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REVIEW ARTICLE

Applications of chlorophyll fluorescence can improve crop production strategies: an examination of future possibilities

Neil R. Baker[1,*] and Eva Rosenqvist[2]

[1] *Department of Biological Sciences, University of Essex, Colchester CO4 3SQ, UK*

[2] *Department of Horticulture, Danish Institute of Agricultural Sciences, Kristinebjergvej 10, PO Box 102, DK-5792 Aarslev, Denmark*

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Abstract

Chlorophyll fluorescence has been routinely used for many years to monitor the photosynthetic performance of plants non-invasively. The relationships between chlorophyll fluorescence parameters and leaf photosynthetic performance are reviewed in the context of applications of fluorescence measurements to screening programmes which seek to identify improved plant performance. The potential role of chlorophyll fluorescence imaging in increasing both the sensitivity and throughput of plant screening programmes is examined. Finally, consideration is given to possible specific applications of chlorophyll fluorescence for screening of plants for tolerance to environmental stresses and for improvements in glasshouse production and post-harvest handling of crops.

Key words: Agricultural production, environmental stress, herbicides, horticultural production, imaging, photosynthesis, photosynthetic induction, post-harvest physiology, screening.

Introduction

The problems associated with improving crop production encountered throughout the world are extremely diverse, for example, potentially ranging from millet production in arid regions to tropical flower production in glasshouses. However, common goals are, frequently, the improvement of crop production by the selection of improved varieties and cultivars to tolerate environmental stresses and by the determination of optimal fertilization and irrigation regimes during crop growth. In glasshouse industries optimization of day-length, light intensity, temperature, and CO_2 concentration are often additional concerns. A further complication for many crop production systems is the necessity to keep produce alive and fresh during the post-harvest period until it reaches the consumer. Post-harvest conditions can impose many stresses that crops have not experienced in the field or glasshouse prior to harvesting, and which the crops are unable to tolerate effectively by the operation of acclimatory responses.

An important feature of crop development and improvement programmes is the effective evaluation of crop growth and performance, ideally using rapid, non-invasive techniques. Major developments in the instrumentation for measuring chlorophyll fluorescence from intact plants and improvements in the understanding of how changes in plant fluorescence characteristics relate to physiological performance have led to a widespread use of chlorophyll measurements in plant physiological studies. Potentially, the technique has many applications in crop production and

* To whom correspondence should be addressed. Fax: +44 (0)1206 873319. E-mail: baken@essex.ac.uk

Abbreviations: 2,4-D, 2,4-dichlorophenoxyacetic acid; DCMU, 3-(3′,4′-dichlorophenyl) 1,1-dimethylurea; F', fluorescence level at any point between F'_o and F'_m; F_i, transient inflection level of fluorescence immediately after exposure of dark-adapted leaf to actinic light; F_m, maximal fluorescence level from dark-adapted leaves; F'_m, maximal fluorescence level from leaves in light; F_o, minimal fluorescence level from dark-adapted leaves; F'_o, minimal fluorescence level of leaves in light; F_p, peak level of fluorescence reached on exposure of dark-adapted leaf to actinic light; F'_q, difference in fluorescence between F'_m and F' ($F'_q = F'_m - F'$); F_v, variable fluorescence level from dark-adapted leaves ($F_v = F'_m - F_o$); F'_v, variable fluorescence level of leaves in light ($F'_v = F'_m - F'_o$); F'_q/F'_m, PSII operating efficiency (the quantum yield of PSII photochemistry for a leaf in light); F'_q/F'_v, the PSII efficiency factor which relates to the ability to maintain PSII reaction centres in the open state; F_v/F_m, maximum quantum efficiency of PSII photochemistry; F'_v/F'_m, PSII maximum efficiency which estimates the maximum quantum efficiency of PSII under given light conditions; *NPQ*, non-photochemical quenching; PSI, photosystem I; PSII, photosystem II; Q_A, primary quinone electron acceptor of PSII.

development programmes, but as yet it has not been widely applied.

It has been known for a considerable time that changes in chlorophyll fluorescence emission from photosynthetic organisms are frequently indications of changes in photosynthetic activity (McAlister and Myers, 1940; Kautsky and Zedlitch, 1941). More recently, the demonstrations that chlorophyll fluorescence measurements could be used to estimate, rapidly and non-invasively, the operating quantum efficiency of electron transport through PSII in leaves (Genty *et al.*, 1989) and that this PSII operating efficiency was related to CO_2 assimilation (Genty *et al.*, 1989, 1990; Harbinson *et al.*, 1990; Krall and Edwards, 1990, 1991; Krall *et al.*, 1991; Cornic and Ghashghaie, 1991; Siebke *et al.*, 1997) have led to the current widespread use of chlorophyll fluorescence for examining photosynthetic performance in leaves in laboratory, controlled environment, and field situations. There is no doubt that measurements of chlorophyll fluorescence, when applied with appropriate care, can provide useful information about leaf photosynthetic performance. An examination of the literature overwhelmingly reveals in excess of 3500 papers on chlorophyll fluorescence, of which *c*. 20% are relevant to eco-physiological performance and a similar proportion can be considered to have implications for agricultural or horticultural issues. However, there are many examples in this literature of inappropriate use and misinterpretation of fluorescence measurements that have frequently led to extravagant and unsubstantiated claims of the potential of fluorescence for evaluating plant performance in a wide range of applications. Consequently, there has been an understandable reluctance by the agricultural, horticultural, and agrochemical industries to include fluorescence techniques in crop improvement programmes.

In this review, the objective is to identify ways in which chlorophyll fluorescence may be used effectively to improve plant selection processes and rapidly evaluate plant performance in agricultural and horticultural crop improvement programmes. It is not the intention to provide a comprehensive review of chlorophyll fluorescence theory and applications, since this has been the subject of other recent reviews (Papageorgiou and Govindjee, 2004).

Fluorescence parameters

There have been very many fluorescence parameters defined in the literature. It is not possible, nor appropriate, to give an extensive review of all of these here. The aim is to provide information on the parameters that can be usefully used in crop improvement programmes to identify differences in plant performance non-destructively and rapidly. Consequently, the focus will be on the fluorescence parameters associated with the induction of fluorescence on exposure of dark-adapted leaves to light and the operation of photosynthesis under growth and other light conditions.

Unfortunately, the literature on analyses of fluorescence induction transients and the processes that quench fluorescence is often extremely confusing because of the diverse, and frequently duplicated, nomenclature that has been used. The majority of the terms in historical and currently used nomenclature have been summarized by Rosenqvist and van Kooten (2003). Although many attempts have been made to establish a single nomenclature (van Kooten and Snell, 1990; Maxwell and Johnson, 2000; Baker *et al.*, 2001), there are still many examples in recent publications of different terms being used to define the same fluorescence level or parameter. In this review the nomenclature suggested by Baker *et al.* (2001) and Barbagallo *et al.* (2003) will be used for consistency.

Fluorescence induction

During the induction of photosynthesis when a dark-adapted leaf is exposed to light, large changes in chlorophyll fluorescence occur (Fig. 1). The rapid changes in fluorescence that occur during the rapid induction to a peak have long been attractive for detecting differences in photosynthetic performance between plants. On immediate exposure to light, fluorescence rises to the minimal level of fluorescence, termed F_o level, which is the fluorescence level obtained when the PSII reaction centres are in the 'open' state (capable of photochemistry since Q_A, the primary quinone acceptor of PSII, is maximally oxidized). The fluorescence then rises rapidly to the transient inflection level, F_i, before reaching a peak level, F_p (Fig. 1). It should be noted that if the actinic PPFD being used to drive the fluorescence induction is saturating and effects maximal closure of PSII reaction centres (maximal reduction of Q_A) at F_p, then the maximal fluorescence level, defined as F_m will be attained. The difference between F_m and F_o is termed the variable fluorescence, F_v. The rapid rise to F_i reflects an increase in the rate of charge stabilization at PSII

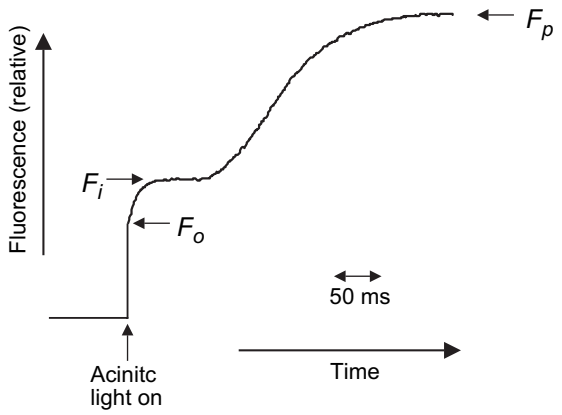

Fig. 1. Chlorophyll fluorescence induction curve from a dark-adapted wheat leaf exposed to weak actinic blue light (30 μmol m^{-2} s^{-1}). The minimal (F_o), transient inflection (F_i), and peak (F_p) levels of fluorescence are shown.

(as the primary quinone acceptor, Q_A, is reduced) that is independent of subsequent changes in the redox state of the plastoquinone pool. Changes in the fluorescence level between F_i and F_p are almost entirely due to increased reduction of the plastoquinone pool, which is largely determined by the relative rates of PSII photochemistry and oxidation of plastoquinol by electron transfer to PSI.

It is not surprising to find that perturbations of photosynthetic metabolism, which can be induced by many biotic and abiotic factors, will modify significantly fluorescence emission kinetic characteristics of plants. However, there is also evidence that many inhibitors of metabolic processes that are not directly involved in photosynthetic metabolism can produce modifications to fluorescence induction kinetics (Blowers, 1989; Percival and Baker, 1991; Crudace, 2000; Barbagallo *et al.*, 2003). It is possible to quantitate the changes in fluorescence induction characteristics resulting from such perturbations by using ratios of fluorescence levels during induction (Habash *et al.*, 1985). Absolute fluorescence values, such as F_o, F_i, F_m, and F_p, are dependent upon both the photochemical activities and the optical properties of the leaf and, consequently, it is essential to remove the variable of leaf optical properties when attempting to compare changes in fluorescence characteristics between different leaf samples. This can be achieved by comparing ratios of fluorescence values. The potential of the use of ratios of the fluorescence induction parameters F_o, F_i, F_m, F_v, and F_p to detect metabolic perturbations has been demonstrated by examining the effects of a number of herbicides that are known not to impact directly on photosynthetic metabolism (Barbagallo *et al.*, 2003). *Arabidopsis* seedlings were treated with the recommended field application rate for Asulam, Bifenox, 2,4-D, Diclofop-methyl, Glyphosate, and Imazapyr and the fluorescence parameters F_v/F_m, $1-(F_o/F_p)$, $1-(F_o/F_i)$, and $1-(F_i/F_p)$ were monitored with time. After 6 h, significant decreases in all of the parameters were detected for plants treated with Asulam, Diclofop-methyl, Glyphosate, and Imazapyr, whereas the changes induced by Bifenox and 2,4-D were not significant. After 48 h, significant decreases in F_v/F_m, $1-(F_o/F_p)$, and $1-(F_i/F_p)$ were observed with all of the herbicides. Experiments with other species (*Alopecurus myosuroides, Avena fatua, Phaseolus vulgaris, Sinapis alba, Triticum aestivum*, and *Zea mays*) have demonstrated the wide applicability of these fluorescence parameters for the detection of herbicide-induced perturbations of metabolism (Habash *et al.*, 1985; Blowers, 1989; Crudace, 2000). Clearly, these fluorescence parameters can be used successfully to detect a number of very different metabolic perturbations in leaves of many species. Although the mechanistic bases for the effects of non-photosynthetic inhibitors on fluorescence emission have not been unequivocally identified, it is likely that inhibition of metabolic reactions not involved directly in photosynthesis will modify the pool sizes of a range of metabolic intermediates, which could influence the rate of synthesis of key intermediates in photosynthetic metabolism and, consequently, interfere with the rate of photosynthesis and fluorescence emission characteristics. Similarly, environmental stress factors would be expected to impact indirectly on fluorescence induction characteristics by perturbing metabolic pools associated with photosynthetic metabolism.

After reaching the peak, a decline in fluorescence occurs, often termed quenching, until a steady-state is reached (Fig. 2). The quenching of F_p to the steady-state level is associated with changes in the photosynthetic apparatus that are associated with the induction of CO_2 assimilation. Although many factors can induce modifications in the kinetics of quenching from F_p, the transients observed can be extremely variable. Consequently, analyses of such transients have not proved as useful as other fluorescence parameters for the rapid and sensitive detection of differences between plants. However, differences in the rate of fluorescence quenching from F_p have been used successfully to identify differences between cultivars' responses to environmental stresses (Flagella *et al.*, 1996; Havaux and Lannoye, 1985).

Modulated fluorescence measurements

The majority of fluorescence measurements are now made using modulated fluorometers with the leaf poised in a known state. The procedures for making such measurements are shown in Fig. 2, together with the fluorescence levels for a leaf in specific states. For a dark-adapted leaf, F_o is determined using a very low PPFD (generally considerably below 1 μmol m^{-2} s^{-1}), which ensures that almost all of the PSII reaction centres are in the open state (capable of photochemistry). When the dark-adapted leaf is exposed to a short actinic light pulse of very high PPFD (generally less than 1 s at several thousand μmol m^{-2} s^{-1}), a maximal level of fluorescence (F_m) is generated as the majority of the PSII reaction centres have been closed (incapable of photochemistry). The ratio of F_v/F_m provides an estimate of the maximum quantum efficiency of PSII photochemistry (Butler, 1978). F_v/F_m has been widely used to detect stress-induced perturbations in the photosynthetic apparatus, since decreases in F_v/F_m can be due to the development of slowly relaxing quenching processes and photodamage to PSII reaction centres, both of which reduce the maximum quantum efficiency of PSII photochemistry.

The potential for the application of fluorescence measurements to study changes in leaf photosynthetic performance increased dramatically with the development of the light addition technique which could resolve fluorescence quenching into photochemical and non-photochemical components (Bradbury and Baker, 1981, 1984). When a leaf in the light-adapted state is exposed to a saturating pulse of very high PPFD, there is an increase in fluorescence from the F' level to a maximal level, F'_m (Fig. 2). The

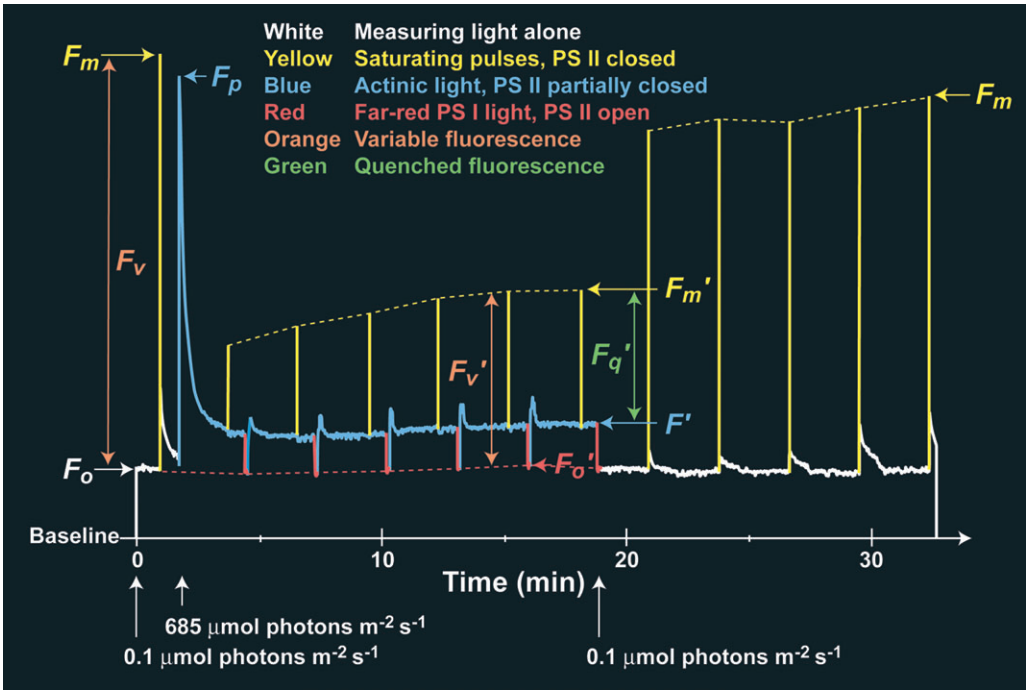

Fig. 2. Protocol for quenching analysis using modulated fluorescence. A dark-adapted leaf is exposed to various light treatments. The flourescence parameters denoted with a prime originate from the illuminated leaf, where energy-dependent, non-photochemical quenching is present. The parameters without a prime are obtained from the leaf in the dark-adapted state, where there is no energy-dependent non-photochemical quenching. The different colours of the trace denote different light treatments. White, weak measuring light alone (0.1 μmol photons m^{-2} s^{-1}) that gives F_o. Yellow, saturating light pulse (⩽1 s duration, >6000 μmol photons m^{-2} s^{-1}) that gives F_m in darkness and F'_m in light. Blue, actinic light that drives photosynthesis (in this case 685 μmol photons m^{-2} s^{-1}) that gives F' (if steady-state has been reached this has often been denoted by F'_s). The actinic light can be produced from a range of sources, for example, sunlight, halogen lamp, light-emitting diodes. The initial peak of fluorescence is denoted as F_p (without prime, since it originates from the nomenclature of the rapid phase of fluorescence induction, see Fig. 1). Red, far-red light (30 μmol photons m^{-2} s^{-1} at 720–730 nm for 4 s) that excites PSI only, and thus oxidizes the plastoquinone and Q_A pools associated with PSII and gives F'_o. Orange, variable fluorescence calculated as $F_v = F_m - F_o$ from the dark-adapted leaf and $F'_v = F'_m - F'_o$ from the illuminated leaf. Green, fluorescence that is quenched from F'_m to F' by PSII photochemistry in the illuminated leaf, calculated as $F'_q = F'_m - F'$. All parameters, except F'_q, F_v, and F'_v, are measured from the baseline.

difference between F'_m and F' is termed F'_q since this is the fluorescence that has been quenched from the maximal level. The saturating light pulse maximally closes the PSII reaction centres and consequently removes any photochemical quenching by open PSII reaction centres. For a healthy leaf operating at steady-state photosynthesis under moderate to high PPFDs, the F'_m level generated by the saturating light pulse will be considerably less than the F_m level generated from a dark-adapted leaf by the same pulse (Fig. 2). This difference is due to the development of light-induced, non-photochemical quenching processes during the induction of photosynthesis in the leaf. The demonstration that the ratio F'_q/F'_m was an estimate of the quantum yield of PSII photochemistry for a leaf at any given light condition (Genty *et al.*, 1989) has led to this parameter being widely used to estimate the operating quantum efficiency of PSII electron transport (hereafter termed the PSII operating efficiency). From a physiological screening standpoint, this is a particularly useful parameter, since it is directly related to the rate at which CO_2 is assimilated by the leaf (Genty *et al.*, 1989, 1990; Harbinson *et al.*, 1990; Krall and Edwards, 1990, 1991; Krall *et al.*, 1991; Cornic and Ghashghaie, 1991; Edwards and Baker, 1993; Siebke *et al.*, 1997). Consequently, measurements of F'_q/F'_m have become widely used to examine perturbations of photosynthetic performance.

The PSII operating efficiency has been shown to be the product of two other important fluorescence parameters, F'_v/F'_m (the maximum efficiency of PSII under the given light conditions, generally determined by the level of quenching in PSII reaction centres and antenna) and F'_q/F'_v (the PSII efficiency factor which relates to the ability to maintain PSII reaction centres in the open state):

$$\frac{F'_q}{F'_m} = \frac{F'_v}{F'_m} \cdot \frac{F'_q}{F'_v} \qquad (1)$$

F'_v is the variable fluorescence of a light-adapted leaf defined as $(F'_m - F'_o)$ (Fig. 2). F'_o is the minimal level of fluorescence when PSII centres are maximally open for the leaf in a light-adapted state. F'_o has frequently been measured by exposing the leaf at F' to weak far-red light in the absence of actinic light (van Kooten and Snel, 1990; Maxwell and Johnson, 2000), since it is assumed that far red light will preferentially excite PSI relative to PSII thus removing electrons from the PSII electron acceptors and opening the PSII reaction centres. Unfortunately, during the

far red light treatment there will almost certainly be relaxation of some non-photochemical quenching as well as an opening of the PSII reaction centres (Oxborough and Baker, 1997). Alternatively, F'_o can be calculated from measured values of F_o, F_m, F'_m, and F_v using the following equation (Oxborough and Baker, 1997):

$$F'_o = \frac{F_o}{\frac{F_v}{F_m} + \frac{F_o}{F'_m}} \quad (2)$$

Empirically this method of calculating F'_o has been shown to be valid, and it is potentially more accurate than using far red light to generate an F'_o level (Oxborough and Baker, 1997).

Non-photochemical quenching (heat dissipation) has been quantified using *NPQ* which compares the light-induced F'_m level to the dark-adapted F_m level (Bilger and Björkman, 1990):

$$NPQ = (F_m/F'_m) - 1 \quad (3)$$

It is important to recognize that *NPQ* assesses increases in non-photochemical quenching in a light-adapted leaf relative to the non-photochemical quenching occurring in the dark-adapted state. Consequently, it is only valid to make comparisons between samples which have the same quenching characteristics in the dark-adapted state; similar values of F_v/F_m would be a good indication of this.

A list of the major fluorescence parameters referred to in this review is given in Table 1, together with a summary of what the parameters are measuring. A more comprehensive consideration of these parameters is given in Baker and Oxborough (2004).

Fluorescence imaging

An important recent development in fluorescence measurements in the context of plant performance and selection is the imaging of fluorescence signals using charge coupled device (CCD) cameras (Oxborough, 2004). The advantage that fluorescence imaging instruments have over conventional fluorometers is that images of fluorescence parameters can be obtained from whole leaves or large numbers of plants. Imaging of leaves has revealed that photosynthetic performance across leaves can be extremely heterogeneous (Genty and Meyer, 1995; Siebke and Weis, 1995*a*, *b*; Bro *et al.*, 1996; Eckstein *et al.*, 1996; Scholes and Rolfe, 1996; Oxborough and Baker, 1997; Leipner *et al.*, 2001; Meng *et al.*, 2001). The power of fluorescence imaging in resolving heterogeneity in photosynthetic function across a leaf can be readily demonstrated by images of PSII operating efficiency from a leaf that has been fed through the petiole with diuron (DCMU), a potent inhibitor of PSII electron transport (Fig. 3; Oxborough, 2004). Prior to uptake into the leaf lamina of the diuron there was little heterogeneity of the PSII operating efficiency across the leaf (Fig. 3A). However, 2 h after feeding with diuron, the

Table 1. *Summary of the major fluorescence parameters*

Fluorescence parameter	Definition	Physiological significance
F	Fluorescence emission from dark-adapted leaf	Provide little information of photosynthetic performance as they are influenced by many factors
F'	Fluorescence emission from leaf adapted to actinic light	
F_o	Minimal fluorescence from dark-adapted leaf	Level of fluorescence when primary quinone electron acceptors of PSII (Q_A) are maximally oxidized (PSII centres are open).
F'_o	Minimal fluorescence from light-adapted leaf	
F_m	Maximal fluorescence from dark-adapted leaf	Level of fluorescence when Q_A is maximally reduced (PSII centres are closed).
F'_m	Maximal fluorescence from light-adapted leaf	
F_v	Variable fluorescence from dark-adapted leaf	Demonstrates the ability of PSII to perform primary photochemistry (photoreduction of Q_A).
F'_v	Variable fluorescence from light-adapted leaf	
F'_q	Difference in fluorescence between F'_m and F'	Photochemical quenching of fluorescence due to open PSII centres
F_v/F_m	Maximum quantum efficiency of PSII photochemistry	Maximum efficiency at which light absorbed by light-harvesting antennae of PSII is converted to chemical energy (Q_A reduction)
F'_q/F'_m	PSII operating efficiency	Estimates the efficiency at which light absorbed by PSII antennae is used for photochemistry (Q_A reduction). At a given light intensity it provides a measure of the quantum efficiency of linear electron transport through PSII. Has previously been termed $\Delta F/F'_m$ and ϕ_{PSII}
F'_v/F'_m	PSII maximum efficiency	Provides an estimate of the maximum efficiency of PSII photochemistry at a given light intensity, which is the PSII operating efficiency if all the PSII centres were open (Q_A oxidized)
F'_q/F'_v	PSII efficiency factor	Is non-linearly related to the proportion of PSII centres that are open (with Q_A oxidized). Relates the PSII maximum efficiency to the PSII operating efficiency. Mathematically identical to the coefficient of photochemical quenching, *qP*
NPQ	Non-photochemical quenching	Estimates the non-photochemical quenching from F_m to F'_m. Monitors the apparent rate constant for non-radiative decay (heat loss) from PSII and its antennae.

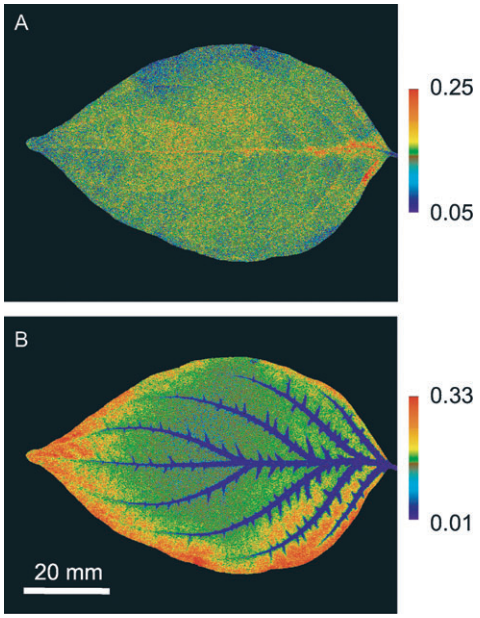

Fig. 3. The effect of petiole feeding of DCMU on the heterogeneity of the PSII operating efficiency across a leaf. The petiole of the leaf was immersed in 10 mM DCMU and images of F'_q/F'_m were taken 60 s (A) and 2 h (B) after exposure to a PPFD of 300 μmol m^{-2} s^{-1}. Coloured bars at the right hand side of the images show the range of values and how they mapped to the colour palette.

PSII operating efficiency in cells close to the vascular tissues, where the herbicide will be initially delivered to the leaf lamina, had decreased to almost zero (Fig. 3B).

Rapid screening of seedling performance

Frequently, a limitation to crop improvement programmes is the lack of rapid screening techniques to identify plants with improved or impaired metabolism and growth. This need within the agrochemical industry, where combinatorial chemistry is now used to generate large numbers of new chemicals, has resulted in the development of new microscreening or high throughput screening which can involve growing plants in 96-well microtitre plates and employing rapid assessment procedures (Berg *et al.*, 1999; Evans, 1999). Conventional glasshouse screening using visual assessment of plants over a period of weeks cannot be used for effective screening of such large numbers of plants. Chlorophyll fluorescence measurements offer great potential for rapidly screening large numbers of small plants. However, a major drawback in the past has been the small sampling area of commercially available fluorometers, since measurements could only be made on individual leaves and, consequently, screening large numbers of plants was extremely time-consuming. Recently, the development of chlorophyll fluorescence imaging systems that can image fluorescence parameters from areas in excess of 100 cm^2 has allowed the application of the technique for the screening of many plants simultaneously (Fig. 4; Barbagallo *et al.*, 2003; Oxborough, 2004).

The efficacy of fluorescence imaging for rapidly identifying metabolic perturbations in large numbers of plants has been demonstrated recently by treatment of *Arabidopsis* and *Agrostis tenuis* seedlings with Imazapyr (Barbagallo *et al.*, 2003). This herbicide inhibits acetolactase synthase and, consequently, inhibits the synthesis of branched chain amino acids (Shaner *et al.*, 1985; Singh *et al.*, 1989). This would not be expected to have any direct effect on photosynthetic electron transport. However, 24 h after spraying with Imazapyr, at a concentration similar to that used for field applications, marked changes in fluorescence induction characteristics were detected with no apparent visual effects on plant growth and development (Barbagallo *et al.*, 2003). Images of the fluorescence parameter F_v/F_m for seedlings growing in a 96 well plate showed that a range of Imazapyr treatments had produced a marked decrease in this parameter after 24 h (Fig. 5), with the magnitude of the decreases being related to the concentration of herbicide applied (Barbagallo *et al.*, 2003).

Besides detecting perturbations to metabolism and physiological performance, fluorescence imaging can be used for rapidly estimating leaf area in seedlings that have planophile leaves which do not significantly overlap, such as *Arabidopsis*. The number of pixels in which chlorophyll fluorescence emission is detected is directly related to the distribution of chlorophyll across the leaf, irrespective of pixel value. Consequently, provided chlorophyll is distributed across the whole of the leaf, as is the case in the majority of plants, the leaf area can be estimated from the area of chlorophyll fluorescence emission (Barbagallo *et al.*, 2003). However, for non-planophile leaf growth, as shown by many monocotyledonous plants like *A. tenuis*, this may not always be the case and careful examination of the relationship between fluorescent leaf area and growth should be made for the system before routinely using fluorescent area to screen for growth differences.

Fluorescence, leaf photosynthetic physiology and environmental stresses

The relationship between the PSII operating efficiency, estimated by F'_q/F'_m, and CO_2 assimilation in leaves allows fluorescence to be used to detect differences in the response of plants to environmental challenges and, consequently, to screen for tolerance to environmental stresses. The rate of leaf CO_2 assimilation is sensitive to a wide range of environmental perturbations (e.g. atmospheric pollutants, high and low temperatures, high light, nutrient deficiency, UV-B irradiation, water deficits), although the sites of limitation of CO_2 assimilation during these various stresses can be quite different (Baker, 1996). F'_q/F'_m estimates directly the efficiency of light use for electron transport by PSII. A major factor determining this efficiency is the ability of the leaf to remove electrons from the quinone acceptors of PSII (the PSII efficiency factor given by

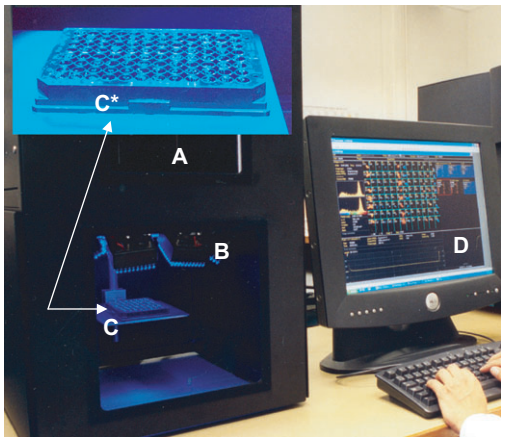

Fig. 4. Fluorescence imaging system (CF*Imager*, Technologica Ltd., Colchester, UK) being used to image simultaneously 96 *Arabidopsis* seedlings being grown on a microtitre plate. (A) Housing for camera and blocking filters. (B) Array of light-emitting diode bricks (total of 16 bricks with 100 LEDs each) that provide measuring and actinic light. (C) Sample platform with 96-well microtitre plate, which is enlarged in C*. (D) CF*Imager* software interface, through which experimental protocols are constructed and run, and fluorescence trace data and images are presented in real time. Examples of the images of fluorescence parameters taken with this system are shown in Fig. 5.

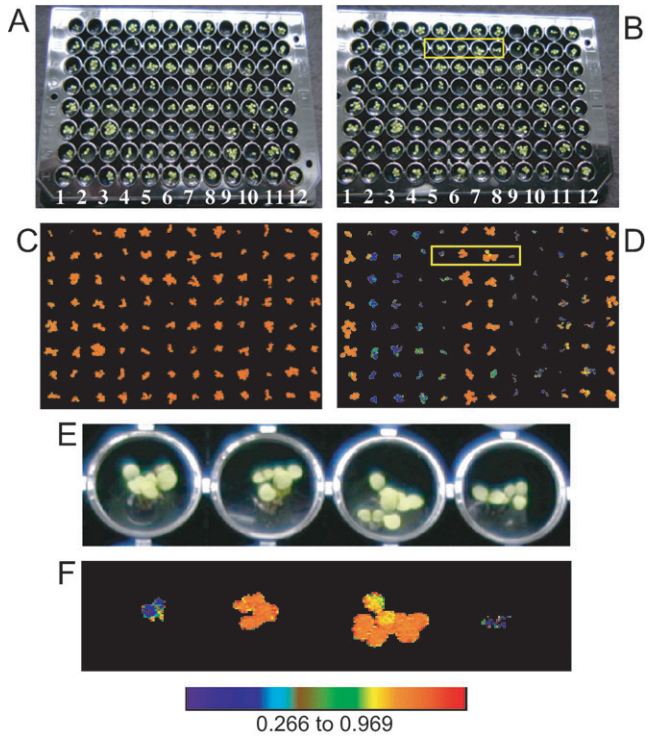

Fig. 5. Detection of the effects of the herbicide, Imazapyr, on plant metabolism using chlorophyll fluorescence imaging prior to the appearance of visual effects on plant growth. (A) 4-d-old *Arabidopsis* plants growing in a 96 well plate immediately before treatment with 0.4 (rows 5 and 11), 0.8 (rows 4 and 10), 4 (rows 3 and 9), and 8 (rows 2 and 8) mM Imazapyr in 50% acetone containing 0.1% Tween; plants in rows 6 and 12 were untreated controls and plants in rows 1 and 7 were treated with 50% acetone containing 0.1% Tween. Plants treated as described for (A) are shown after 24 h in (B). Images of the chlorophyll fluorescence parameter, F_v/F_m, for the plants shown in (A) and (B) are shown in (C) and (D), respectively. (E, F) are enlargements of the plants and images of F_v/F_m outlined by the yellow boxes in (B) and (D), respectively. The data in the images of F_v/F_m shown in (C), (D), and (F), respectively, have been mapped to the colour palette shown below (F). Modified from Barbagallo *et al.* (2003) with permission.

F'_q/F'_v), which is directly related to the rate at which the products of photosynthetic electron transport (NADPH and ATP) are consumed. The importance of this sink limitation in determining electron transport rate is nicely demonstrated during the induction of CO_2 assimilation in a maize leaf (Fig. 6). Images of F'_q/F'_m, F'_v/F'_m, and F'_q/F'_v show that the changes in F'_q/F'_m are similar to the changes in F'_q/F'_v, but not F'_v/F'_m. Consequently, changes in PSII efficiency are being determined by the ability to transfer electrons away from PSII and not by non-photochemical quenching in the PSII antennae.

Decreases in the rate of consumption of NADPH and ATP can result from decreases in carboxylation efficiency, the rate of regeneration of ribulose 1,5-*bis*phosphate, the supply of CO_2 via the stomata to the sites of carboxylation, or the transport of carbohydrates out of the cells (Fig. 7). Such restrictions on PSII electron transport can result in an increase in the proton electrochemical potential difference across the thylakoid membrane, resulting in an increase in non-photochemical quenching in the PSII antennae, which will be detected by a decrease in F'_v/F'_m and serve to reduce the rate of excitation of the PSII reaction centres and prevent the PSII quinone acceptors becoming highly reduced. With increasing levels of stress, the increases in non-photochemical quenching can be insufficient to maintain the PSII electron acceptors partially oxidized and then photodamage to PSII will occur unless alternative electron acceptors, such as oxygen, are used (Ort and Baker, 2002). Consequently, the ability to maintain the PSII quinone acceptors partially oxidized is a key factor in tolerating environmental stresses (Rosenqvist, 2001; Ort and Baker, 2002) and this can be screened for by monitoring F'_q/F'_v. Oxygen can act as an electron acceptor by accepting electrons from PSI to produce superoxide, which is rapidly dismutated to hydrogen peroxide, and which, in turn, is converted to water by an ascorbate peroxidase. This process is often termed the Mehler-peroxidase reaction or the water–water cycle (Fig. 7; Ort and Baker, 2002). Oxygen can also be reduced in the processes of photorespiration (Fig. 7; Ort and Baker, 2002).

It will next be reviewed how fluorescence studies have been applied to investigate the effects of major abiotic stresses on photosynthesis during crop production and to identify cultivars that are tolerant to such stresses. The review is not intended to be exhaustive, but to identify some useful approaches that could be used as the bases for future programmes seeking to improve the stress tolerance of crops.

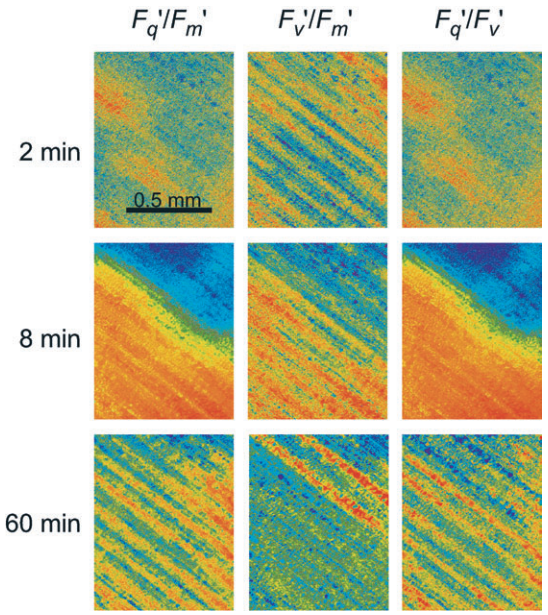

Fig. 6. Regulation of the PSII operating efficiency by ability to consume electrons in photosynthetic metabolism. Images of F'_q/F'_m (PSII operating efficiency), F'_v/F'_m (PSII maximum efficiency), and F'_q/F'_v (PSII efficiency factor) from a maize leaf during induction of photosynthesis after exposure to a PPFD of 815 μmol m^{-2} s^{-1} for 2, 8, and 60 min. Note the similarity between F'_q/F'_m and F'_q/F'_v, but not F'_v/F'_m, for the three sampling times. Modified from Baker *et al.* (2001) with permission.

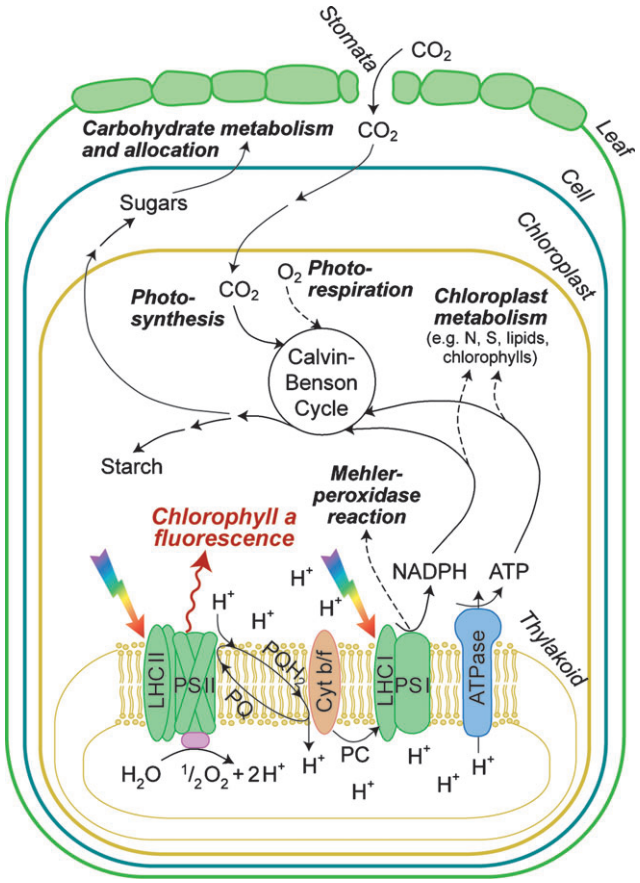

Fig. 7. Schematic representation showing the interactions of the main processes in C_3 photosynthesis in higher plants. Photophosphorylation starts with the absorption of light by the light-harvesting antenna complexes (LHCI and LHCII) associated with photosystem II (PSII) and photosystem I (PSI) in the chloroplast thylakoid membrane. This drives electron transport from water via a series of electron carriers to NADP, producing reducing power (NADPH) and a H^+ electrochemical potential difference across the membrane. Dissipation of this proton motive force by the passage of H^+ back across the membrane through the ATPase drives the production of ATP. Ribulose 1,5-*bis*phosphate carboxylase/oxygenase (Rubisco) catalyses the assimilation of CO_2 with ribulose 1,5-*bis*phosphate (RubP) in the carboxylation reaction of the Calvin–Benson cycle in the stroma of the chloroplast. The diffusion of atmospheric CO_2 into the leaf is regulated by the stomata in the epidermis. Other reactions of the Calvin–Benson cycle utilize NADPH and ATP to produce triose phosphates which are required for the synthesis of carbohydrates. NADPH and ATP are also used in a range of other metabolic activities (e.g. nitrogen and sulphur metabolism, lipid and pigment synthesis) in the chloroplast. Rubisco can also catalyse the photorespiratory oxygenation of RubP, which involves consumption of NADPH and ATP by the Calvin–Benson cycle. O_2 can also be directly photoreduced by electron transfer from PSI to produce superoxide which is then rapidly dismutated to hydrogen peroxide, which in turn is detoxified to water; this process is often termed the Mehler-peroxidase reaction or the water–water cycle. Cyt *b/f*, cytochrome b_6/f complex; PC, plastocyanin; PQ, plastoquinone; PQH_2, plastoquinol.

Drought

In many regions of the world anthropogenic climate change is resulting in extended periods of high temperatures and reduced water supply for crops (Houghton *et al.*, 2001; McCarthy *et al.*, 2001). Often coupled to problems of groundwater pollution (Postel, 2000), this decrease in water availability is increasing the potential for drought in many crop production systems.

Decreases in the relative water content (*RWC*) of leaves initially induce stomatal closure, imposing a decrease in the supply of CO_2 to the mesophyll cells and, consequently, resulting in a decrease in the rate of leaf photosynthesis (Fig. 7; Williams *et al.*, 1999; Lawlor and Cornic, 2002). Such stomatal effects on photosynthesis will not impact on the efficiency of the primary photochemical events of PSII or modify the associated fluorescence induction parameters, such as F_v/F_m, as has been demonstrated in apple (Massacci and Jones, 1990), cashew (Blaikie and Chacko, 1998), coffee (Lima *et al.*, 2002), lavender and rosemary (Nogués and Alegre, 2002), papaya (Marler and Mickelbart, 1998), potato (Jeffries, 1994), tepary and common bean (Castonguay and Markhart III, 1991), and willow (Ögren, 1990). The stomatal limitations imposed on photosynthesis will be accompanied by a decrease in the rate of consumption of ATP and NADPH for CO_2 assimilation (Fig. 7), which could result in decreases in the rate of linear electron transport and, consequently, in F'_q/F'_m. However, operation of the water–water cycle and, in C_3 plants, an increase in photorespiration under the stress conditions may maintain rates of electron transport similar to those observed in non-stressed leaves despite the rate CO_2 assimilation decreasing (Leegood and Edwards, 1996; Flexas *et al.*, 1998, 2002; Noctor *et al.*, 2002; Ort

and Baker, 2002). This would result in little or no change in F'_q/F'_m. Such buffering effects of electron sinks other than CO_2 assimilation have been identified in field-grown grapevine during a slowly imposed drought, where a 75% decrease in stomatal conductance resulted in a 54% decrease in CO_2 assimilation, but only a 19% decrease in the estimated electron transport rate (Flexas *et al.*, 2002).

Although mild drought stress does not affect the maximum efficiency of PSII primary photochemistry, decreases in intracellular CO_2 concentration resulting from stomatal closure can modify the kinetics of photosynthetic induction (Ögren and Öquist, 1985; Ögren, 1990). In willow, the largest differences in the fluorescence induction kinetics between leaves of mild drought-stressed and well-watered plants were observed at low light (180 μmol m^{-2} s^{-1}) and high (5%) atmospheric CO_2 (Ögren, 1990). The parameter found to give the greatest difference between the mild drought-stressed and control leaves under these induction conditions was F' (normalized on F_p) after 25 s. The relative rate of fluorescence quenching in the later stages of the induction of photosynthesis in dessicated leaves has been used successfully to distinguish between drought-resistant and sensitive cultivars of wheat (Havaux and Lannoye, 1985).

With increasing water loss, inhibition of photosynthetic metabolism can occur and can result in a decline in photosynthetic potential which, when sufficiently large to overcome stomatal limitation, will result in a further decrease in CO_2 assimilation rate (Lawlor and Cornic, 2002). When drought stress is severe, decreases in the rate of utilization of ATP and NADPH in photosynthetic metabolism will not be compensated for by increases in water–water cycling and photorespiration, or other electron sinks, and, consequently, decreases in F'_q/F'_m will occur (Fracheboud and Leipner, 2003). Although such decreases in F'_q/F'_m are often considerably less than the decreases in CO_2 assimilation (Bota *et al.*, 2001; Flexas *et al.*, 2002; Maroco *et al.*, 2002), monitoring of F'_q/F'_m should prove useful for rapid screening of tolerance to severe water stress. In severely droughted grapevine leaves, which had a water potential of −4.5 MPa and negligible CO_2 assimilation, significant rates of electron transport were still observed, although these were considerably less then in well-watered leaves (Bota *et al.*, 2001).

Salinity

Salinization of soils is becoming an increasing problem in production systems where high rates of fertilization and irrigation are employed in climates with high evapotranspiration. The initial effects of increasing soil salinity are very similar to those observed when plants are exposed to drought. Reductions in leaf water potential will reduce stomatal conductance and eventually inhibit photosynthetic metabolism, which will result in changes in F'_q/F'_m as discussed in the previous section. Salinity appears not to affect PSII primary photochemistry initially, as demonstrated by the lack of change in F_v/F_m when maize was grown in high concentrations of NaCl (Shabala *et al.*, 1998). As with drought, fluorescence induction characteristics are modified by salinity, and have the potential to be used for screening for salt-tolerant varieties (Smillie and Nott, 1982).

Freezing

At freezing temperatures, leaf metabolism is severely inhibited and the potential for slowly recovering down-regulation and photodamage to PSII is great. Consequently, it is not surprising that measurements of F_v/F_m have been used successfully to identify differences in freezing tolerance (Lindgren and Hällgren, 1993; Binder and Fielder, 1996). In white spruce F_v/F_m showed a sigmoid correlation to the visible damage to needles after freezing at different temperatures (Binder and Fielder, 1996). In lodgepole pine and Scots pine a linear correlation was observed between F_v/F_m and needle damage, and F_v/F_m could be used to estimate the critical temperature for 50% needle damage after freezing (Lindgren and Hällgren, 1993).

Many evergreen and hardy deciduous nursery stock plants are lifted during the autumn and are kept bare-rooted in cold store during the winter before being planted in the spring. In Douglas fir it was found that if F'_q/F'_m of needles was high at the time of lifting in the autumn, then survival of plants was low when planted out in the spring (Perks *et al.*, 2001). Clearly, plants which had effectively down-regulated photosynthetic electron transport in the autumn were better fitted to begin growth after the winter period of cold storage. Both F_v/F_m and F'_q/F'_m measured at the end of the cold storage showed a sigmoid relationship to root growth potential (Perks *et al.*, 2001). Besides being used to establish optimum lifting time before winter storage, F_v/F_m has been used to evaluate the effects of various cultivation techniques on improving survival after freezing (Percival *et al.*, 1999). Regular application of Ca^{2+} in the nutrient solution was shown to improve survival after freezing in both the freezing-tolerant white poplar and the susceptible hornbeam, with F_v/F_m showing higher correlation to survival of plants than ion leakage from roots or leaves, which are the standard assays to test for winter hardiness.

Chilling

The response of crops to low temperatures is generally dependent upon the climatic conditions of the regions from where the plants originated. Crops originating in temperate zones are usually much more tolerant of decreases in temperature than subtropical and tropical species, and depressions in growth and metabolism are rapidly reversed when temperatures rise. However, many crops grown in temperate regions have subtropical and tropical origins and are very sensitive to cool temperatures since they have not

developed effective acclimatory responses to cool temperatures which are found in plants native to temperate climates. The growth and physiology of crops of subtropical and tropical origin can be perturbed by exposure to temperatures as high as 15 °C. A widespread response of chilling-sensitive crops to chilling temperatures is the rapid development of inhibition of photosynthesis (Ort, 2002), which, even at moderate light intensities, can result in down-regulation and photodamage to PSII.

A primary effect of reductions in temperature is the inhibition of photosynthetic carbon metabolism (Leegoood and Edwards, 1996; Allen and Ort, 2001; Ort, 2002) which results in a decrease in the sink for the products of electron transport (ATP and NADPH) and F'_q/F'_m (Brüggermann and Linger, 1994; Andrews *et al.*, 1995; Gray *et al.*, 1997; Fracheboud and Leipner, 2003). Generally, this decrease in the rate of utilization of photoreductants and ATP, which results in a decrease in the PSII efficiency factor (F'_q/F'_v), is also accompanied by an increase in the non-photochemical dissipation of excitation energy as down-regulation of PSII occurs, which is reflected by decreases in F'_v/F'_m (Groom and Baker, 1992; Brüggermann and Linger, 1994; Andrews *et al.*, 1995; Gray *et al.*, 1997; Fracheboud and Leipner, 2003). Differences in chilling tolerance have been identified between chilling-acclimated and non-acclimated *Lycopersicon peruvianum* from the chill-induced decrease in F'_q/F'_v (Brüggermann and Linger, 1994).

Inevitably, chilling stress also results in decreases in F_v/F_m and this has been widely used to screen for differences in tolerance to chilling. A particularly interesting example was its use to evaluate the progeny of crosses between chilling-tolerant and high-yielding rice cultivars in Nepal (Sthapit *et al.*, 1995).

For many crops chilling during leaf development can result in chlorotic leaves. A striking example of this problem has been observed in maize where chloroplasts in leaves grown at 14 °C exhibit incomplete development and photosynthetic capacity is limited (Baker and Nie, 1994). The cause of this appears to be an inhibition of the accumulation of a number of chloroplast-encoded thylakoid proteins compared with the nuclear-encoded chloroplast proteins (Nie and Baker, 1991). A similar effect on accumulation of chloroplast-encoded proteins has been observed when tomato leaves are chilled in the dark (Ort, 2002). Such chill-induced perturbations of chloroplast development result in very large reductions in the values of F_v/F_m, F'_q/F'_m, and F'_v/F'_m (Fryer *et al.*, 1995). Consequently, these fluorescence parameters could potentially be used to screen for cultivars tolerant to chilling during leaf development. However, caution is needed when attempting to use F'_q/F'_m and F'_v/F'_m in such screens, since acclimation of maize leaves to growth at low temperatures can result in a relative increase in electron flux to acceptors other than CO_2, most probably to oxygen, and this results in elevated values of F'_q/F'_m (Fryer *et al.*, 1998). However, the relative increases in F'_q/F'_m are associated with an increase in the ability to scavenge reactive oxygen species (Fryer *et al.*, 1998) and, consequently, can be considered to reflect tolerance to the chilling stress. When this is a possible complication, the relationship between F'_q/F'_m and the quantum efficiency of CO_2 assimilation of the leaves should be examined (Fryer *et al.*, 1998).

High temperatures

High temperature stress can restrict crop growth and productivity (Boyer, 1982) and is likely to become an increasingly important factor with the changing climate. Inactivation of PSII and thylakoid disorganization are considered key features of high temperature stress and have been followed by monitoring the sharp rise in F_o as a function of temperature that indicates the critical temperature for PSII inactivation (Smillie and Nott, 1979; Havaux, 1993). Both the rise in F_o and a decrease in F_v/F_m have been used to determine differences in the response of potato cultivars (Havaux, 1995) and species of birch (Ranney and Peet, 1994) to high temperatures. As CO_2 assimilation and electron transport are inhibited at high temperatures, measurements of F'_q/F'_m and F'_v/F'_m also have potential for use in screens to identify tolerance to high temperatures.

Nutrition

Nitrogen is one of the most important and managed nutrients in plant production. For many species, a strong correlation exists between total leaf nitrogen and CO_2 assimilation at high irradiance (Evans, 1989), consequently, fluorescence is an obvious method to probe nitrogen status. Decreasing nitrogen content of apple leaves was associated with decreases in F'_q/F'_m, F'_q/F'_v, and F'_v/F'_m (Cheng *et al.*, 2000), although nitrogen contents have to reach very low levels before F_v/F_m is affected. However, it is likely that changes in the status of many other nutrients in leaves will have little effect on fluorescence characteristics. For example, reduction of sulphur levels in leaves of sugar beet had to reach starvation levels before any changes in F'_q/F'_m, F'_q/F'_v, and F'_v/F'_m were observed (Kastori *et al.*, 2000). Consequently, careful studies of the effects of deficiency of specific nutrients on leaf fluorescence characteristics are required before using fluorescence parameters to screen for nutrient deficiencies.

In acidic soils aluminium toxicity can affect crop production. Differences in the fluorescence induction parameters $(F_p - F_i)/F_i$, F_i/F_p, and F_v/F_p (see Fig. 1 for definition) can be used to identify wheat cultivars with tolerance to aluminium (Moustakas *et al.*, 1995). Decreases in F_v/F_o have been used to detect aluminium stress in cultivars of sorghum (Peixoto *et al.*, 2002) and *Citrus* species (Pereira *et al.*, 2000). F_v/F_o was used in these studies to amplify the rather small changes observed in F_v/F_m and enable more readily detection of differences

between cultivars; for example, a decrease in F_v/F_m from 0.8 to 0.6 corresponds to a decrease in F_v/F_o from 4.0 to 1.5. However, it should be noted that F_v/F_o, unlike F_v/F_m, is non-linearly related to the maximum quantum efficiency of PSII photochemistry.

The use of sewage sludge and incinerator flyash from domestic waste processing for improving the nutrient status of soils has resulted in the build-up of heavy metals in soils (Tan *et al.*, 1999). Decreases in F_v/F_m have been shown to distinguish differences in the sensitivity of ornamental plants to heavy metals in such soils (Tan *et al.*, 1999). The use of chlorophyll fluorescence to study effects of cadmium, copper, and mercury on photosynthesis has been recently reviewed (Popovic *et al.*, 2003).

Glasshouse production

The commercial production of vegetables and ornamental pot plants is a high technology business involving balancing the optimization of plant production with the control of energy costs. Optimization of the daily carbon gain by the crops in the summer and energy saving in the winter can be achieved by dynamic climate control (Hansen *et al.*, 1996; Aaslyng *et al.*, 2003). It is becoming clear that many subtropical, tropical, and temperate species can cope and grow effectively under much more dynamic climatic conditions than had traditionally been thought to be the case by the horticultural industry. However, a potential limitation to effective optimization of carbon gain by crops is a lack of non-invasive techniques to monitor this parameter in a commercial glasshouse environment. From the above sections it can be seen that fluorescence measurements can be useful in rapidly evaluating the effects of climatic factors, as well as water and nutrient regimes, on plant performance. Consequently, application of fluorescence techniques to study the responses of plants to dynamic climate control have great potential in the future development of systems for optimizing crop production and cost saving in glasshouses.

The use of elevated CO_2 is widespread throughout the glasshouse horticultural industry. Elevation of CO_2 can increase the temperature optimum for photosynthesis in C_3 plants (Leegood and Edwards, 1996) and increase the critical temperature for damage to PSII, which has been monitored from decreases in F_v/F_m and increases in F_o (Taub *et al.*, 2000). Also, the ability of plants to acclimate to different light conditions can be monitored by changes in F'_q/F'_m, F'_q/F'_v, and *NPQ* (Rosenqvist, 2001), and can potentially provide information for the management of shade screens and supplementary lighting during production. Fluorescence has already proved valuable in investigating the effects of changing climatic conditions (light intensity, temperature, and CO_2) on hybrids of the CAM plant *Phalaenopsis* (Lootens and Heursal, 1998).

An extremely stressful time in the life of potted plants is the propagation phase. Most ornamental cultivars are propagated from cuttings. Fluorescence imaging has been used on stem cuttings from roses to evaluate the role of the leaf in relation to the quality of the propagation (Costa, 2002).

Post-harvest physiology

In commercial horticultural production, crops are generally either harvested and transported as fresh produce, such as fruits and vegetables, or transported from the sites of production to market as intact plants, such as potted or bare-rooted ornamental and landscape plants. During harvest and transport, plants and produce may be exposed to a range of stress factors that are not normally encountered during the growth of the crop and which can be potentially damaging. Harvested vegetables and cut flowers can experience drought stress after harvest if not handled properly. Fruits, vegetables, and flowers originating in subtropical and tropical regions may be damaged by the chilling temperatures used during storage. Many fruits are exposed to high temperature treatments to remove any infestations prior to transport. Fresh fruit and vegetables are often transported in packages with modified atmosphere to delay senescence; O_2 is reduced and CO_2 elevated to reduce the rate of metabolic processes linked to the onset of senescence (DeEll and Toivonen, 2003). Potted plants can be subjected to many days in darkness during transport and are frequently kept in very low light levels in the homes of customers.

Fluorescence can be used to detect perturbations caused by handling and transport of green crops (DeEll and Toivonen, 2003) and even to detect damage in fruits that have chlorophyll in their peels (Nedbal *et al.*, 2000). For example, fluorescence imaging of lemons demonstrated that different types of peel damage had different fluorescence signatures (Nedbal *et al.*, 2000). Damaged regions that became mouldy after a few days had low F_v/F_m values, whereas damaged regions that did not develop mould exhibited much higher F_v/F_m values. Changes in the mouldy areas when F_v/F_m was low were followed by using F_o/F_p, which is inversely and non-linearly related to F_v/F_m, but will show greater changes than F_v/F_m when systems have low PSII photochemical activities. This study demonstrated that fluorescence imaging could be used for automatic sorting of lemons for future fruit quality (Nedbal *et al.*, 2000).

Fluorescence has also been successfully used to determine the optimum O_2 and CO_2 concentrations to be used in modified atmosphere packages (MAP) used for the transport and storage of vegetables. Measurements made through the unbroken packaging during storage of broccoli showed that decreases F_v/F_m and F'_q/F'_m correlated with the production of ethanol, ethyl acetate, and the development of odour, which are all characteristic of deteriorating quality (Toivonen and DeEll, 2001).

Many harvested crops are refrigerated during transport and storage. Many crops are very chilling-sensitive and it has already been discussed above how fluorescence has been used successfully to identify sensitivities and tolerances to chilling conditions. However, fluorescence has also been used to evaluate new storage practices, such as hydrocooling of cucumber (DeEll *et al.*, 2000) and treatment of green bell pepper with the antioxidant diphenylamine to decrease chill-induced pitting (Purvis, 2002). Fluorescence has also been used to investigate the effects of storage at chilling temperatures on cut flowers. Although a linear relationship was found between F_v/F_m and days to wilting for cut kangaroo paw flowers stored at 0 °C and 1 °C, this was not the case for flowers stored at 2 °C and above, where flowers showing only small changes in F_v/F_m exhibited wilting (Joyce and Shorter, 2000). Clearly, this indicates that factors not affecting primary PSII photochemistry are important in maintaining flower quality at these temperatures and that this fluorescence parameter should not be used to assess quality. However, it is quite possible that measurements of F'_q/F'_m may prove to be a much more useful indicator of the physiological status of cut flowers.

Concluding remarks

It is evident that chlorophyll fluorescence can be a very sensitive probe of the physiological status of leaves, which can provide very rapid assessment of plant performance in a wide range of situations. A key factor for the successful application of chlorophyll fluorescence measurements in crop improvement programmes will no doubt be the careful selection of appropriate fluorescence parameters to identify changes in plant performance. This process can often involve considerable investigation of the systems in question in order to provide a satisfactory calibration of the changes in fluorescence parameters with key plant performance indicators. However, once a satisfactory calibration has been achieved, fluorescence will provide an extremely powerful analytical tool for plant selection. Although the attractiveness of fluorescence for screening programmes has been considerably enhanced by the recent development of fluorescence imaging systems that can image quite large areas and allow simultaneous measurement of many plants, further improvements in the size of the surface areas that can be imaged will no doubt be made in the future. Coupling of such technical developments in fluorescence imaging systems with automated sampling systems should enable the agricultural and horticultural industries to improve the quality and rate of many of their plant screening programmes.

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References

Aaslyng JM, Lund JB, Ehler N, Rosenqvist E. 2003. IntelliGrow: a greenhouse component-based climate control system. *Environmental Modelling and Software* **18,** 657–666.

Allen DJ, Ort DR. 2001. Impacts of chilling temperatures on photosynthesis in warm-climate plants. *Trends in Plant Science* **6,** 36–42.

Andrews JR, Fryer MJ, Baker NR. 1995. Characterization of chilling effects on photosynthetic performance of maize crops during early season growth using chlorophyll fluorescence. *Journal of Experimental Botany* **46,** 1195–1203.

Baker NR. (ed.) 1996. *Photosynthesis and the environment*. Dordrecht: Kluwer Academic Press.

Baker NR, Nie G-Y. 1994. Chilling sensitivity of photosynthesis in maize. In: Bajaj YPS, ed. *Biotechnology of maize*. Berlin: Springer-Verlag, 465–481.

Baker NR, Oxborough K. 2004. Chlorophyll fluorescence as a probe of photosynthetic productivity. In: Papageorgiou G, Govindjee, eds. *Chlorophyll fluorescence: a signature of photosynthesis.* Dordrecht: Kluwer Academic Publishers (in press).

Baker NR, Oxborough K, Lawson T, Morison JIL. 2001. High resolution imaging of photosynthetic activities of tissues, cells and chloroplasts in leaves. *Journal of Experimental Botany* **52,** 615–621.

Barbagallo RP, Oxborough K, Pallett KE, Baker NR. 2003. Rapid, non-invasive screening for perturbations of metabolism and plant growth using chlorophyll fluorescence imaging. *Plant Physiology* **132,** 485–493.

Berg D, Tiejten K, Wollweber D, Hain R. 1999. From genes to targets: impact of genomics on herbicide discovery. *Proceedings of the BCPC Conference – Weeds* 1999 **2,** 491–500.

Bilger W, Björkman O. 1990. Role of the xanthophyll cycle in photoprotection elucidated by measurements of light-induced absorbance changes, fluorescence and photosynthesis in leaves of *Hedera canariensis*. *Photosynthesis Research* **25,** 173–185.

Binder WD, Fielder P. 1996. Chlorophyll fluorescence as in indicator of frost hardiness in white spruce seedlings from different latitudes. *New Forests* **11,** 233–253.

Blaikie SJ, Chacko EK. 1998. Sap flow, leaf exchange and chlorophyll fluorescence of container-grown cashew (*Anacardium occidentale* L.) trees subjected to repeated cycles of soil drying. *Australian Journal of Experimental Agriculture* **38,** 305–311.

Blowers MH. 1989. Applications of chlorophyll fluorescence to study the penetration of herbicides into leaves. PhD thesis, University of Essex, Colchester, UK.

Bota J, Flexas J, Medrano H. 2001. Genetic variability of photosynthesis and water use in Balearic grape vine cultivars. *Annals of Applied Biology* **138,** 353–361.

Boyer J. 1982. Plant productivity and the environment. *Science* **218,** 443–448.

Bradbury M, Baker NR. 1981. Analysis of the slow phases of the *in vivo* chlorophyll fluorescence induction curve. Changes in the redox state of photosystem II electron acceptors and fluorescence emission from photosystems I and II. *Biochimica et Biophysica Acta* **635,** 542–551.

Bradbury M, Baker NR. 1984. A quantitative determination of photochemical and non-photochemical quenching during the slow phase of the chlorophyll fluorescence induction curve of bean leaves. *Biochimica et Biophysica Acta* **765,** 275–281.

Bro E, Meyer S, Genty B. 1996. Heterogeneity of leaf CO_2 assimilation during photosynthetic induction. *Plant, Cell and Environment* **19,** 1349–1358.

Brüggemann W, Linger P. 1994. Long-term chilling of young tomato plants under low light. IV. Differential responses of chlorophyll fluorescence quenching coefficients in *Lycopersicon* species of different chilling sensitivity. *Plant and Cell Physiology* **35,** 585–591.

Butler WL. 1978. Energy distribution in the photochemical apparatus of photosynthesis. *Annual Review of Plant Physiology* **29,** 345–378.

Castonguay Y, Markhart III AH. 1991. Saturated rates of photosynthesis in water-stressed leaves of common bean and tepary bean. *Crop Science* **31,** 1605–1611.

Cheng L, Fuchigami LH, Breen PJ. 2000. Light absorption and partitioning in relation to nitrogen content in 'Fuji' apple leaves. *Journal of the Americal Society of Horticultural Science* **125,** 581–587.

Cornic G, Ghashghaie J. 1991. Effect of temperature on net CO_2 assimilation and photosystem II quantum yield on electron transfer of French bean leaves (*Phaseolus vulgaris* L.) during drought stress. *Planta* **183,** 178–184.

Costa JMRC. 2002. The role of the leaf in growth dynamics and rooting of leafy stem cuttings of rose. PhD thesis. University of Wageningen, The Netherlands.

Crudace AJ. 2000. The investigation of the *in vivo* behaviour of a maize herbicide—Isoxaflutole. PhD thesis. University of Essex, Colchester, UK.

DeEll JR, Toivonen PMA. 2003. Use of chlorophyll fluorescence in postharvest quality assessments of fruits and vegetables. In: DeEll JR, Tiovonen PMA, eds. *Practical applications of chlorophyll fluorescence in plant biology*. Boston: Kluwer Academic Publishers, 201–242.

DeEll JR, Vigneault C, Lemerre S. 2000. Water temperature for hydrocooling field cucumbers in relation to chilling injury during storage. *Postharvest Biology and Technology* **18,** 27–32.

Eckstein J, Beyschlag W, Mott KA, Ryel RJ. 1996. Changes in photon flux can induce stomatal patchiness. *Plant, Cell and Environment* **19,** 1066–1074.

Edwards GE, Baker NR. 1993. Can CO_2 assimilation in maize leaves be predicted accurately from chlorophyll fluorescence analysis? *Photosynthesis Researach* **37,** 89–102.

Evans DA. 1999. How can technology feed the world safely and sustainably. In: Brooks GT, Roberts TR, eds. *Pesticide chemistry and bioscience: the food-environment challenge*. London, UK: Royal Society of Chemistry, 3–24.

Evans JR. 1989. Photosynthesis and nitrogen relationships in leaves of C_3 plants. *Oecologia* **78,** 9–19.

Flagella Z, Campanile RG, Ronga G, Stopelli MC, Pastore D, De Caro A, Di Fonzo N. 1996. The maintenance of photosynthetic electron transport in relation to osmotic adjustment in durum wheat cultivars differing in drought resistance. *Plant Science* **118,** 127–133.

Flexas J, Bota J, Escalona JM, Sampol B, Medrano H. 2002. Effects of drought on photosynthesis in grapevines under field conditions: an evaluation of stomatal and mesophyll limitations. *Functional Plant Biology* **29,** 461–471.

Flexas J, Escalona JM, Medrano H. 1998. Down-regulation of photosynthesis by drought under field conditions in grapevine leaves. *Australian Journal of Plant Physiology* **25,** 893–900.

Fracheboud Y, Leipner J. 2003. The application of chlorophyll fluorescence to study light, temperature and drought stress. In: DeEll JR, Tiovonen PMA eds. *Practical applications of chlorophyll fluorescence in plant biology*. Boston: Kluwer Academic Publishers, 125–150.

Fryer MJ, Andrews JR, Oxborough K, Blowers DA, Baker NR. 1998. Relationship between CO_2 assimilation, photosynthetic electron transport and active oxygen metabolism in leaves of maize in the field during periods of low temperature. *Plant Physiology* **116,** 571–580.

Fryer MJ, Oxborough K, Martin B, Ort DR, Baker NR. 1995. Factors associated with the depression of photosynthetic quantum efficiency in maize at low growth temperatures. *Plant Physiology* **108,** 761–767.

Genty B, Briantais J-M, Baker NR. 1989. The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochimica et Biophysica Acta* **990,** 87–92.

Genty B, Harbinson J, Baker NR. 1990. Relative quantum efficiencies of the two photosystems of leaves in photorespiratory and non-photorespiratory conditions. *Plant Physiology and Biochemistry* **28,** 1–10.

Genty B, Meyer S. 1995. Quantitative mapping of leaf photosynthesis using chlorophyll fluorescence imaging. *Australian Journal of Plant Physiology* **22,** 277–284.

Gray GR, Cauvin L-P, Sarhan F, Huner NPA. 1997. Cold acclimation and freezing tolerance. A complex interaction of light and temperature. *Plant Physiology* **114,** 467–474.

Groom QJ, Baker NR. 1992. Analysis of light-induced depressions of photosynthesis in leaves of a wheat crop during the winter. *Plant Physiology* **100,** 1217–1223.

Habash D, Percival MP, Baker NR. 1985. Rapid chlorophyll fluorescence technique for the study of penetration of photosynthetically active herbicides into leaf tissue. *Weed Research* **25,** 389–395.

Hansen JM, Ehler N, Karlsen P, Høgh-Schmidt, Rosenqvist E. 1996. Decreasing the environmental load by a photosynthetic-based system for greenhouse climate control. *Acta Horticulturae* **440,** 105–110.

Harbinson J, Genty B, Baker NR. 1990. The relationship between CO_2 assimilation and electron transport in leaves. *Photosynthesis Research* **25,** 213–224.

Havaux M. 1993. Rapid photosynthetic adaptation to heat stress triggered in potato leaves by moderately elevated temperatures. *Plant, Cell and Environment* **16,** 461–467.

Havaux M. 1995. Temperature sensitivity of the photochemical function of photosynthesis in potato (*Solanum tuberosum*) and a cultivated Andean hybrid (*Solanum×juzepczukii*). *Journal of Plant Physiology* **146,** 47–53.

Havaux M, Lannoye R. 1985. Drought resistance of hardy wheat cultivars measured by a rapid chlorophyll fluorescence test. *Journal of Agricultural Sciences Cambridge* **104,** 501–504.

Houghton JT, Ding Y, Griggs DJ, Noguer M, van der Linden PJ, Dai X, Maskell K, Johnson CA. 2001. *IPCC report on climate change 2001: the scientific basis*. New York: Cambridge University Press.

Jefferies RA. 1994. Drought and chlorophyll fluorescence in field-grown potato (*Solanum tuberosum*). *Physiologia Plantarum* **90,** 93–97.

Joyce DC, Shorter AJ. 2000. Long term, low temperature storage injures kangaroo paw cut flowers. *Postharvest Biology and Technology* **20,** 203–206.

Kastori R, Plesnicar M, Arsenijevic-Maksimovic I, Petrovic N, Pankovic D, Sakac Z. 2000. Photosynthesis, chlorophyll fluorescence and water relations in young sugar beet plants as affected by sulfur supply. *Journal of Plant Nutrition* **23,** 1037–1049.

Kautsky H, Zedlitz W. 1941. Fluoreszenzkurven von Chloroplasten-Grana. *Naturwissenschaften* **29,** 101–102.

Krall JP, Edwards GE. 1990. Quantum yields of photosystem. II. Electron transport and CO_2 fixation in C_4 plants. *Australian Journal of Plant Physiology* **17,** 579–588.

Krall JP, Edwards GE. 1991. Environmental effects on the relationship between quantum yield of carbon assimilation and *in*

vivo PSII electron transport in maize. *Australian Journal of Plant Physiology* **18,** 267–278.

Krall JP, Edwards GE, Ku MSB. 1991. Quantum yield of photosystem II and efficiency of CO_2 fixation in *Flaveria* (Asteraceae) species under varying light and CO_2. *Australian Journal of Plant Physiology* **18,** 369–383.

Lawlor DW, Cornic G. 2002. Photosynthetic carbon assimilation and associated metabolism in relation to water deficits in higher plants. *Plant, Cell and Environment* **25,** 275–294.

Leegood RC, Edwards GE. 1996. Carbon metabolism and photorespiration: temperature dependence in relation to other environmental factors. In: Baker NR, ed. *Photosynthesis and the environment*. Dordrecht: Kluwer Academic Publishers, 191–221.

Leipner J, Oxborough K, Baker NR. 2001. Primary sites of ozone-induced perturbations of photosynthesis in leaves: identification and characterization in *Phaseolus vulgaris* using high resolution chlorophyll fluorescence imaging. *Journal of Experimental Botany* **52,** 1689–1696.

Lima ALS, DaMatta FM, Pinheiro HA, Totola MR, Loureiro ME. 2002. Photochemical responses and oxidative stress in two clones of *Coffea canephora* under water deficit conditions. *Environmental and Experimental Botany* **47,** 239–247.

Lindgren K, Hällgren J-E. 1993. Cold acclimation fo *Pinus contorta* and *Pinus sylvestris* assessed by chlorophyll fluorescence. *Tree Physiology* **13,** 97–106.

Lootens P, Heursel J. 1998. Irradiance, temperature, and carbon dioxide enrichment affect photosynthesis in *Phalaenopsis* hybrids. *HortScience* **33,** 1183–1185.

Marler TE, Mickelbart MV. 1998. Drought, leaf gas exchange, and chlorophyll fluorescence of field-grown papaya. *Journal of the American Society of Horticultural Science* **123,** 714–718.

Maroco JP, Rodrigues ML, Lopes C, Caves MM. 2002. Limitation to leaf photosynthesis in field-grown grape vine under drought—metabolic and modelling approaches. *Functional Plant Biology* **29,** 451–459.

Massacci A, Jones HG. 1990. Use of simultaneous analysis of gas-exchange and chlorophyll fluorescence quenching for analysing the effects of water stress on photosynthesis in apple leaves. *Trees* **4,** 1–8.

Maxwell K, Johnson GN. 2000. Chlorophyll fluorescence—a practical guide. *Journal of Experimental Botany* **51,** 659–668.

McAlister ED, Myers J. 1940. The time-course of photosynthesis and fluorescence observed simultaneously. *Smithsonian Institute Miscellaneous Collections* **99,** 1–37.

McCarthy JJ, Canziani OF, Leary NA, Dokken DJ, White KS. 2001. *IPCC report on climate change 2001: impacts, adaptation and vulnerability*. New York: Cambridge University Press.

Meng Q, Siebke K, Lippert P, Baur B, Mukherjee U, Weis E. 2001. Sink-source transition in tobacco leaves visualized using chlorophyll fluorescence imaging. *New Phytologist* **151,** 585–596.

Moustakas M, Ouzounidou G, Lannoye R. 1995. Aluminium effects on photosynthesis and elemental uptake in an aluminium-tolerant and non-tolerant wheat cultivar. *Journal of Plant Nutrition* **18,** 669–683.

Nedbal L, Soukupová J, Whitmarsh J, Trtílek M. 2000. Post-harvest imaging of chlorophyll fluorescence from lemons can be used to predict fruit quality. *Photosynthetica* **38,** 571–579.

Nie G-Y, Baker NR. 1991. Modifications to thylakoid composition during development of maize leaves at low growth temperatures. *Plant Physiology* **95,** 184–191.

Noctor G, Veljovic-Jovanovic S, Driscoll S, Novitskaya L, Foyer CH. 2002. Drought and oxidative load in the leaves of C_3 plants: a predominant role for photorespiration? *Annals of Botany* **89,** 841–850.

Nogués S, Alegre L. 2002. An increase in water deficit has no impact on the photosynthetic capacity of field-grown Mediterranean plants. *Functional Plant Biology* **29,** 621–630.

Ögren E. 1990. Evaluation of chlorophyll fluoescence as a probe for drought stress in willow leaves. *Plant Physiology* **93,** 1280–1285.

Ögren E, Öquist G. 1985. Effects of drought on photosynthesis, chlorophyll fluorescence and photoinhibition susceptibility in intact willow leaves. *Planta* **166,** 380–388.

Ort DR. 2002. Chilling-induced limitations on photosynthesis in warm climate plants: contrasting mechanisms. *Environmental Control in Biology* **40,** 7–18.

Ort DR, Baker NR. 2002. A photoprotective role for O_2 as an alternative electron sink in photosynthesis? *Current Opinion of Plant Biology* **5,** 193–198.

Oxborough K. 2004. Using chlorophyll *a* fluorescence imaging to monitor photosynthetic performance. In: Papageorgiou G, Govindjee, eds. *Chlorophyll fluorescence: a signature of photosynthesis.* Dordrecht: Kluwer Academic Publishers (in press).

Oxborough K, Baker NR. 1997. Resolving chlorophyll *a* fluorescence images of photosynthetic efficiency into photochemical and non-photochemical components—calculation of q_P and F'_v/F'_m without measuring F'_o. *Photosynthesis Research* **54,** 135–142.

Papageorgiou G, Govindjee. (eds) 2004. Chlorophyll fluorescence: a signature of photosynthesis. Dordrecht: Kluwer Academic Publishers (in press).

Peixoto PHP, Da Matta, FM, Cambraia J. 2002. Responses of the photosynthetic apparatus to aluminium stress in two sorghum cultivars. *Journal of Plant Nutrition* **25,** 821–832.

Percival MP, Baker NR. 1991. Herbicides and photosynthesis. In: Baker NR, Percival MP, eds. *Herbicides*. Amsterdam: Elsevier, 1–26.

Percival GC, Boyle C, Baird L. 1999. The influence of calcium supplementation on the freezing tolerance of woody plants. *Journal of Arboriculture* **25,** 285–291.

Pereira WE, de Siqueira DL, Martínez CA, Puiatti M. 2000. Gas exchange and chlorophyll fluorescence in four citrus rootstocks under aluminium stress. *Journal of Plant Physiology* **157,** 513–520.

Perks JP, Monaghan S, O'Reilly C, Osborne BA, Mitchell DT. 2001. Chlorophyll fluorescence characteristics, performance and survival of freshly lifted and cold stored Douglas fir seedlings. *Annals of Forest Science* **58,** 225–235.

Popovic R, Dewez D, Juneau P. 2003. Applications of chlorophyll fluorescence in ecotoxicology: heavy metals, herbicide and air pollutants. In: DeEll JR, Tiovonen PMA, eds. *Practical applications of chlorophyll fluorescence in plant biology*, Boston: Kluwer Academic Publishers, 151–184.

Postel SL. 2000. Entering an era of water scarcity: the challenges ahead. *Ecological Applications* **100,** 941–948.

Purvis AC. 2002. Diphenylamine reduces chilling injury of green bell pepper fruit. *Postharvest Biology and Technology* **25,** 41–48.

Ranney TG, Peet MM. 1994. Heat tolerance of five taxa of birch (*Betula*): physiological responses to supraoptimal leaf temperatures. *Journal of the American Society of Horticultural Science* **119,** 243–248.

Rosenqvist E. 2001. Light acclimation maintains the redox state of PSII electron acceptor Q_A within a narrow range over a broad range of light intensities. *Photosynthesis Research* **70,** 299–310.

Rosenqvist E, van Kooten O. 2003. Chorophyll fluorescence: a general description and nomenclature. In: DeEll JR, Tiovonen PMA, eds. *Practical applications of chlorophyll fluorescence in plant biology*, Boston: Kluwer Academic Publishers, 31–77.

Scholes JD, Rolfe SA. 1996. Photosynthesis in localized regions of oat leaves infected with crown rust (*Puccinia*

coronata): quantitative imaging of chlorophyll fluorescence. *Planta* **199,** 573–582.

Shabala SN, Shabala SI, Martynenko AI, Babourina O, Newman IA. 1998. Salinity effect on bioelectric activity, growth, Na^+ accumulation and chlorophyll fluorescence of maize leaves: a comparative survey and prospects for screening. *Australian Journal of Plant Physiology* **25,** 609–616.

Shaner D, Stidham M, Muhitch M, Reider M, Robson P. 1985. Mode of action of the imidazolinones. *Proceedings of the BCPC Conference—Weeds 1985* **1,** 147–154.

Siebke K, Weis E. 1995*a*. Imaging of chlorophyll *a* fluorescence in leaves: topography of photosynthetic oscillations in leaves of *Glechoma hederacea*. *Photosynthesis Research* **45,** 225–237

Siebke K, Weis E. 1995*b*. Assimilation images of leaves of *Glechoma hederacea*: analysis of non-synchronous stomata related oscillations. *Planta* **196,** 155–165.

Siebke K, von Caemmerer S, Badger M, Furbank RT. 1997. Expressing an RbcS antisense gene in transgenic *Flaveria bidentis* leads to an increased quantum requirement for CO_2 fixed in photosystems I and II. *Plant Physiology* **105,** 1163–1174.

Singh BK, Newhouse KE, Stidham, MA, Shaner DL. 1989. Acetohydroxyacid synthase-imidazolinone interaction. In: Copping LC, Dalziel J, Dodge AD, eds. *Prospects for amino acid biosynthesis inhibitors in crop protection and pharmaceutical chemistry*. BCPC Monograph No. 42. Farnham, UK: British Crop Protection Council, 87–95.

Smillie RM, Nott R. 1979. Heat injury in leaves of alpine, temperate and tropical plants. *Australian Journal of Plant Physiology* **6,** 135–141.

Smillie RM, Nott R. 1982. Salt tolerance in crop plants monitored by chlorophyll fluorescence *in vivo*. *Plant Physiology* **70,** 1049–1954.

Sthapit BR, Witcombe JR, Wilson JM. 1995. Methods of selection for chilling tolerance in nepalese rice by chlorophyll fluorescence analysis. *Crop Science* **35,** 90–94.

Tan LP, He J, Lee SK. 1999. Physiological responses of certain ornamental plants to sludge and artificial topsoils derived from flyash, sludge and rengam series subsoil. *Journal of Plant Nutrition* **22,** 987–999.

Taub DR, Seemann JR, Coleman JS. 2000. Growth in elevated CO_2 protects photosynthesis against high-temperature damage. *Plant, Cell and Environment* **23,** 649–656.

Toivonen PMA, DeEll JR. 2001. Chlorophyll fluorescence, fermentation product accumulation, and quality of stored broccoli in modified atmosphere packages and subsequent air storage. *Postharvest Biology and Technology* **23,** 61–69.

van Kooten O, Snel JFH. 1990. The use of chlorophyll fluorescence nomenclature in plant stress physiology. *Photosynthesis Research* **27,** 121–133.

Williams MH, Rosenqvist E, Buchhave M. 1999. Response of potted miniature roses (*Rosa*×*hybrida*) to reduced water availability during production. *Journal of Horticultural Science and Biotechnology* **74,** 301–308.