shown some promise as providing an effective reference surface [46].

2.2. Aspects Relating to Angles of View and Illumination, Distance and Imager Field of View (FOV)

Recent advances in technology, especially the development of relatively inexpensive thermal imagers have allowed measurements to be conducted over larger areas (and hence more plots) than is possible with point measurements. With images, however, it is important to note that different parts of the image will be viewed at different angles in relation to the sun: this variation in the angle between the direct solar beam and the view angle has important implications. An image appears brightest where the sun is directly behind the observer as there are no shadows visible in the image and only highly reflecting sunlit leaves or soil can be seen: this is known as the "hotspot" as the overall reflectance is highest at this angle. The variation in apparent reflectance as the angle between the sun and the observer changes is described by the Bidirectional Reflectance Distribution Function (BRDF) [10]. A similar phenomenon occurs for thermal images where the observed temperature (as measured by the emitted thermal radiation) is also highest in the hotspot because little of the cooler shaded area is visible [47]. As the angle between the sun and the observer increases the proportion of visible shaded leaf or soil increases and the average temperature observed tends to decrease as compared with the hotspot view [10]. Although many studies have investigated the impact of view angle on apparent temperature of different canopies [48–51] in most cases the conclusions have related to observation of canopies with less than full canopy cover where temperature variation is dominated by the changing proportion of background viewed at different angles (see discussion below of background contamination).

The imager field of view (FOV) determines the range of variation in view angle across an image with reference to the solar beam. Wide angle lenses lead to greater variation in the BRDF across an image resulting, for example, in a wide range of the fraction of shaded pixels for such images and a lack of comparability of observed temperatures across an image. Although the BRDF can substantially impact measurements where the view-illumination angle varies substantially between different plots in any image for a phenotyping experiment, such problems can be minimised by taking multiple overlapping images and deriving a genotype mean from many individual replicate plots with differing view angles [29].

A particular aspect of illumination is the selection of view angle; a question that is particularly relevant for row crops. Is it better to image the sunlit side (for which canopy temperature changes more for a given change in conductance [25]) or the shaded side for which canopy temperature is less sensitive to angle of orientation of the leaves [27,52]? It is uncertain in any situation which of these opposing priorities will be most satisfactory in any specific situation. Although in some cases, such as an example where water status in cotton was mapped using oblique angle images, it has been reported that mapping was successful at different angles [42], more generally it is necessary to account for angle and BRDF for accurate work. Similar considerations apply to the choice of viewing angle for continuous field crops, with the commonest approach being to view the canopy obliquely from the sunlit side, though it is necessary to determine the most appropriate strategy for each particular study. An oblique view angle is generally recommended rather than a nadir view so as to minimise the chance of viewing soil (with it's usually rather different temperature) when the canopy is sparse.

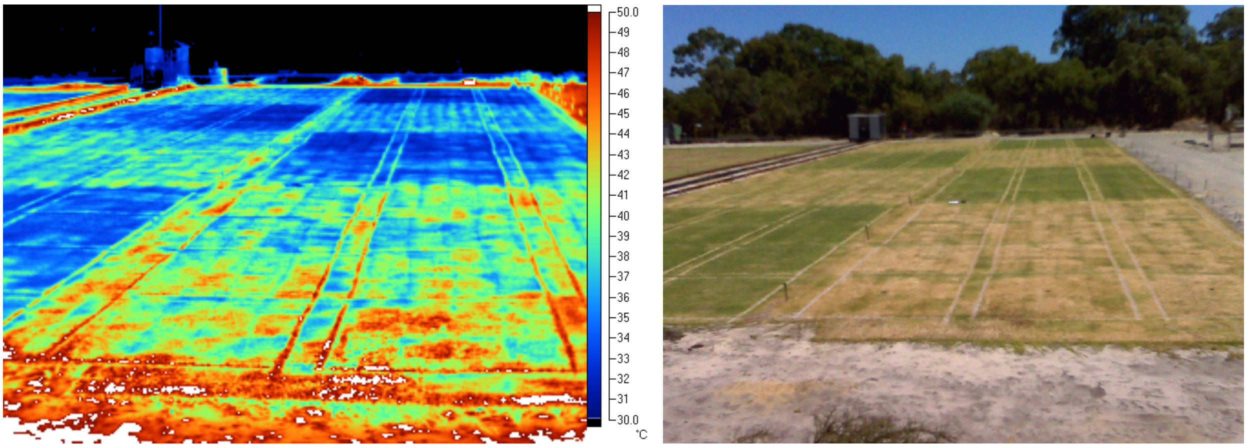
The camera-object distance clearly affects the spatial resolution, as pixel size increases proportionately to distance. Larger pixels have an increasing probability of being mixed, containing both leaf and background soil in any one pixel. With mixed pixels it is difficult to determine the temperature of the leaf component of the image which is what is required for phenotyping. The leaf temperature can be approximated from the mean pixel temperature if one can estimate independently the fraction of the pixel occupied by leaf (for example using a spectral vegetation index as a measure of vegetation cover [10]) and one has an estimate of the temperature of the background soil. This and other approaches to overcoming this mixed-pixel problem are discussed in detail by [53]. An advantage of smaller pixels is that it is possible to have a greater number of pixels representing solely the object of interest; this improves the accuracy of its temperature estimation.

Another aspect of the camera-object distance that needs consideration is the fact that the apparent temperature varies with camera-object distance because of contamination of the further objects by thermal emission from the intervening air path [10]. An example of this effect is given in Figure 2 for a turfgrass experiment in Western Australia, where it is clear that the apparent temperature of replicate treatments is significantly higher in the nearer plots (Figure 2). A possible solution to this distance problem might be to correct each pixel's temperature by using a standardised distance-related correction factor derived by regression of apparent temperature against distance for an image where stomatal conductances are known to be constant at all distances (as in Figure 2).

2.3. Canopy Aerodynamics

The plant or canopy aerodynamic characteristics, such as crop height or aerodynamic roughness, can substantially affect the results of thermal imagery through their effects on boundary layer resistance and canopy energy balance. For example it has been noted on several occasions that there can be consistent canopy temperature differences between tall and short genotypes in screening trials, even where stomatal differences were not apparent [54,55]. For example, genotypic differences in grain yield have been shown to be associated with warmer canopies [56,57] when analyzed without using height as a covariate but higher yield was associated with cooler canopies when statistics included height as a covariate before flowering [54]. Similarly it is likely that the aerodynamic properties, and hence temperatures of canopies, will also change when flowering shoots start to extend above the general canopy, though there have been relatively few rigorous studies of this effect [58]. Similarly, differences in canopy albedo will also affect canopy temperature, with more reflective canopies (e.g., as a result of more waxy leaves or even greater prevalence of awns) being expected to be cooler than equivalent less reflective canopies. Until such effects are better understood it would be is wise to restrict genotypic comparisons to lines with similar phenology and morphology.

Figure 2. Image of a turf grass experiment with four replicate blocks of well-irrigated (cool) and four less-irrigated (warmer) plots in Perth, Western Australia (T_{air} = 30.3 °C; relative humidity = 19%) [59] taken with a Fluke Ti32 thermal camera, showing the gradient of apparent temperature with distance with the average temperature of the less-irrigated plots ranging from 40.7 °C for the nearest to 36.2 °C for the furthest. Similar results were obtained if the plots were viewed from the opposite end (data not shown [59]) confirming that the variation was an effect of viewing distance and not a soil effect.



2.4. Sensitivity and Reproducibility of Thermography

In order to understand genetics and select for genotypes for breeding over different locations, it is essential to be able to evaluate large numbers of genotypes under contrasting field conditions. Thermal methods have the significant advantage that they view an ensemble of leaves rather than single leaves so they can give a more reliable estimate of crop stomatal conductance than does porometry on single leaves [27]. Useful characters for plant breeders tend to be those with consistent expression on different days, times of day and at different sites; imaging approaches have the capacity to provide the large scale phenotyping capacity that facilitates the collection of the necessary data. It has been confirmed that different cameras can produce similar screening results, thus enhancing the potential capacity of phenotyping systems; for example in one case on vineyards temperatures recorded by two cameras (SnapShot 525, a 120 × 120 pixel line scan imager in the 8–12 μm wavebands and ThermaCAM SC2000, a long-wave imager with a 320 × 240 pixel sensor) were highly correlated (0.94) [35]. Other experiments have confirmed that the thermal ranking of genotypes can be maintained over measurements made on different days, times of day and at different sites and temperature-related quantitative trait loci (QTLs) identified [25,29,60,61]. For high-throughput phenotyping it is not usually necessary to estimate stomatal conductance accurately (e.g., using reference surfaces or models) and relative measures are usually adequate though it remains critical to ensure that data are not biased by background noise due to soil or woody parts.

3. Infra-Red Thermography for Plant Stress Detection

3.1. Screening for Abiotic Stress

3.1.1. Screening for Drought and Salinity

IRT has been widely used to study stomatal responses to drought and salinity stress and to select for stomatal mutants especially those involving altered abscisic acid (ABA) metabolism affecting stomatal closure. In an early laboratory study for example, Raskin and Ladyman [62] isolated an ABA-insensitive "cool" mutant in barley, while both ABA synthesis and ABA-insensitivity genes have been isolated using similar approaches in Arabidopsis [22,24]. This early work provides the basis for extrapolation of thermal screening to the field. Although ABA is a critical hormone involved in the coordination of many stress responses [63], thermal imaging only provides information on those responses involving stomatal closure. Unfortunately it is not necessarily obvious when selecting genotypes for drought tolerance whether one should be selecting those lines that maintain high stomatal conductance or whether rapid stomatal closure and consequent water conservation phenotypes may be more relevant. Indeed in many cases there will be an optimum stomatal conductance that balances the higher water use efficiency as stomata close and the increasing photosynthesis as stomata open: this optimum will depend on environment and water availability [64]. In addition to the many laboratory studies, both infrared thermometry and infrared thermography have been widely used in field studies for both irrigation scheduling [17,18,42,65] and for genetic screening [54,56,66–70]. Although infrared thermometry can be used as a cheaper alternative for screening for drought tolerance [56,67] the much greater time and labour requirement means that it is nothing like as well suited for high-throughput systems as is thermography, especially when automated image analysis procedures are used.

A comparable abiotic stress response to that under drought is also shown under salinity conditions due to osmotic stress. Studies on barley and wheat, for example, have shown a strong relationship between salt concentration of the medium and stomatal conductance or leaf temperature, highlighting the potential use of IRT for screening genotypes for stomatal traits related to salt tolerance [71].

An alternative potential approach to the use of thermal imaging has been proposed that is based on the idea that the temperature variation between neighbouring leaves increases with drought stress [26,72,73]. This approach depends on the idea that with randomly oriented leaves in a canopy some will intercept more radiation than others, and hence will heat up more: the temperature range observed increases as latent heat loss becomes a decreasing component of the overall energy balance so that more stressed canopies will show greater variance in leaf temperature. Unfortunately this approach does not work in regular canopies such as many grape vine canopies [27].

3.1.2. Heat and Frost Stress

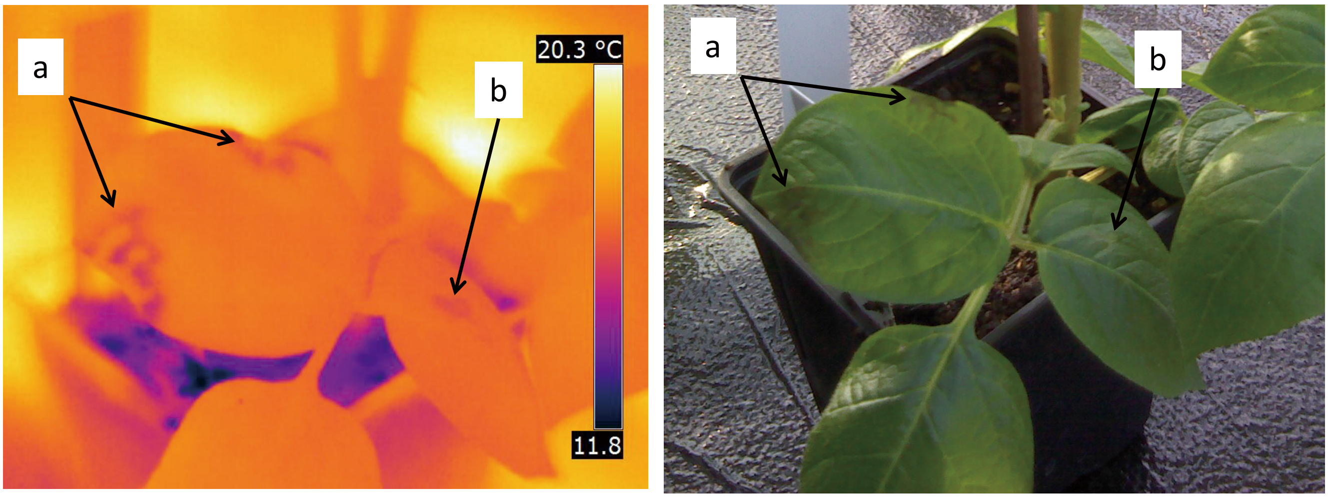
With expected climate change scenarios [74], it is forecast that heat stress is likely to become an increasingly serious factor affecting world agriculture. Infrared thermometry and thermography have been proposed as useful tools for monitoring crops for their tolerance of high temperature stresses [56,75], though thermal responses to heat stress are not particularly different from thermal responses to other abiotic stresses. Canopy temperature has been used as an indicator of heat tolerance [67] and heat tolerant lines in wheat have been shown to possess lower canopy temperature than susceptible lines [76]. Under drought conditions cooler canopy may be linked to increased root depth, but under hot conditions this can be due to improved root capacity permitting higher transpiration rate [76,77]. Therefore, canopy temperature can usefully be used as an indicator for improved physiology under a range of stress conditions.

Infra-red thermography also provides a particularly powerful tool for studying the freezing process in plants because the exotherm generated as water turns to ice heats up the tissue in a way that can be readily detected by IRT [77–83]. This allows one to follow the dynamics of freezing in different tissues and genotypes so that it should be possible to devise screening procedures to detect genotypic differences in sensitivity to freezing temperatures, and also to investigate the role of nucleating agents such as the ice-nucleating bacteria in determining frost sensitivity. As an example the roles of extrinsic and intrinsic nucleating agents have been investigated in beans, where the rates of propagation of freezing events and their relationship to plant structure have been successfully visualised [78,84].

3.2. Screening for Biotic Stress

Infection by various pathogens can lead to a number of physiological changes including stomatal closure and alterations in water status as well as changes in respiration and other metabolic processes that should be detectable by thermal sensing. The dynamic and spatial variation in stomatal conductance, and hence temperature, is particularly useful for the non-invasive detection of disease before visual symptoms are apparent [85–89]. Thermal imaging can be used as a diagnostic in cases where the dynamics of thermal response are characteristic of specific diseases with some diseases causing transient decreases in water loss while others may cause increases [8,90]. Figure 3 illustrates an example where the presence of potato blight could be detected by using thermal imaging with confirmed validation of affected area in top part of leaf and pre-symptomatic area highlighted as an example for diagnosis before visual symptoms were apparent. Although primarily used in the laboratory, thermography can also help in field scale monitoring of disease and for forecasting epidemics and prediction of yield loss at an early stage and possibly also for targeted, plant- or site-specific fungicide application as and when needed. Although thermography can potentially be a useful component of monitoring or diagnostic systems for biotic stresses, its application to high-throughput screening for resistance to any of the specific biotic stresses discussed below will require careful design of the screening protocol in order to avoid confounding by genotypic differences in stomatal behaviour unrelated to the biotic stresses. As thermography is highly sensitive to environmental conditions during measurement, and as thermal effects of disease are generally small, it will be crucial to ensure that effective normalisation of data is adopted, while there will commonly also be a need to combine multiple sensors such as thermal, hyperspectral and fluorescence [91–94] to allow effective discrimination of disease from other possible factors affecting temperature. For some diseases, further information may be obtained from the temperature dynamics by means of frequent observations on the same material. For convenience we discuss below the applications of thermography to above-ground and soil-borne diseases separately, even though the distinction is not always clear cut.

Figure 3. Illustration of potato late blight detection by using of thermal imaging. Area marked "a" shows confirmed blight area for validation. Area marked "b" highlighting pre-symptomatic area and its temperature variation for diagnosis before visual symptoms are very prominent. Images taken with a FLIR E50 thermal camera (T_{air} = 18 °C; relative humidity~50%), with the average temperature of the infected area averaging 16.1 °C and dead tissue in infected area averaging 15.4 °C.



In addition, it should in principle, also be possible to detect pest infestations using thermal imaging where pests affect canopy transpiration, and there have even been a few reports where large scale and very damaging infestations have been detected remotely (e.g., [95]), in general the sensitivity for phenotyping is likely to be rather small if only thermal sensing is used as many other factors also affect temperature so that a multi-sensor approach will almost certainly be needed for a practical method [96].

3.2.1. Pathogens Affecting above Ground Parts

Thermography can be useful in monitoring plant disease development based on the local temperature changes resulting from either plant defence mechanisms or disease impact. Both spatial and temporal variation in temperature can be detected with the temperature response depending on the type of infection. Temperatures may decrease in diseased parts where the disease causes an increase in water loss due to processes such as cuticle damage, changes in underlying leaf cell membrane permeability or to cell death, or else temperatures may increase where the disease closes stomatal closure as a result of decreased xylem water flow or the release of certain closure-inducing chemicals [8,86,88,90,97–99].

The hypersensitive response followed by induced cell death is illustrated in tobacco infected by tobacco mosaic virus (TMV), where the characteristic temperature dynamics can be diagnostic [90,92]. In this case an increase in temperature resulting from stomatal closure was apparently caused by an accumulation of salicylic acid at the infection site; this was followed by a decrease in temperature resulting from cell death as visible necrotic lesions developed. Similarly, IRT has also been used under controlled conditions to identify areas infected by downy mildew before the visible symptoms appeared [99] and under field conditions to detect *Plasmopara viticola* in grapevine [100] and *Venturia inaequalis*, in apple [101].

3.2.2. Soil Borne Diseases

Relatively little research has focused on use of the IRT for soil borne diseases such as root rots and wilts, though as they restrict the water uptake by roots they would in turn cause stomatal closure and increased canopy temperature [89]. Leaf temperature can not only be used to detect the presence of disease but also to monitor disease severity in individual plants either under controlled or field conditions without the need for destructive visual inspection of roots. For example, cucumber plants infected with *Fusarium oxysporium* showed increased temperatures as compared with controls well before the symptoms appeared visually [102], and these responses were related to stomatal closure induced by ABA in leaves. Root rot severity in beans has also been correlated with an increase in temperature [103]. Thermography has even been used using airborne sensors (when combined with narrow band hyperspectral and fluorescence studies) for the study of infection by *Verticillium dahliae* (causing *Verticillium wilt*) [104]. Thermal sensing, especially when used in combination with other optical sensing systems, therefore appears to hold promise as a tool for the screening of disease resistant cultivars.

3.3. Screening for Crop Yield and Quality

There is much interest in the development of methods for high-throughput screening of variety trials for crop yield. Although thermography has primarily been suggested as a tool for the study of variability in stomatal conductance, there are indications that temperature differences can, on occasion, be associated directly with commercial yield in a number of crops including cotton [105], wheat [56,57,70], rice [106,107] and potato [29]. As canopy temperature is primarily an indicator of stomatal conductance any association with yield tends to be indirect, though there has been an expectation that higher yields would be associated with higher conductance and hence higher photosynthesis. It is, however, necessary to treat these results with some caution, as under drought conditions it may be possible that the higher yielding genotypes may be those that conserve water by stomatal closure [2,108].

In a rather different approach, applicable specifically to crops with massive fruits, such as citrus or apple, thermal imagery has been used to count fruits as an indicator of crop yield [109,110]. The basis of such measurements has been the use of image analysis algorithms to identify fruit as those parts of an image that are warmer than the general canopy after exposure to sunlight. We would suggest that such approaches would need some supplementary RGB imagery and/or thermal dynamics to increase their reliability to a level that would be useful in practice. It is also worth noting that thermal imaging has been proposed as a tool for assessment of quality and especially the detection of internal bruising for massive fruits and root crops, by making using of differing thermal time-constants of healthy and damaged tissues [111,112].

4. Conclusions and the Future

Phenotypic and crop monitoring tools are still at an early stage of development in collating data from large number of genotypes and varieties under field conditions and further improvement will depend on improvements in automation of data collection and analysis. A number of initial steps have been made in the automation of image analysis, particularly those based on the use of vegetation indices [113] or temperature thresholding [27,42,114] to allow automatic separation of canopy from background soil or sky. Similar approaches are available for airborne and satellite imagery [10]. All these approaches allow one to identify a vegetation mask from a multispectral image allow one to extract only the canopy temperature from an overlaid thermal image using remote sensing software (e.g., ENVI (Exelis Visual Information Solutions, Boulder, CO, USA), ERDAS-IMAGINE (Intergraph Corporation Part of Hexagon, Huntsville, AL, USA) or Matlab (Mathworks, Cambridge, UK)). Automatic registration of optical and infrared images has been used to construct an automated irrigation control system where plant water information was sensed by thermal imaging [115–117]. Wang and colleagues developed fully automatic image registration algorithms for the alignment of optical and IR image pairs, where Pearson's cross-correlation between a pair of images was used as the similarity measure, and have made it available as a packaged software application [115,116]. Other workers have developed procedures based on unsupervised classification of colour images to analyse images where thermal and visible images were obtained synchronously [117]. Such methods drastically reduce time required for image analysis and eliminate any subjectivity due to operator inputs, but although there have been a number of recent examples of the use of semi-automated analysis procedures for temperature studies [29,69,118], there is still a need for the development of more user-friendly and robust automation. More work is needed so that automation is applied for image capture and its analysis not only to exclude the variability due to background noise but integrate this with soil spatial analysis and meteorological data to generate appropriate stress indices at different locations and among different genotypes for phenotyping. In conclusion we have shown the enormous potential of thermal sensing for high-throughput screening, not just for abiotic stresses but also for biotic stresses because it is sensitive and can be rapid and non-destructive. The key precautions that must be adopted if thermography is to provide a reliable high-throughput phenotyping tool have been outlined, especially the need to account for variation resulting from environmental variation (e.g., differences in exposure, incident radiation, *etc*.) and the use of appropriate normalisation techniques. Although the automation of the processing and analysis is being currently addressed, further improvement is still needed including the wireless networks and their commercialisation.

Unfortunately, a single sensor approach will always have limitations as stress is a complex trait and is not just influenced by one physiological or morphological component. In order to well define stress, a multi-sensor approach is needed where IRT is just one component and used in conjunction with other sensing techniques including fluorescence and spectral reflectance or absorbance. With advances in technology, the precision of imaging is improving and costs decreasing so that tools applicable to plant biology can be generated. The advancement of tools for plant biology will help plant scientists to ensure that agricultural production is sufficient to satisfy needs of human population increase under changing climatic scenarios by improving the phenotypic prediction and link with the genotypic composition of sustainable breeding.

Acknowledgments

We gratefully acknowledge the financial support of the Scottish Government Rural and Environmental Science and Analytical Services (RESAS) Division and thank colleagues at The James Hutton Institute for critical reviewing of the manuscript. We are also grateful to Brian Harrower for supplying plants infected with late blight.

Author Contributions

Ankush Prashar and Hamlyn Jones, both contributed equally to the overall conception and writing of the article.

Conflicts of Interest

The authors declare no conflict of interest.

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