technical focus

Imaging techniques and the early detection of plant stress

By the time that it displays visible symptoms of stress, a plant can already be adversely affected. Current imaging techniques allow presymptomatic (Box 1) monitoring of changes in the physiological state of plants non-destructively. In this respect, thermal, reflectance and fluorescence imaging have proved their potential by detecting stress-related changes in the pattern of light emission from plant leaves. These techniques can be applied on scales ranging from microscopic observation to airborne remote sensing. Using these for crop monitoring would allow us to alleviate stress at an early stage, so avoiding irreversible damage and thus substantially reducing yield losses.

Several imaging techniques have been used to detect the early signs of stress by monitoring changes in water status, photosynthetic efficiency, accumulation of secondary metabolites or structural modifications[1–3]. Thermography, reflectance detection and fluorescence imaging are currently the most highly evolved of these techniques.

Thermography

Thermography allows the visualization of differences in surface temperature by detecting emitted infrared radiation [long-wave infrared (8–14 μm)]. Computer software transforms these radiation data into thermal images in which temperature levels are indicated by a false-colour gradient. Thermal imaging is well known for its use in human and animal medicine[4].

The temperature of plant leaves is determined by environmental factors and transpirational cooling (the outward latent heat flux)[5]. By contrast, metabolic activity has a marginal role in the energy budget[6]. Only so-called 'thermogenic' flowers, mostly members of the *Araceae* family, can regulate their temperature by controlled metabolic heating. The timing of this thermogenic effect and its precise location in the flower structure has been studied extensively using thermography[7]. As described below, changes in leaf temperature in non-thermogenic plants mainly results from alterations of transpiration in response to particular stresses. Thermography has also been used to study freezing processes and local leaf water content.

Transpiration and cooling

Modifications in the water status of a plant caused by adverse conditions lead to changes in leaf transpiration as a result of active regulation of stomatal aperture[1]. The associated changes in patterns of leaf cooling can be monitored instantly and remotely by thermographic imaging (Fig. 1).

This technique has a clear advantage over direct transpiration measurements by porometric techniques, which can only indicate the average increase in humidity of air circulated over an enclosed leaf. Porometric techniques either require repeated point measurements (diffusion porometry) or constant enclosure of a leaf in a measuring cuvette (gas exchange measurements). Such point or area measurements can be used for screening purposes but cannot reveal spatial heterogeneity. Imaging techniques intrinsically possess a high spatial resolution and thus the ability to visualize patterns and gradients in the parameters under study. Obvious drawbacks of direct transpiration techniques are the laborious, repetitive measurements on populations of plants and the possible interference with the natural behaviour of leaves caused by contact.

The flexibility of screening by thermographic imaging has been shown by the isolation of mutants affected in transpirational control from barley[8] and, recently, *Arabidopsis* (J. Giraudat, pers. commun.). In conventional breeding programmes aimed at selecting plants based on water use efficiency, thermography could improve the speed and effectiveness of monitoring transpiration. In a study on transpiration by conifer seedlings, carried out to assess vigour before planting[9], infrared thermography was found to have the potential to handle many seedlings in a short time by virtue of its non-contact approach. Moreover, thermography has the advantage that it can be automated[10]. Therefore, from a

Box 1. Glossary of terms

Biotrophic pathogen
A pathogen that lives inside intact cells.

Fluorescence transient
The evolution of fluorescence emission upon illumination of dark-adapted leaves.

Hypersensitive response (HR)
Reaction of a resistant plant to an incompatible pathogen, characterized by the death of infected cells, eventually forming necrotic flecks of dead tissue.

Hyperspectral imaging
Simultaneous measurements performed in narrow spectral bands of the order of tens of nanometres.

Multispectral imaging
Simultaneous imaging in different spectral regions (e.g. far infrared, near infrared, visual).

Necrotrophic pathogen
A pathogen that lives on dead cells.

Non-photochemical quenching ($\Delta F_m/F_m'$)
The decrease in chlorophyll fluorescence caused by photoprotective thermal dissipation of absorbed light energy. This parameter is calculated as ($F_m -$ ′ ÷ ′, where F_m is the fluorescence measured during the first saturating light pulse at the start of the fluorescence induction transient and F_m' is that measured during a later flash of saturating light. Thus, $\Delta F_m/F_m'$ represents the ratio of quenched to remaining fluorescence.

Non-saturating illumination (actinic light)
Illumination with an intensity typically <1000 μmol − −.

Photochemical quenching (Φ_{II})
The decrease in chlorophyll fluorescence caused by photochemical reactions leading to CO_2 fixation. Calculated as (F_m' − × ÷ ′, where F_m' and F_s are the fluorescences measured under saturating and non-saturating illumination, respectively, and R is the ratio of saturating to non-saturating light. This parameter is the ratio of the difference between 'maximum' fluorescence at saturating light and steady-state fluorescence at non-saturating light to the 'maximum' fluorescence at saturating light. A high Φ_{II} means efficient photosynthesis.

Presymptomatic
In this context, before the appearance of visual symptoms.

Saturating illumination
Illumination with an intensity typically >2000 μmol m− −1.

Variable chlorophyll fluorescence ratio (Ratio of fluorescence decay: Rfd)
This is defined as ($F_m -$ ÷ F_s, where F_m is the maximum fluorescence intensity attained early during the fluorescence induction transient and F_s the final steady-state value of fluorescence reached upon activation of photosynthesis.

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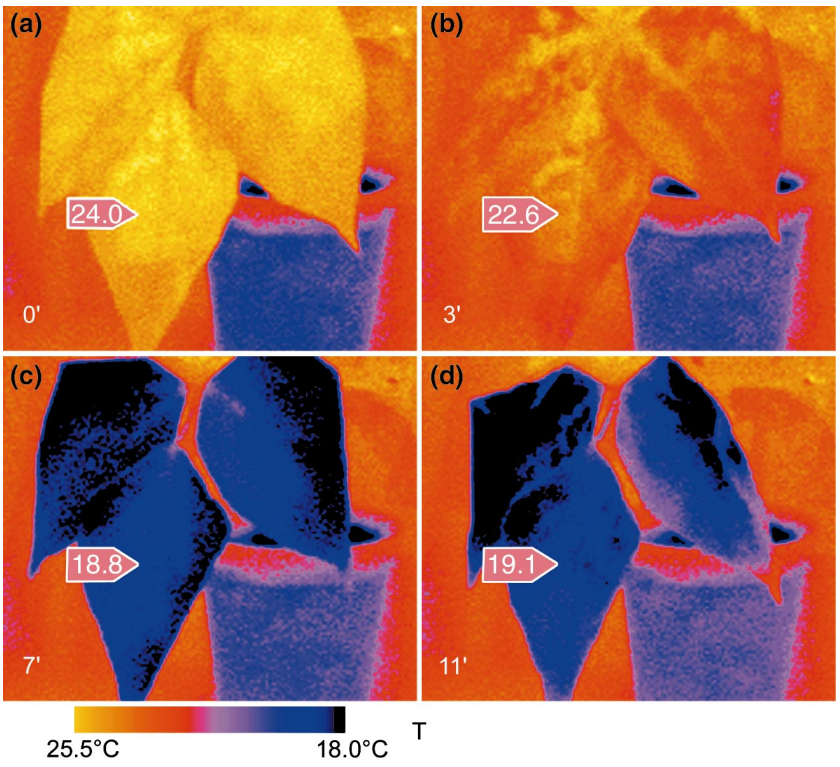

Fig. 1. False-colour thermal infrared images of a first trifoliate leaf of French bean showing the 'Iwanov' effect. This response results from severing the leaf from the plant and consequently from its water supply, which was done 10 s after image (a) was taken. First, a rapid cooling is apparent, characterized by initial stomatal opening (b,c). The onset of rewarming, which is starting in (d), is due to stomatal closure as the leaf water status declines. Time indication is in minutes; the average temperature of a 1 cm^2 region on the leaf surface is shown on each image by the arrow. *Modified, with permission, from Ref. 11.*

biotechnological perspective, it can be used as an efficient method to screen populations of transgenic or mutant plants.

Stomatal conductance is a measure of transpiration. Values for this parameter calculated from thermographic measurements were shown to correlate well with values obtained using a diffusion porometer[11]. This will probably further stimulate the use of thermography in the remote assessment of plant transpiration. In addition, the combination of gas exchange and thermographic measurements is a powerful approach to 'calibrate' thermographic data before undertaking large-scale screening projects. A setup that allows simultaneous thermographic and gas exchange measurements from the same leaf area has been developed to prove the linearity of the inverse relationship between leaf temperature and transpiration[12].

Ever since portable thermal imaging systems became available, thermographic research has been carried out on two scales. On the field scale, the feasibility of thermographic detection of emerging pathogen attacks by remote sensing was established several decades ago[1,13]. In the early 1990s, a hypothesis was proposed that linked stomatal closure caused by limited damage before the disease was visible with increased canopy temperature[13]. At the plant scale, this hypothesis was proved to be true for different plant–pathogen systems[10,14]. In a study on the hypersensitive response (HR) of resistant tobacco plants to tobacco mosaic virus (TMV) infection, a presymptomatic increase in leaf surface temperature was visualized[10]. By contrast, in anterior experiments under less strictly controlled conditions, detection was only possible after appearance of visible symptoms[10].

By using continuous gas exchange measurements, the increase was shown to correlate with localized stomatal closure, possibly driven by HR-associated synthesis of the phenolic compound salicylic acid. The thermal effect expanded rapidly compared with the slower extension of colocalized cell death (Fig. 2). The final sizes of thermal and subsequent visual effects were found roughly to coincide, supporting the notion that the thermal effect provides a 'preview' of the final extension of necrosis. In a subsequent study on tobacco and *Arabidopsis* cell death mutants that spontaneously form lesions resembling a pathogen-induced HR, a thermal effect was detected before visual damage became apparent[15]. Moreover, areas of impending cell death contrasted strongly with the surrounding unaffected tissue because of the evaporation of leaking cell contents and concomitant cooling. Thermographic monitoring could thus be applied to guide precise sampling in studies concerning the evolution of *in vivo* plant cell death and associated oxidative stress. Such applications can be envisaged for all of the imaging techniques here described.

Many substances, including atmospheric pollutants, can affect stomatal aperture. The closing effect of SO_2 and NO_2 on stomata and the heterogeneous distribution of the effect on the leaf were visualized by thermography[16]. In this study, the presumed relationship between stomatal aperture and local leaf temperature was confirmed by direct visualization with electron microscopy. Also on the microscopic scale, continuous recording (over a period of several days) of stomatal movements in a gas exchange chamber[17] would probably allow the reaction of individual stomata to environmental stresses and infections to be seen. A thermographic setup with a resolution of 0.002°C was developed to assess the metabolic effect of pharmacological compounds on animal cell cultures[18]. It might be possible to use this system to screen for changes in metabolic reactions of plant cell cultures upon stress.

Freezing and heating

As mentioned above, transpiration causes leaf cooling because latent heat is used to evaporate water. By contrast, the freezing of water releases latent heat and produces an increase in temperature. Freezing processes in plants are efficiently visualized by thermography and can be used to test frost protection strategies[19]. Transient heating permits thermographic detection of local differences in heat capacity, which are linked to tissue structure or composition, by monitoring the speed of either warming or subsequent cooling. This 'active' thermographic technique has been used in medical applications, in post-harvest processing and in agricultural robotics[4,20,21]. The method has been further extended using a promising approach in order to visualize water distribution in plant leaves[12].

The technique allows monitoring of the temporal evolution of water content, which is impossible with the commonly used destructive methods. By feeding data from a thermal infrared image sequence of a periodically heated leaf into a mathematical model, images were generated that indicate the distribution of heat capacity over the leaf surface. Veins have a higher heat capacity than the surrounding tissue owing to a higher water content per unit leaf area. Therefore, they show a slower reaction

to temperature changes, as visualized by a larger phase shift between the applied periodic heating and the measured increase in leaf temperature (Fig. 3). The mapping of heat capacity by 'active' thermography has the potential to monitor changes in leaf thickness during growth, albeit under strictly controlled conditions. This technique might also allow the visualization of internal structural heterogeneity of plant leaves resulting from responses to infection.

In conclusion, active thermography permits non-destructive determination of local leaf water content, whereas passive thermography measurements can be used to estimate stress-related changes in the amount of water transpired (Table 1). Although passive thermography can be used to locate emerging disease outbreaks, as shown by its current use in aerial remote sensing at the field scale (H. Nicolas, pers. commun.), it does not characterize the stressor. To identify the pathogen implicated, additional tests must be carried out. When deploying thermography at field scale, its sensitivity to changing environmental conditions should be taken into account[1,13]. Thermography under controlled conditions (e.g. monitoring diseases in greenhouses) is less affected by these factors and therefore certainly applicable.

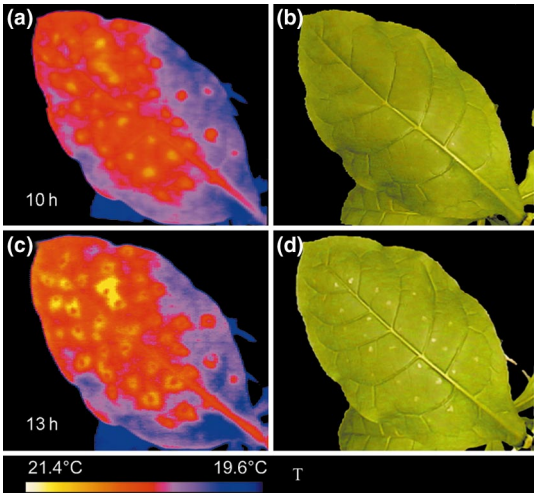

Fig. 2. Thermographic visualization of the local resistance response of tobacco to tobacco mosaic virus infection as spots of higher temperature on an attached leaf. (a,b) Images taken 10 h after a temperature shift. (c,d) The expansion of the thermal effect and cell death 3 h later. Images a and c show pseudo-colour thermal images; b and d show the real-colour reflection images. A movie of the evolution of thermal and visual symptoms, and a description of the setup can be seen at http://www.plantgenetics.rug.ac.be/~lacha (Ref. 10). To synchronize the appearance of cell death at all infected locations, plants were grown for 38 h at 32°C and then transferred to 21°C.

Reflectance and near-infrared imaging

In addition to influencing stomatal resistance, infections, toxic compounds and adverse growth conditions can also induce changes in surface and internal leaf structure, cause the accumulation of secondary metabolites or lead to the breakdown of photosynthetic pigments[1,2]. Structural alterations modify the reflection of light from plant leaves or canopies. Factors leading to a decrease in light absorption automatically increase light reflection and vice versa. These changes can be visualized by reflectance imaging, either in the visible spectrum or at near-infrared wavelengths undetectable by the human eye (0.7–1.3 μm)[1,2] (Table 1). As an example, treatment with the phytotoxic air pollutant ozone resulted (in some species) in an increase in reflectance of visible light, which is associated with visible damage, or in a presymptomatic decrease in near-infrared reflectance[22].

Near-infrared imaging permitted disease-affected trees to be told from healthy neighbours[23,24], and high-resolution near-infrared images enabled the detection of in-field spatial variability in soil type, crop nutrient stress and yield[25]. Information about these factors is important, because soil temperature, water status and nitrogen availability can mask the effect of pathogen attacks and thus hamper their detection. Obtaining an overview of spatial in-field variability would thus be a prerequisite for site-specific disease management. This will be important in the context of precision agriculture, where different imaging techniques could be combined into a multispectral visualization approach (H. Nicolas, pers. commun.). Hyperspectral reflectance imaging detected herbicide-induced stress in pine several days before the first visible signs of damage[26]. By contrast, thermography could not detect any presymptomatic change in temperature, which was attributed to rapidly varying environmental conditions. In summary, reflectance imaging clearly permits a wide variety of stresses to be detected. Hyperspectral reflectance imaging is a promising way to detect specific signatures for a particular stress and a given plant species[2].

Fluorescence imaging

Visible light energy absorbed by plant leaves is either used for photochemical reactions (and thus ultimately for CO_2 assimilation) or dissipated as heat and fluorescence[3]. Although the proportion of these three different processes changes as part of the normal function of the leaf, stress also has profound effects. Under adverse circumstances, patterns of chlorophyll fluorescence imaged upon excitation by visible or UV light have an indicator function[27] (Table 1). Upon stress, chlorophyll can also be shielded from the excitation light by the accumulation of light-absorbing secondary metabolites[28]. Importantly, the intensity of the fluorescence emission is much lower than that of the excitation light reflected by the leaf. Thus, special care has to be taken to discriminate selectively between the fluorescence emission wavelengths and the reflection of excitation wavelengths from the leaves. This is even more of a challenge when detecting the fluorescence induced by the continuous visual spectrum of solar light[28].

Basics

On a laboratory scale, a so-called fluorescence transient is generally induced in dark-adapted leaves. In these circumstances, the fluorescence emission rises rapidly from a minimal level and attains a maximum (F_m) shortly after the start of illumination. Thereafter, the signal declines because of quenching, which can be mediated by both photochemical (use of light energy for CO_2 assimilation) and non-photochemical (photoprotective) reactions, until a steady-state value (F_s) is reached[29,30]. Fluorescence measurements during saturating light pulses, which cause photochemical processes to come to a temporary halt, are used to quantify differences in non-photochemical fluorescence quenching ($\Delta F_m/F_m'$). In combination with measurements in non-saturating light, the photochemical quenching (Φ_{II}) is calculated. This is one of several ways of extracting information from the polyparametric fluorescence signal[29]. Physiological parameters characterizing the studied stress factor must be determined in order to calibrate the fluorescence intensity of the image pixels for subsequent interpretation[28]. Fluorescence imaging approaches have been used to monitor abiotic stresses and viral or fungal infections.

Using UV-laser illumination (laser-induced fluorescence imaging system, or LIFIS)[3,27] permits the simultaneous capture of fluorescence emission from four spectral bands (blue, green, red and far-red) by excitation with a single wavelength (e.g. neodymium-yttrium aluminium garnet (Nd–YAG) laser, $\lambda = 355$ nm). An increase in blue and green fluorescence is an early indicator of abiotic and biotic stress that is correlated with the accumulation of phenolic secondary metabolites in the epidermal cell layers[27] (Fig. 4). The combined blue and green fluorescence

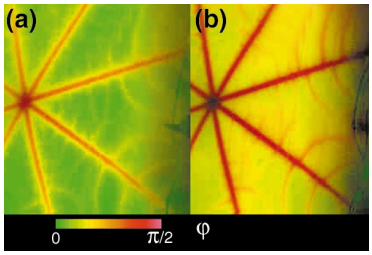

Fig. 3. Images showing the distribution of water content in a *Ricinus* leaf, calculated from data from active thermography experiments using periodic fluxes of heat radiation. The images represent two parameters. The magnitude of the phase shift φ between the period of leaf temperature increase and the period of input heat flux is indicated by the colour bar. The local intensity of the image (amplitude parameter) is a measure of the local temperature increase. The input heat-flux period was set to 4 min (a) or 2 min (b). From the pictures, it is clear that veins have a stronger phase shift than interveinal tissue, which indicates a higher heat capacity and thus a higher water content, resulting in slower warming. Shorter heating intervals lead to a more pronounced phase shift and a lower intensity.

12 http://klimt.iwr.uni-heidelberg.de/~bkuemmer/thermography/riziper.html.

Table 1. Visualization techniques and monitored parameters

Imaging technique	Energy input	Energy output	Visualized parameter	Ref.
Passive thermography	Stomatal aperture determines energy loss	Long-wave infrared radiation 8–14 μm	Transpiration	10,11
Active thermography	Heat pulses (long-wave infrared radiation)	Long-wave infrared radiation 8–14 μm	Heat capacity indicating water content	12
Near-infrared reflectance imaging	Near-infrared light	Near-infrared reflection 0.7–1.3 μm	Near-infrared scatter (reflection)	2
Reflectance imaging	Visible light	Visible reflection 400–700 nm	Visible scatter	2
Fluorescence imaging	Visible light	Fluorescence 600–800 nm	Chlorophyll fluorescence	27
UV-induced fluorescence imaging	UV light	Fluorescence 600–800 nm 400–600 nm	Chlorophyll fluorescence Blue-green fluorescence	27

emission can also be used as an internal standard to quantify the variation of chlorophyll fluorescence during short-term heat stresses or herbicide treatments. In addition, blue–green fluorescence intensity is inversely dependent on leaf temperature and has been proposed as a remote sensing thermometer[28]. Thermography could be used to calibrate these fluorescence measurements to detect increases in leaf temperature reminiscent of water stress.

Heavy metals

Chlorophyll contents can be assessed by determining the ratio of the emission in the red and far-red bands[27]. Photosynthetic activity is proportional to the decline in chlorophyll fluorescence during the fluorescence transient, as indicated by the variable chlorophyll fluorescence ratio (Rfd) (Fig. 4). Visible-light-induced chlorophyll fluorescence imaging of leaves on cadmium-treated bean and copper-treated tobacco plants revealed spatial heterogeneity in Rfd before symptoms linked to stress caused by this heavy metal became apparent[31]. In addition, UV-laser-induced fluorescence indicated an increase of phenolic compounds as a reaction to cadmium uptake (R. Valcke, pers. commun.). Similarly, apple fruit deterioration has been detected before the presence of visual symptoms using UV-laser-induced fluorescence imaging (R. Valcke, pers. commun.).

Virus infection

Early fluorescence imaging techniques have already been successful in presymptomatic monitoring of fluorescence patterns on virus-infected susceptible plants, long before any visible damage appeared[27]. More recently, chlorophyll fluorescence imaging has also enabled the early visualization of differential patterns of photosynthesis around sites of viral infection in such plants[32]. In another study, the extent of infection was already apparent before symptoms developed[33], which is comparable to the results obtained by thermographic monitoring of TMV infection on a resistant plant[10].

Infection of susceptible tobacco with TMV results in the development of visible chlorotic mosaic symptoms. The earliest indication of infection in tissues that ultimately became chlorotic was a reduction in chlorophyll fluorescence, which was caused by virus-induced non-photochemical quenching and was not associated with chlorophyll breakdown[34]. By contrast, thermography was unable to detect any difference in surface temperature of infected susceptible plants, indicating that transpiration (and stomatal aperture) presumably remained unaffected[10]. Fluorescence imaging would be well suited to study the evolution of chlorophyll activity during early senescence of (mutant) plants. Classical spectrophotometric techniques are destructive and can only measure chlorophyll content without reference to its activity.

Fungus infection

Chlorophyll fluorescence imaging was also successful in monitoring fungal infections, which are by far the most yield-limiting biotic stresses in cereal production. Biotrophic pathogens, such as rusts and mildews, generally induce a decrease in photosynthesis[35]. Incipient lesions at the loci of fungal rust infection of *Phaseolus* bean were visualized by an increase in chlorophyll fluorescence emission[30]. However, this phenomenon was limited to the initial stages of the fluorescence transient. Importantly, infected leaves are extremely heterogeneous: they consist of invaded tissue, affected but uninvaded tissue and unaffected tissue.

Rust-infected oat leaves are a typical example of this heterogeneity. Quantitative fluorescence imaging was used to elucidate local changes in plant metabolism[36]. A clear temporal evolution was seen in the pattern of non-photochemical quenching ($\Delta F_m/F_m'$) from the centre of the infection zone towards its edges, under steady-state conditions (Fig. 5). Moreover, even for the uninfected part of the leaf, this parameter was significantly different when comparing control and infected leaf. The evolution of Φ_{II} was less striking than the changes in $\Delta F_m/F_m'$ and did not follow a complementary pattern, presumably because of damage to the photosynthetic system at these stages of the infection.

The same approach was used to image the upper side of *Arabidopsis* leaves infected with *Albugo candida*. Presymptomatic patterns of $\Delta F_m/F_m'$ and Φ_{II} were shown to be colocalized with the subsequent expansion of infection[37].

Importantly, there were no visual signs of disease on the upper leaf side at these early time points (Fig. 5). In this example, the patterns of these two quenching parameters were complementary, indicating that the light energy that was not used for CO_2 assimilation (as shown by a decrease in Φ_{II}) was dissipated as heat (indicated by an increase in $\Delta F_m/F_m'$). CO_2 assimilation was determined simultaneously by gas-exchange measurements. These measurements and independent fluorescence data obtained with a portable fluorometer were used to calibrate the Φ_{II} measurements.

Limitations

Chlorophyll imaging systems can achieve high resolution, which is necessary to study the spatial heterogeneity of leaves. However, the area that can be analysed is generally restricted to a few square centimetres. Recently, a cheap system using stroboscopes has been designed to permit measurements of chlorophyll *a* fluorescence on large leaf areas or concurrently on multiple samples[38].

Most of the described fluorescence imaging approaches are laboratory-based. For rapid screening of plant populations, non-imaging point measurements using portable fluorometers are currently the appropriate tool[29]. In the future, portable fluorescence imaging could be used for the field-scale assessment of infections, even for those that leave no visible trace[32]. As is the case for thermography, using fluorescence imaging on the field scale can only provide a non-specific indication of stress. Additional in-field tests will thus be necessary in order to identify the stress factor.

Multispectral approaches to stress detection

The three imaging techniques described above each have their pros and cons as well as their specific applicability. Thermography monitors plant transpiration remotely. This process is linked with nutrient uptake by the roots and, ultimately, with crop productivity, but it also reflects water use efficiency. Adverse effects that lead to a decrease in photosynthesis, which clearly limits crop yield, are readily visualized by fluorescence or reflectance imaging. In order to provide an early warning signal for a wide range of stress factors, a combination of the available techniques is the approach of the future.

As a preliminary example, thermography in combination with hyperspectral reflectance imaging was recently used for aerial remote sensing. Each of the techniques permitted cartography of disease outbreaks at a different stage of growth (H. Nicolas, pers. commun.). Fluorescence imaging, although needing supplemental illumination (UV, white or red light), should enable early detection in all cases of stress in which photosynthesis is locally impaired and/or secondary metabolites accumulate. Simultaneous laboratory-scale measurements of infection processes using thermography and chlorophyll fluorescence would make it possible to define the applicability of both techniques to the detection of different categories of plant–pathogen interactions (e.g. biotrophic, necrotrophic).

At the microscopic level, confocal fluorescence imaging of leaves infected by pathogens transformed with green fluorescent protein or red fluorescent protein enables real-time colocalization of pathogens and symptoms[33]. Reactions to numerous biotic and abiotic stresses are characterized by changes in the concentration of Ca^{2+}, a ubiquitous second messenger[39]. The evolution of local changes in Ca^{2+} can be revealed by visualizing bioluminescence in transformed plants expressing aequorin, a protein that emits light upon binding Ca^{2+}. Although limited to transformed plants[3], this method could be complemented by microscopic imaging of chlorophyll *a* fluorescence, also permitting detection of early cellular responses to stress[40].

Multispectral imaging in plant sciences is generally linked to remote sensing. The precise alignment of images taken in the different spectral regions could become increasingly important at the laboratory scale. Other techniques, such as *in vivo* nuclear magnetic resonance imaging[1,9], also hold promise for integration into a multispectral approach. It is important that the data obtained by imaging techniques are calibrated against established, classical measuring procedures. By doing so, a reliable interpretation can be guaranteed, enabling appropriate decision-making in field-scale applications.

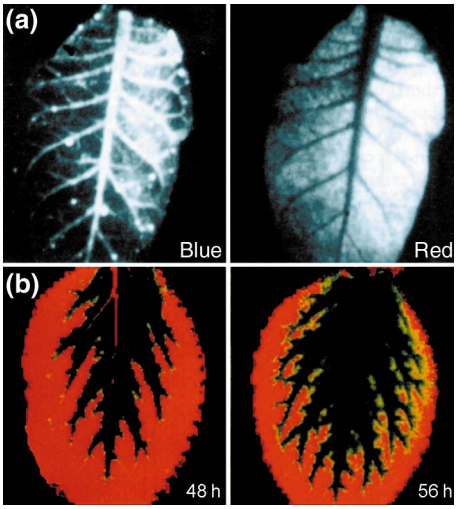

Fig. 4. (a) Grey-scale intensity images of blue and red fluorescence from a tobacco leaf affected by white fly. At the sites of punctures made by the insects, phenolic compounds accumulate, as shown by spots of higher intensity in the blue fluorescence image. When comparing the intensities of both images, red fluorescence is associated with interveinal tissue (this indicates chlorophyll content). By contrast, blue fluorescence emanates mainly from the veins of the leaf. (b) False-colour images showing the decrease in variable chlorophyll fluorescence ratio (Rfd; see Box 1) during the progressive uptake of the herbicide diuron (DCMU) by attached *Digitalis* leaves. The Rfd is an indicator of photosynthetic activity. When DCMU inhibits photosynthetic electron transport, photochemical quenching of photosynthesis does not occur and the decrease in fluorescence from maximum to steady-state emission diminishes. Red represents a high Rfd and hence normal photosynthesis; dark blue indicates fully inhibited photosynthesis and thus a low Rfd. Images were taken at 48 h and 56 h after treatment. *Reproduced, with permission, from Ref. 27.*

Possibilities for remote sensing on the field scale

Comparing infected loci on individual leaves is similar to detecting stress areas in healthy canopies. Diseases characterized by spot-wise initiation[13] and subsequent spreading of the symptoms could be detected by remote sensing and accurately treated. Quantification of infection is generally more efficient by the aforementioned imaging techniques than by visual assessment, even when initial visual symptoms are present[1]. Both thermal images and non-photochemical quenching images can 'magnify' colocalized visual symptoms (Figs 2 and 5), which would ease the detection and confinement of disease outbreaks at field scale. The use of satellite imaging (e.g. the multispectral Landsat Thematic Mapper) permits an overview of vast areas but is limited by insufficient resolution[1,13]. In this respect, aerial measurements are better and can be applied in a flexible way.

Imaging of chlorophyll fluorescence emission of plant canopies under natural conditions could take advantage of the changes in light intensity during a day, because the evolution of fluorescence emission as a function of light intensity indicates the vitality of plant leaves[41]. Multiangular remote sensing, which measures reflectance under multiple view angles, might also be useful to detect stress at an early stage by revealing changes in leaf area and leaf angle distribution (H. Jones, pers. commun.). Combining these techniques into a multispectral approach, possibly also including radar and microwave imaging[1], would permit site-specific crop management. This should enable

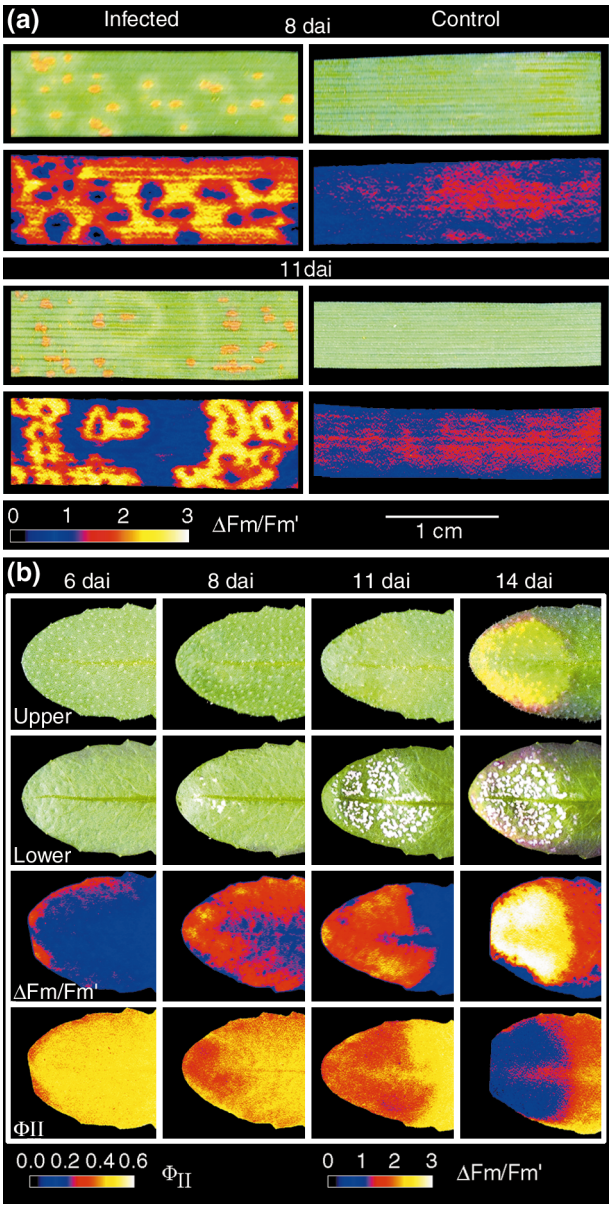

Fig. 5. (a) Natural-colour reflection images (upper row for each time point) and pseudo-colour images of non-photochemical fluorescence quenching ($\Delta F_m/F_m'$; see Box 1) (lower images for each time point) derived from steady-state fluorescence measurements of oat leaves showing crown rust caused by *Puccinia coronata*. The $\Delta F_m/F_m'$ has a high spatial heterogeneity associated with the infection loci and changes profoundly during the infection process. Compared with the natural-colour reflection images, the $\Delta F_m/F_m'$ images of the infected leaves show alterations over the whole leaf [8 days after infection (8 dai)] or over a larger area of the leaf (11 dai), when taking the images of the control leaf as a reference. This could be advantageous in remote detection of disease outbreaks at field scale. *Modified with permission from Ref. 37.* (b) Natural-colour reflection images (top) and images of non-photochemical fluorescence quenching ($\Delta F_m/F_m'$) and photochemical efficiency (Φ_{II}). The images are of *Arabidopsis* infected with white blister rust (caused by *Albugo candida*) at 6, 8, 11 and 14 dai. The fluorescence parameters were calculated from steady-state fluorescence measurements. Both fluorescence quenching image series show local changes, colocalized with white blister formation at the lower side of the leaf, before disease symptoms on the upper side become clearly apparent. *Modified with permission from Ref. 36.* The imaging setup used[36,37] allowed the contributions of photochemical and non-photochemical quenching to be separated by independent calculation of Φ_{II} and $\Delta F_m/F_m'$ from images taken under saturating and non-saturating illumination. The reflection images of the upper and lower leaf side were taken after detaching the leaves, with the sole purpose of visualizing disease extension, and are not indicative of the fluorescence measuring conditions.

an objective identification of needs and thus avoid excessive pesticide and fertilizer treatment[2,13,28].

Future perspectives

An increased demand for remote monitoring of temperature in industrial processes has led to the development of simpler, cheaper thermal imaging systems. This should open the way to an increasing number of thermographic applications in agricultural robotics[21]. Chlorophyll fluorescence imaging can become a general biosensing technique (fluorosensing) for stress detection in plants by applying it at field scale using either long-range laser or solar excitation[28]. Phenotyping of transgenic plants, post-harvest monitoring of fruits and vegetables, and the early detection of infections in greenhouses are all within reach. Robot-mounted multispectral vision systems, either ground-based or airborne, could become an asset in 21st-century precision agriculture.

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An inordinate fondness for Northern blots

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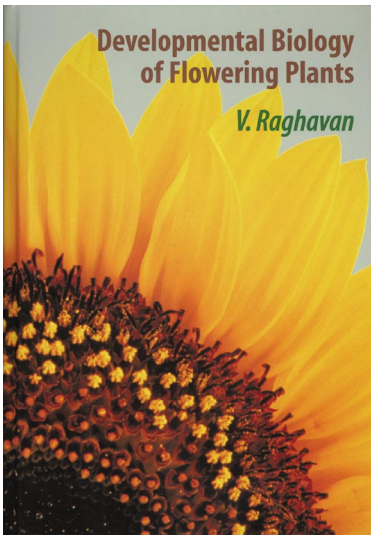

With well over 300 000 known species, beetles form by far the largest order of animals, but the reason for their evolutionary success has remained enigmatic to date[1]. When the famous British biologist John B.S. Haldane was asked by some theologians what insights his studies of creation had given him into the nature of the Creator, he is said to have replied 'An inordinate fondness for beetles'.

I am tempted to use similar aphorisms to explain what insights my reading of Val Raghavan's book have given me into the intentions of the author. A major drawback of the book is the type and quality of the figures (157 illustrations in total), and I asked myself how did the author make his selection. One idea that came to mind is an 'inordinate fondness' for raw data, especially Northern blots. Northern blots are the series of fuzzy-black bands on dirty-grey backgrounds that give information about the spatial or temporal expression pattern of a gene. They can be extremely revealing in a specific experimental context. However, they are bad in outlining general concepts, and even worse in conveying enthusiasm about developmental biology to students.

Writing a book for students of plant biology at the advanced undergraduate and graduate level was a major intention of the author. In my view, such a book should have two major goals. First,