THERMAL AND OTHER REMOTE SENSING OF PLANT STRESS

H. G. Jones[1*] and P. Schofield[1]

[1]Division of Plant Sciences, University of Dundee at SCRI, Invergowrie, DUNDEE DD2 5DA, Scotland

Summary. In this paper we outline the ways in which thermal and spectral remote sensing can be used to diagnose and monitor effects of environmental stresses on plants. Following an introduction to the theory and practice of using thermal sensing to study plant water relations and stresses involving stomatal closure, the discussion is widened to cover a range of imaging technologies. Particular emphasis is placed on the relationship between various stresses and responses detectable using imaging technologies. Various approaches for the combination of thermal remote sensing with spectral remote sensing, especially to distinguish plant from background, and otherwise to enhance the diagnostic powers of the technology, are outlined. The advantage of using a multi-sensor approach for stress diagnosis is highlighted and some possible applications summarized.

Key words: chlorophyll fluorescence, multi-angular sensing, spectral sensing, stress sensing, thermography.
Abbreviations: CWSI – Crop water stress index; ROS – reactive oxygen species.

*Corresponding author, e-mail: h.g.jones@dundee.ac.uk

INTRODUCTION

Imaging is a powerful technique for visualising, diagnosing and quantifying plant stresses. Here we give a broad introduction to the approaches that are now becoming available for the use of imaging techniques in studies of stress physiology and for application in crop management and especially in precision agriculture. The content of this paper draws heavily on the output from an EU-Research Training Network ("STRESSIMAGING" - HPRN-CT-2002-00254) which aimed to bring together contrasting imaging technologies to study a range of plant stresses; we acknowledge especially the contributions of all partners in this programme.

As illustrated in Fig. 1, there are wide ranges both of stresses that affect plant functioning and of ways in which the effects of these stresses can be detected my remote imaging. In principle we do not usually image the stress itself – what we most frequently study is a built-in plant response to the stress. For example, we can study stomatal aperture by the effect that it has on leaf temperature, but we need to remember that this response (changing stomatal aperture) can arise from a wide range of alternative stresses including those as different as drought, flooding, salinity, mineral toxicity or infection. Conversely, it is also important to note that any individual stress can affect a wide range of different processes. For example, drought not only leads to stomatal closure, but it decreases photosynthesis rate, causes reduced growth and leaf wilting, and can lead to loss of key pigments such as chlorophyll. All of these responses have the potential of being separately identified and used for stress diagnosis.

The key point to note is that many different stresses have common intermediate responses (signals) that can potentially be detected; therefore it is difficult to distinguish the causal stresses from a single signal alone. Particularly important among these intermediary responses are the secondary stresses such as enhanced production of free radicals and other reactive oxygen species (ROS) characteristic of oxidative stress. Where there is a need to diagnose or quantify the occurrence of different stresses this complexity suggests that it would be preferable to use a multi-sensor approach.

As we shall see in more detail below, and illustrated in Fig. 1, each imaging technique available is usually most appropriate for the study of specific individual intermediate responses (signals). The ambiguity, therefore, of any signal is more commonly related to the possible multiple causes of that specific signal, than to the sensing part of the measurement.

Thermal sensing

Thermal sensing is primarily used to study plant water relations, and specifically stomatal conductance, because a major determinant of leaf temperature is the rate of evaporation or transpiration from the leaf. The cooling effect of transpiration arises because a substantial amount of energy (the latent heat of vaporization, λ) is required to convert liquid water to water vapour, and this energy is then taken away from the leaf in the evaporating water and therefore cools it. In rare cases leaf temperature may be affected by other physiological processes: for example the heat generated (the exotherm) as water in a leaf freezes can be readily imaged (e.g. Wisniewski et al*.,* 1997), while in extreme cases of particularly high respiratory rates (e.g. as found in the *Arum* spadix) raised temperatures can be used as a measure of these increased respiration rates (Seymour, 1999). In most cases, however, the heat generated by respiration is too small in quantity to have a detectable effect on leaf temperature.

The main problem with the use of thermal sensing to estimate stomatal conductance or transpiration rate is that leaf temperature (T_l) at any time depends in a complex manner on air temperature (T_a), air humidity (e), wind speed (u) and absorbed net radiation (R_n) as given by equation 1 (see Leinonen et al., 2006)

$$T_l - T_a = [r_{HR}(r_{aW} + r_s)\gamma R_{ni} - \rho c_p r_{HR} D] / [\rho c_p[\gamma(r_{aW} + r_s) + s r_{HR}]] \quad (1)$$

where r_s is the leaf resistance to water vapour (equal to the reciprocal of the stomatal conductance) and assumed to be dominated by the stomatal resistance component (s m^{-1}), r_{aW} the boundary layer resistance to water vapour (s m^{-1}), R_{ni} the net isothermal radiation (the net radiation that would be absorbed by a leaf if it were at air temperature, W m^{-2},), ρ the density of air (kg m^{-3}), c_p the specific heat capacity of air (J kg^{-1} K^{-1}), s the slope of the curve relating saturating water vapour pressure to temperature (Pa $^{\circ}C^{-1}$), γ the psychrometric constant (Pa K^{-1}) and r_{HR} the parallel resistance to heat and radiative transfer (s m^{-1}) and *D* the air vapour pressure deficit (Pa). This equation can be rearranged (Leinonen et al., 2006) to give the following expression for stomatal resistance

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

The behaviour of this equation is illustrated schematically in Fig. 2.

Although it is in principle feasible to measure all these required environmental variables together with leaf temperature and therefore to calculate the conductance from this (Leinonen et al., 2006) it is easier to make use of reference model leaves that are either wetted and transpiring at the maximum rate for the environment, or dry and non-transpiring (Jones, 1999a, b). In this case the stomatal resistance can be estimated from

$$r_s = (r_{aW} + (s/\gamma)\, r_{HR}) \cdot (T_l - T_{wet}) \,/\, (T_{dry} - T_l) \qquad (3)$$

where the first term depends primarily on wind speed. A detailed review of the theory and application of thermal imaging to the study of plant water relations and for the diagnosis and monitoring of disease has been presented by Jones (2004).

For water stress, it is common to assume that one can define a crop water stress index (CWSI) according to Idso et al. (1981) as

$$CWSI = (T_{canopy} - T_{nws})/(T_{max} - T_{nws}) \qquad (4)$$

where T_{max} is the temperature of a dry surface and T_{nws} is the empirical "non-water-stressed baseline" temperature (that of a well watered crop transpiring at the potential rate in the same enviroment). This index is effectively a measure of stomatal opening rather than correctly of the water stress itself.

We are currently investigating the opportunities for automation of thermal imaging, combined with visible (or red/infrared reflectance) sensing, for application to automated scheduling of irrigation. A particularly suitable target crop is the hardy nursery stock industry where it is important to ensure that all pots are well watered, but to avoid over-irrigation with the associated problems of excess runoff and wastage of scarce water resources. In principle such a system would combine the automated identification of plant/no-plant as achieved by Leinonen and Jones (2004) with an appropriate algorithm for detection of stomatal closure as an indicator of water need.

Fluorescence imaging

Even more information about the stress responses of a leaf may be obtained from the fluorescence emission. The main wavebands involved in the fluorescence emission from a green leaf when excited by UV-A radiation are in the blue at 440 nm, in the green at 520 nm, in the red at 690 nm and in the far red at 740 nm. Those peaks in the red and far-red are primarily associated with chlorophyll-*a* fluorescence which is directly related to the photosynthetic process, while the shorter wavelength emissions are predominantly associated with fluorescence from other, primarily phenolic, compounds in the leaves, especially from ferulic and chlorogenic acid bound to the cell walls (e.g. Lichtenthaler and Miehé, 1997; Buschmann et al., 2000). At any wavelength the fluorescence intensity is influenced by the concentration of the emitting substance, the internal optics of the leaf including factors that affect the partial re-absorption of the fluorescence, and especially for photosynthesis-related fluorescence the energy partitioning between the photosystems and the energy quenching processes in the chloroplast.

In practice the raw amount of fluorescence at any time is a relatively unhelpful signal as it is very dependent on illumination and a range of structural characteristics of the leaf, with the absolute magnitude being a rather arbitrary measure. Therefore it is useful to normalise the data and to derive from images analogues of the classic chlorophyll fluorescence parameters that are normally obtained from either the fluorescence transients on illumination or those obtained using modulated fluorescence techniques (Maxwell and Johnson, 2000; Nedbal et al., 2000). It is commonly found that the most useful chlorophyll fluorescence parameters are the ratio of variable to maximal fluorescence after acclimation in the dark (F_v/F_m) which indicates the potential maximum efficiency of photosystem II, and the quantum yield of photosystem II which is given by instantaneous ratio of F_v/F_m during steady state photosynthesis (Maxwell and Johnson, 2000). These ratios can be derived from pairs of fluorescence images to indicate variation in these properties over the surface of leaves.

Most research on chlorophyll fluorescence has been concentrated on the use of single point-measurements, though in recent years there have been a number of fluorescence imaging systems that have become available (e.g. Lichtenthaler and Miehé, 1997; Nedbal *et al*., 2000). These imaging systems have become particularly useful, not only for the study of photosynthesis responses to stress, but also for their application to the study of a wide range of biotic stresses, as both multicolour and chlorophyll fluorescence data can be used to demonstrate and distinguish the early stages of infection by fungi, viruses and bacteria before symptoms are visible in standard reflectance images (Chaerle et al., 2006; Pineda et al., 2008; Rodriguez-Moreno et al., 2008). Similarly, multicolour fluorescence techniques have also been used for diagnosis of nutrient status of leaves (e.g. Langsdorf et al., 2000).

Reflectance imaging

We normally view leaves by reflected light and the colour detected provides a good indicator of leaf health. The mean reflectance spectrum depends on the relative composition of all the pigments in the leaf including chlorophylls, carotenoids, flavonoids and so on. This, especially the changes in leaf chlorophyll content, is a good indicator of stress, as the decreasing chlorophyll content as leaves senesce can be readily detected spectrophotometrically. Much greater discriminatory power, however, is available if we take account of the distribution of the colour across the leaf. Many diseases and many mineral deficiencies or toxicities show characteristic patterning of colour. For example while Nitrogen deficiency leads to a general loss of chlorophyll (and hence yellowing of the leaf), other deficiencies lead to clear patterns, with Zinc deficiency leading to interveinal browning, and Magnesium deficiency leading to yellowing only in the interveinal areas, while Sulphur deficiency leads to purpling of the veins. A very nice summary of typical deficiency symptoms in tomato may be found in the web companion to Taiz and Zeiger (2002) while deficiency symptoms for citrus are shown in Futch and Tucker (2001). A wide range of other images may be found using a web search.

Similarly many plant diseases are conventionally diagnosed from their characteristic symptom patterning with often clear distribution of colour changes either near or remote from the veins or the leaf edges, combined with colour changes which may be diagnostic for specific diseases. Unfortunately it requires a skilled observer to diagnose disorders from the visual pattering of leaves; it would be of particular interest to develop automated approaches to such diagnosis through image analysis or decision support systems. Some initial steps are being taken but the technology requires substantial development before automation of such approaches can become reliable.

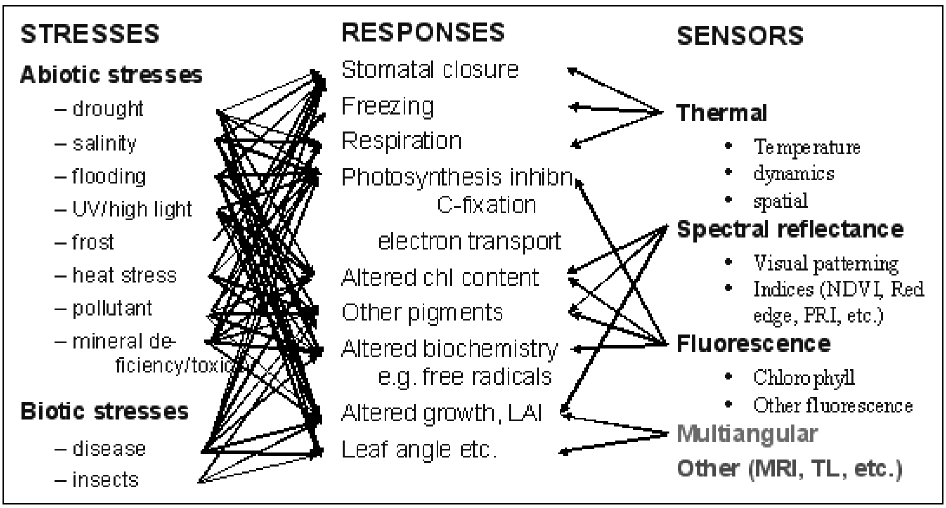

Fig. 1. An illustration of the relationships between primary stresses and the intermediate responses and secondary stresses, together with an indication of which of these intermediate responses are detected by different remote sensing technologies.

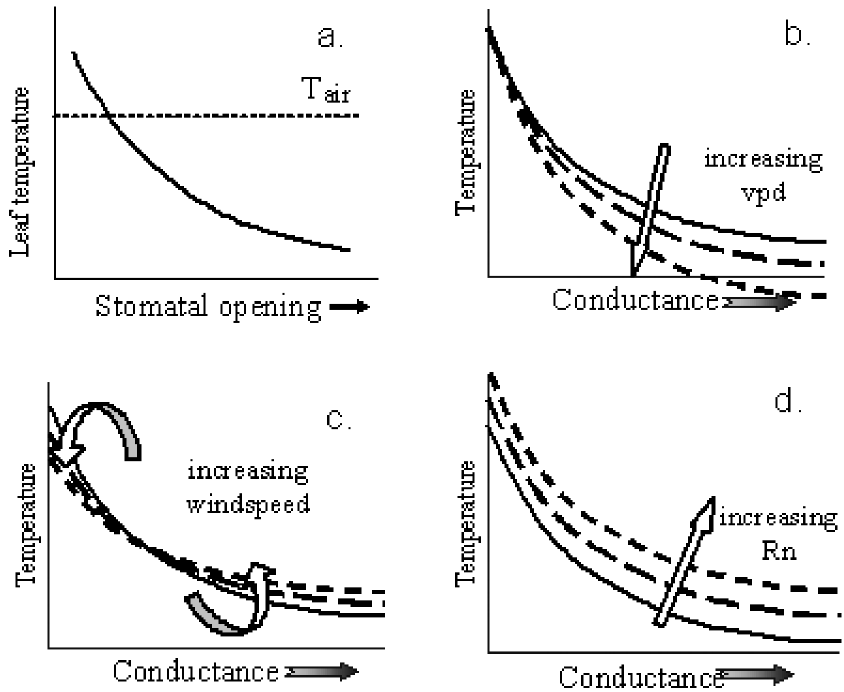

Fig. 2. A schematic diagramme showing the dependence of the relationship between temperature and stomatal conductance on environmental factors. (a) the general relationship, (b) the dependence on vapour pressure deficit of the atmosphere (vpd), (c) the dependence on windspeed and (d) the dependence on net radiation absorbed (Rn).

Multi-sensor imaging for diagnosis

Because any individual imaging sensor provides only limited information, indicating changes in only one or two intermediary responses, but that response can be caused by a wide range of primary stresses (see Fig. 1), our ability to diagnose the particular primary stress is greatly enhanced by the combination of two or more imaging technologies. For example thermal imaging responds primarily to changes in evaporation rate, which are generally caused by changes in stomatal aperture, but stomatal closure can

Table 1. An abstract of a table outlining the possible use of multi-sensor imaging for the diagnosis of different stresses, outlining the major responses tpo a number of stresses (contents from Chaerle, L., Leinonen, I., Lenk, S., Van Der Straeten, D., Jones, H.G. and Buschmann, C. unpublished).

Stress type	Thermography	Reflectance	Fluorescence
Abiotic stresses			
Water stress	Temperature rise (primary response - especially in 'isohydric plants')[1]	Leaf angle distribution changes - sensed by multiangular sensing[2] Increase of reflectance[3]	increase in blue-green fluorescence, decrease of Chl-F[4], decrease of variable Chl-F[5] or photochemical yield[5]
Nitrogen deficiency	Tendency to rise (but may relate to the reduced leaf area effect)[6]	Detectable by increasing yellow colour of leaves [6]; Increasing reflectance at visible wavelengths, especially green and red[7] **Specific patterns of colour change**	higher blue-green fluorescence and higher Chl-F at 690 nm[8]
Gaseous pollutants (NO_2, SO_2, O_3)	Rise (result of stomatal closure with often increased stomatal heterogeneity) [9]	Increase of reflectance in green and red regions[3]	decrease of maximum quantum yield of PS II Fv/Fm[10]
Biotic stresses			
Fungal infection	Temperature decrease[12] temperature increase[13]	Increase of reflectance in red region and SWIR regions **Specific patterns of colour change**	increase of Chl-F[11,12] decrease of variable Chl-F[13]; decrease of PS II-efficiency
Viral infection	TMV: Initial temperature rise, followed by fall as cell death occurs[14]	**Specific patterns of colour change**	increase of Chl-F and blue-green fluorescence[14] and variation in variable Chl-F[15]
Bacterial infection	*Erwinia elicitor*: Presymptomatic temperature fall[16]	**Specific patterns of colour change**	Higher quantum yield, corresponding with a reduction in steady state Chl-F [17]

[1] Jones (2004); [2] Casa and Jones (2003); [3] Carter (1993); [4] Lichtenthaler and Miehé (1997); [5] Meyer and Genty (1999); [6] Nilsson (1995); [7] Carter and Knapp (2001); [8] Langsdorf et al. (2000); [9] Omasa (1981a, b); [10] Gielen et al. (2006); [11] Chaerle et al. (2004); [12] Charele et al. (2007a); [13] Meyer et al. (2001); [14] Chaerle et al. (1999); [15] Osmond et al. (1998); [16] Boccara et al. (2001); [17] Berger et al. (2004).

be a result of stresses as different as drought, flooding, salinity stress, fungal infection or pollutants. In order to distinguish between these possible causes one needs further information. Therefore there has been recent emphasis on the development of multi-sensor imaging to aid in stress diagnosis and monitoring. The approaches can range from simple combination of, say, thermal and reflectance sensors (e.g. Chaerle et al., 2001; Leinonen and Jones, 2004), or visible reflectance and fluorescence sensors (Lenk et al., 2007), through to combined fluorescence, reflectance and thermal imaging sensors (Chaerle et al., 2003; Chaerle et al., 2006; Chaerle et al., 2007a).

DISCUSSION AND CONCLUSIONS

It is clear from the above that there is enormous potential for combining different imaging technologies for the diagnosis and quantification of both abiotic and biotic stresses in plants. By combining information from a wide range of sensors, each of which detects a different basic physiological response, it should be possible to greatly enhance our sensitivity at diagnosing and quantifying different stresses. A small abstract from such a Table is presented in Table 1 (from Chaerle, Leinonen, Lenk, Van Der Straeten, Jones and Buschmann unpublished).

Acknowledgements: We are very grateful to various colleagues including Ilkka Leinonen, Laury Cherle, Claus Buschmann, Ashley Wheaton and Brian Loveys for their contributions to aspects of the work described, and to funding agencies including EU Research Training Network (Human Potential Programme - HPRN-CT-2002-00254), UK Defra HortLink project WaterLink2 (project HL0168) and the Australian Grape, Wine Research and Development Corporation.

References

Berger, S., M. Papadopoulos, U. Schreiber, W. Kaiser, T. Roitsch, 2004. Complex regulation of gene expression, photosynthesis and sugar levels by pathogen infection in tomato. Physiol. Plant., 122, 419-428.

Boccara, M., C. Boue, M. Garmier, R. De Paepe, A.C. Boccara, 2001. Infra-red thermography revealed a, role for mitochondria in pre-symptomatic cooling during harpin-induced hypersensitive response. Plant J., 28, 663-670.

Buschmann, C., G. Langsdorf, H.K. Lichtenthaler, 2000. Imaging of the blue, green, and red fluorescence emission of plants: An Overview Photosynthetica, 38, 483-491.

Carter, G.A. (1993) Responses of leaf spectral reflectance to plant stress. Am. J. Bot., 80, 239-243.

Carter, G.A., Knapp A.K., 2001. Leaf optical properties in higher plants: linking spectral characteristics to stress and chlorophyll concentration. Am. J. Bot., 88, 677-684.

Casa, R., H.G. Jones, 2004. Retrieval of crop canopy properties: a comparison between model inversion from hyperspectral data and image classification. Int. J. Remote Sensing, 25, 1119-1130.

Chaerle, L., W. Van Caeneghem, E. Messens, H. Lambers, M. Van Montagu, D. Van Der Straeten, 1999. Presymptomatic visualization of plant-virus interactions by thermography. Nature Biotechnol., 17, 813-816.

Chaerle, L., F. De Boever, M. Van Montagu, D. Van Der Straeten, 2001. Thermographic visualization of cell death in tobacco and *Arabidopsis*. Plant Cell and Environ., 24, 15-25.

Chaerle, L., K. Hulsen, C. Hermans, R.J. Strasser, R. Valcke, M. Hofte, D. Van Der Straeten, 2003. Robotized time-lapse imaging to assess in-planta uptake of phenylurea herbicides and their microbial degradation. Physiol. Plant., 118, 613-619.

Chaerle, L., D. Hagenbeek, E. De Bruyne, R. Valcke, D. Van Der Straeten, 2004. Thermal and chlorophyll-fluorescence imaging distinguish plant-pathogen interactions at an early stage. Plant, Cell Physiol., 45, 887-896.

Chaerle, L., M. Pineda, R. Romero-Aranda, D. Van Der Straeten, M. Baron, 2006. Robotized thermal and chlorophyll fluorescence imaging of pepper mild mottle virus infection in *Nicotiana benthamiana*. Plant, Cell Physiol., 47, 1323-1336.

Chaerle, L., D. Hagenbeek, X. Vanrobaeys, D. Van Der Straeten, 2007a.

Early detection of nutrient and biotic stress in *Phaseolus vulgaris*. Int. J. Remote Sensing, 28, 3479-3492.

Chaerle, K., I. Leinonen, H.G. Jones, D. Van Der Straeten, 2007b. Monitoring and screening plant populations with combined thermal and chlorophyll fluorescence imaging. J. Exp. Bot., 58, 773-784.

Futch, S., D. Tucker, 2001. A Guide to citrus nutritional deficiency and toxicity identification HS-797. HS-797. Florida Cooperative Extension Service.

Gielen, B., K. Vandermeiren, N. Horemands, D. D'Haese, R. Serneels, R. Valcke, 2006. Chlorophyll a fluorescence imaging of ozone-stressed *Brassica napus* L. plants differing in glucosinolate concentrations. Plant Biol., 8, 698-705.

Idso, S.B., R.D. Jackson, P.J. Pinter, R.J. Reginato, J.L. Hatfield, 1981. Normalizing the stress-degree-day parameter for environmental variability. Agric. Met., 24, 45-55.

Jones, H.G., 1999a. Use of infrared thermometry for estimation of stomatal conductance in irrigation scheduling. Agric. Forest Met., 95, 135-149.

Jones, H.G., 1999b. Use of thermography for quantitative studies of spatial and temporal variation of stomatal conductance over leaf surfaces. Plant Cell and Environ., 22, 1043-1055.

Jones, H.G., 2004. Application of thermal imaging and infrared sensing in plant physiology and ecophysiology. Adv. Bot. Res., 41, 107-163.

Langsdorf, G., C. Buschmann, M. Sowinska, F. Banbani, F. Mokry, F. Timmermann, H.K. Lichtenthaler, 2000. Multicolour fluorescence imaging of sugar beet leaves with different nitrogen status by flash lamp UV-excitation. Photosynthetica, 38, 539-551.

Leinonen, I., H.G. Jones, 2004. Combining thermal and visible imagery for estimating canopy temperature and identifying plant stress. J. Exp. Bot., 55, 1243-1231.

Leinonen, I., O.M. Grant, C.P.P. Tagliavia, M.M. Chaves, H.G. Jones, 2006. Estimating stomatal conductance with thermal imagery. Plant, Cell and Environ**.,** 29, 1508-1518.

Lenk, S., L. Chaerle, E.E. Pfundel, G. Langsdorf, D. Hagenbeek, H.K. Lechtenthaler, D. Van Der Straeten, C. Buschmann, 2007.

Multispectral fluorescence and reflectance imaging at the leaf level and its possible applications. J. Exp. Bot**.,** 58, 807-814.

Lichtenthaler, H.K., J. Miehé, 1997. Fluorescence imaging as a diagnostic tool for plant stress. Trends in Plant Science, 2, 316-320.

Maxwell, K., G.N. Johnson, 2000. Chlorophyll fluorescence - a practical guide. J. Exp. Bot., 51, 659-668.

Meyer, S., B. Genty, 1999. Heterogeneous inhibition of photosynthesis over the leaf surface of *Rosa rubiginosa* L. during water stress and abscisic acid treatment: induction of a metabolic component by limitation of CO_2 diffusion. Planta, 210, 126-131.

Meyer, S., K. Saccardy-Adji, F. Rizza, B. Genty, 2001. Inhibition of photosynthesis by *Colletotrichum lindemuthianum* in bean leaves determined by chlorophyll fluorescence imaging. Plant, Cell and Environ., 24, 947-955.

Nedbal, L., J. Soukupova, D. Kaftan, J. Whitmarsh, M. Trtilek, 2000. Kinetic imaging of chlorophyll fluorescence using modulated light. Photosynthesis Research, 66, 3-12.

Nilsson, H.E., 1995. Remote sensing and image analysis in plant pathology. Ann. Rev. Phytopath., 33, 489-527.

Omasa, K., Y. Hashimoto, I. Aiga, 1981a. A quantitative analysis of the relationships between O_3 sorption and its acute effects on plant leaves using image instrumentation. Environmental Control in Biol., 19, 85-92.

Omasa, K., Y. Hashimoto, I. Aiga, 1981. A quantitative analysis of the relationships between SO_2 or NO_2 sorption and their acute effects on plant leaves using image instrumentation. Environ. Control in Biology, 19, 59-67.

Osmond, C.B., P.F. Daley, M.R. Badger, U. Lüttge, 1998. Chlorophyll fluorescence quenching during photosynthetic induction in leaves of *Abutilon striatum* Dicks. infected with *Abutilon Mosaic Virus*, observed with a field-portable imaging system. Botanica Acta, 111, 390-397.

Pineda, M., J. Soukupova, K. Matouš, L. Nedbal, M. Baron, 2008. Conventional and combinatorial fluorescence imaging of tobamovirus-infected plants. Photosynthetica (*in press*).

Rodríguez-Moreno L., M. Pineda, S. Soukupová, A.P. Macho, C.R. Beuzón, M. Barón, C. Ramos, 2008. Early detection of bean infection by *Pseudomonas syringae* in asymptomatic leaf areas using chlorophyll fluorescence imaging Photosynthesis Res., 96, 27-35.

Seymour, R.S., 1999. Pattern of respiration by intact inflorescences of the thermogenic arum lily *Philodendron selloum*. J. Exp. Bot., 50, 845-852.

Taiz, L., E. Zeiger, 2002. Plant Physiology. Sinauer Associates, Sunderland, MA 01375, USA (see web companion at http://4e.plantphys.net).

Wisniewski, M., S.E. Lindow, E.N. Ashworth, 1997. Observations of ice nucleation and propagation in plants using infrared video thermography. Plant Physiol., 113, 327-334.