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Measurements of the Quantum Yield of Carbon Assimilation and Chlorophyll Fluorescence for Assessment of Photosynthetic Performance of Crops in the Field [and Discussion]

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Measurements of the quantum yield of carbon assimilation and chlorophyll fluorescence for assessment of photosynthetic performance of crops in the field

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An apparatus is described for the rapid measurement in the field of the quantum yield of CO_2 assimilation, ϕ, and chlorophyll fluorescence kinetics from attached leaves exposed to ambient CO_2 concentrations. This apparatus was used to measure ϕ and the ratio of variable to maximal fluorescence, F_V/F_M, of fully expanded leaves of a maize crop in northeast Essex at monthly intervals throughout the growing season. The quantum yield of CO_2 assimilation and F_V/F_M increased from May to August and then decreased in September. A linear correlation between ϕ and F_V/F_M was observed for the leaves.

The relations between light, temperature, the quantum yield of CO_2 assimilation and fluorescence emission kinetics of leaves of a maize crop during the early growing season were also examined. Decreases in ϕ associated with chilling temperatures and high light were observed and identified from analyses of fluorescence kinetics as being attributable to photoinhibitory damage of the photosynthetic apparatus.

The possibility of using measurements of ϕ and fluorescence kinetics for screening the photosynthetic performance of crops is considered. Studies with winter rape demonstrated that changes in ϕ during the growing season were correlated with changes in the efficiency of light-energy conversion to dry matter by the crop.

Introduction

It is increasingly evident that most canopy photosynthesis in crops occurs at light levels below those required to saturate photosynthesis (Inoue *et al.* 1968; Beuerlein & Pendleton 1971; Hatfield & Carlson 1978; Ort & Baker 1988; Baker *et al.* 1989). This fact can account for the strong correlations often observed for crops between total dry matter production of the crop and the total amount of radiation intercepted by it (Monteith 1977; Gallagher & Biscoe 1978). Consequently, the quantum yield of photosynthesis, defined as the efficiency with which absorbed photons can be utilized for CO_2 assimilation, has emerged as an important parameter for assessment of photosynthetic performance of individual leaves and crops (Baker *et al.* 1989). Although a method, using commercial instrumentation, is available for the rapid measurement of the apparent quantum yield of O_2 evolution of leaf discs exposed to saturating CO_2 (Walker & Osmond 1986), no convenient method exists for the routine determination of the absolute quantum yield of CO_2 assimilation of leaves attached to plants in the field and exposed to atmospheric CO_2 concentration.

Measurements and analyses of chlorophyll fluorescence emission from leaf tissue, made in conjunction with measurements of gas exchange, have proved valuable in elucidating the physiological and biochemical bases for changes in the ability of the leaves to assimilate CO_2 (Walker *et al.* 1983; Ireland *et al.* 1984, 1985, 1986, 1987, 1988; Furbank & Walker 1985; Sivak & Walker 1986; Walker & Osmond 1986). In this paper we describe an apparatus and

method for measuring both the absolute quantum yield of CO_2 assimilation and chlorophyll fluorescence kinetics from attached leaves exposed to atmospheric CO_2 concentration in the field. We also examine the possible relations between the quantum yield of photosynthesis, chlorophyll fluorescence parameters and crop photosynthetic productivity.

Materials and methods

Plant material

Zea mays (cv. LG11) and *Brassica napus* (cv. Bienvenu) were grown from seed in field plots at Colchester (northeast Essex, U.K.) during the summer and autumn/winter respectively. The planting density of maize was 10 plants m^{-2}, whereas rape seeds were sown at 9 cm intervals in rows spaced 15 cm apart.

Light-integrating spherical leaf chamber

The design of the leaf chamber is shown in figure 1. The leaf chamber was constructed from two hemispheres manufactured in aluminium. The internal walls of the hemispheres were coated with a high reflectance (more than 99%) white paint (Kodak Eastman). A small volume (approximately 50 cm^3) gas-exchange compartment within the sphere was formed by the insertion of a Teflon-coated acrylic plastic window across each of the hemispheres. Gas flow into the chamber was through a series of small inlet holes (diameter 0.5 mm) via a common manifold whereas the gas outlet holes are larger (diameter 1.0 mm). A hermetic seal between the two hemispheres was formed by a pair of silicone-rubber '**O**' rings (0.35 mm thickness; Silicone Products Ltd, Blackburn, U.K.), which clamp over the leaf blade or petiole but are

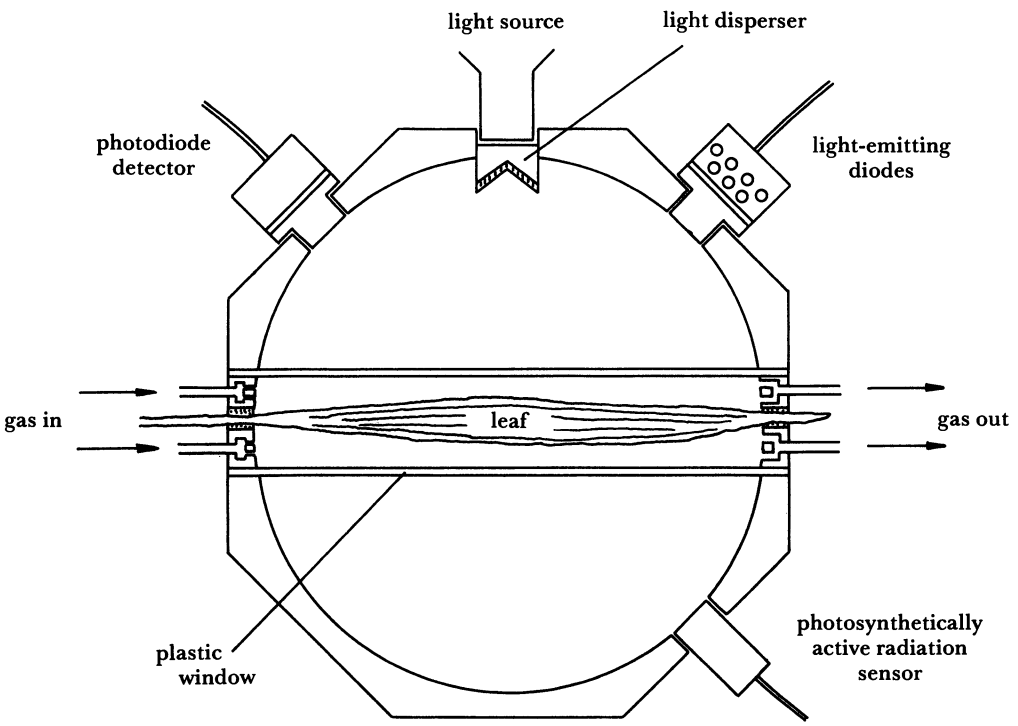

Figure 1. Cross section of light-integrating spherical leaf chamber used to determine the absolute quantum yield of carbon assimilation of crop leaves.

sufficiently soft to prevent physical damage. A port at the top of the chamber allows the input of actinic light which was provided by a DC powered 12 V, 75 W quartz-iodide lamp cooled by a 5 amp fan. It is important that the light flux should be uniform within the chamber and the incoming radiation was therefore scattered by an inverted perspex cone, which is mirrored on the inside to prevent central focusing. The three other ports house a non-actinic, low-intensity light source, a light-detecting diode and a quantum sensor respectively. The quantum sensor employed was a cosine-corrected calibrated detector of photosynthetically active photon flux density (PPFD) (Macam, Photometrics Ltd, Livingston, U.K.). The chamber was supported by a clamp that allowed the two hemispheres to be brought together accurately under an adjustable mechanical pressure, forming a seal over the selected leaf.

The principles of the measurement of the absorbed light flux by a leaf in the integrating sphere have been described previously by Idle & Proctor (1983).

Gas-exchange system

A controlled-rate gas flow to the chamber was provided by a gas supply unit incorporating a mass flow controller and powered by a 12 V DC source (ADC Ltd, Hoddesdon, U.K.). Air was collected 5 m above the ground surface by a vertical pipe. The gas flow was then divided into an analysis flow, which passed through the chamber, and a reference flow, which bypassed the chamber. Both flows were finally passed through a portable infrared gas analyser (ADC Ltd) for measurement of both absolute and differential CO_2 concentrations. The rate of CO_2 exchange by an enclosed leaf was then determined by using the standard equations for an open gas-exchange system (Long 1986).

Determination of absolute quantum yield of CO_2 *assimilation*

The absolute quantum yield of CO_2 assimilation was determined by measuring the CO_2 assimilation rate at a series of light fluxes and plotting the rate against the calculated absorbed flux. The quantum yield is given by the slope of the linear phase of this response and was obtained by a linear regression analysis. However, at low light levels (less than 50 μmol quanta $m^{-2}\ s^{-1}$), there is frequently an apparent elevation of the CO_2 assimilation rate resulting in a nonlinearity in the response around the light compensation point. This event, which is termed the Kok effect, is believed to be due to a light-dependent inhibition of respiration (Sharp *et al.* 1984). The quantum yield should be determined at light levels above the light compensation point where the response is linear and according to previous recommendations (Sharp *et al.* 1984; Jarvis & Sandford 1986). In this study, quantum yield was calculated from the slope of the response determined by five data points between absorbed photon fluxes of 50 and 150 μmol quanta $m^{-2}\ s^{-1}$ (see figure 2). However, when many routine measurements of quantum yield are required in the field, three data points taken between the absorbed photon fluxes of 50 and 120 μmol quanta $m^{-2}\ s^{-1}$ have proved satisfactory to give accurate estimations of quantum yield; such a procedure reduces significantly the measuring time for each leaf.

Measurement and analyses of chlorophyll fluorescence kinetics

Chlorophyll fluorescence from leaves in the leaf chamber was measured by using a modulated fluorimeter that has been described previously (Ogren & Baker 1985). The principles of this measurement of modulated fluorescence and details of the apparatus have been presented previously (Ogren & Baker 1985; Hipkins & Baker 1986; Schreiber *et al.*

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1986). Analyses of the photochemical and non-photochemical components of the quenching of chlorophyll fluorescence were determined by addition of a second, continuous excitation (Bradbury & Baker 1984).

Determination of radiant energy conversion efficiency

A randomized block design was used to sample *B. napus* plants at 5 or 7 day intervals throughout the autumn and winter. Roots were washed free of soil and the plants dried at 80 °C to a constant weight. Canopy-light interception was determined by using tube solarimeters (TSM, Delta-T Devices Ltd), which measure radiation between 400 and 3000 nm. It was assumed that 50 % of the radiation incident upon the canopy was within the 400–700 nm waveband (Szeicz 1974). The penetration of photosynthetically active radiation through the canopy was estimated from the ratio of total radiation above and below the canopy by using values for radiative transfer in a plant canopy, and the reflection of photosynthetically active radiation by the canopy was taken as 9 % (Ross 1975). The efficiency of conversion of radiant energy to dry matter, in grams per megajoule, was then calculated.

Results and discussion

On 1 May 1986 maize seeds were sown in the field plot, and throughout the growing season the light–response curve of CO_2 assimilation and the characteristics of fluorescence induction of the youngest, fully expanded leaves were measured at monthly intervals. Representative curves for the light response of CO_2 assimilation during 100 days from sowing the seed are shown in figure 2. Both the quantum yield of carbon assimilation (ϕ) and the rate of CO_2 assimilation at a PPFD of 850 μmol quanta $m^{-2}\,s^{-1}$, which, although not saturating, was

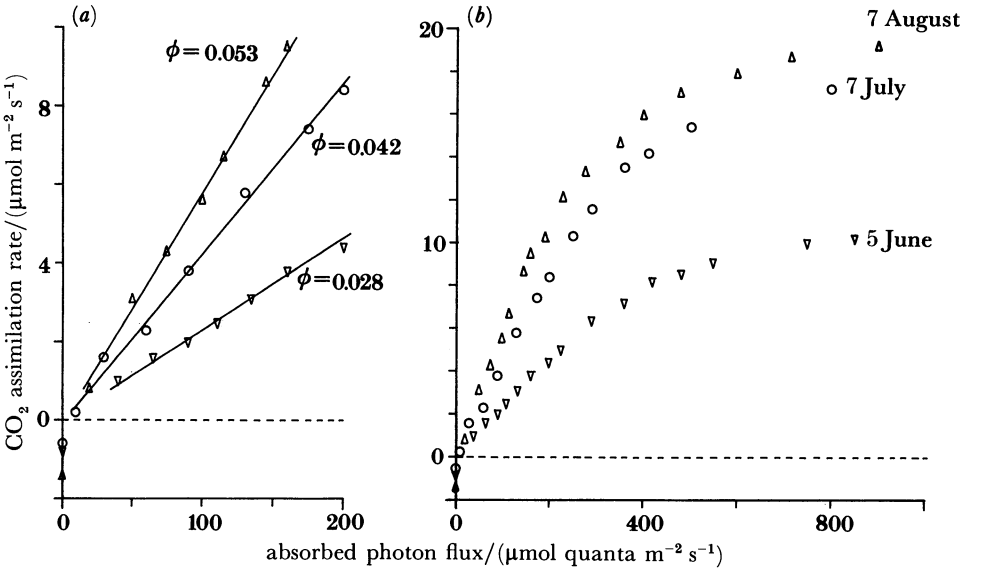

FIGURE 2. Representative responses of the rate of CO_2 assimilation per unit leaf area to absorbed photon flux for the youngest, fully expanded leaves of a maize crop sampled on 5 June (▽), 7 July (○) and 7 August (△). These dates are 36, 68 and 100 days respectively from sowing the seed on 1 May 1986, in a field plot in northeast Essex. The response over an absorbed photon flux range of 50–200 μmol quanta $m^{-2}\,s^{-1}$ is shown in (*a*), and the response up to an absorbed photon flux of 1000 μmol quanta $m^{-2}\,s^{-1}$ is given in (*b*). The quantum yield of CO_2 assimilation (ϕ) is given for each leaf in (*a*).

approaching saturation intensity, were low at the beginning of the growing season and increased with time. The mean ϕ on 5 June, 36 days after sowing the seed, was 0.028 ± 0.006, whereas ϕ measured for leaves of plants grown in a glasshouse at *ca.* 23 °C during the same period varied between 0.055 and 0.060. Clearly a depression of ϕ was induced by the field conditions during the first month of growth. On 7 July ϕ had increased to 0.042 ± 0.004, and by 7 August had further increased to 0.055 ± 0.007, a value similar to that observed for glasshouse-grown maize leaves. This was the maximum value obtained for ϕ of leaves of the maize crop during the growing season and was significantly lower than the maximum value of 0.65 expected for C_4 grasses using the NADP-dependent malic enzyme as their major C_4 decarboxylase (Pearcy & Ehleringer 1984). From measurements of the fluorescence induction kinetics of leaves that had been dark-adapted for 30 min, the ratio of variable to maximal fluorescence (F_V/F_M) at the peak, P, of the induction curve was determined with a PPFD at both leaf surfaces of *ca.* 600 μmol quanta m^{-2} s^{-1}. During the growing season F_V/F_M increased from between 0.3 and 0.4 on 5 June to 0.61–0.68 on 7 August, and then decreased to between 0.50 and 0.60 on 12 September (figure 3). A linear correlation ($r^2 = 0.97$) was observed between ϕ and F_V/F_M for the maize leaves during the growing season, with the extrapolated fitted regression line going near to the origin (figure 3).

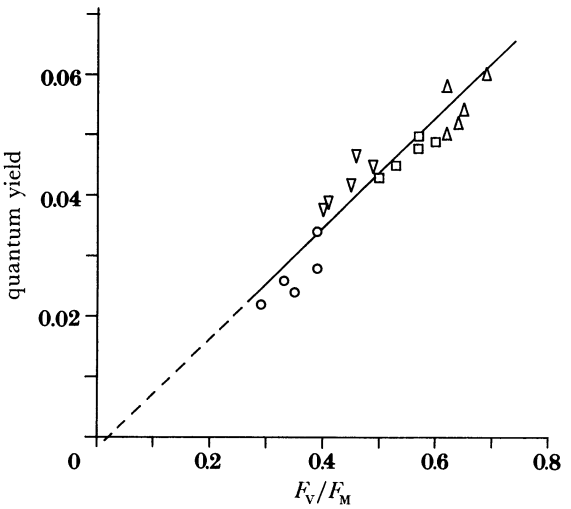

FIGURE 3. Relation between the quantum yield of CO_2 assimilation and the ratio of variable to maximal fluorescence, F_V/F_M, for the youngest, fully expanded leaves of a maize crop during the growing season. Leaves were sampled on 5 June (○), 7 July (▽), 7 August (△) and 12 September (□), which are respectively 36, 68, 100 and 135 days after sowing the seed on 1 May 1986.

Linear correlations have been observed previously between F_V/F_M measured at 77 K and the quantum yield of O_2 evolution for a range of species (Björkman & Demmig 1987). The data presented in figure 3 further support the suggestion that the quantum yield of photosystem II photochemistry can relate directly to the quantum yield of carbon assimilation in leaves. However, caution should be exercised in assuming that such a linear relation exists in all situations, because recent observations of F_V/F_M and ϕ in leaves of *Cyperus longus* and *Spartina cynosuroides* during recovery from photoinhibition suggest that a curvilinear relation exists between these two parameters (C. R. Ireland, S. P. Long and N. R. Baker, unpublished data).

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During June 1985, a study was made of the relations between changes in ϕ and fluorescence emission characteristics of leaves of a maize crop growing in northeast Essex and the environmental variables PPFD and temperature, with a view to assessing whether low-temperature-induced photoinhibition of photosynthesis occurred in maize leaves in the field. Air temperatures at dawn were close to 10 °C throughout the study period, except on 15 and 17 June when they dropped to 5 °C (figure 4). These low dawn temperatures occurred on days with bright early morning sunshine, and above average PPFD values (figure 4). No significant change in ϕ was observed (t, $p > 0.5$) until 14 June (figure 4). On 15 June a significant drop in ϕ (t, $p < 0.05$) occurred and this was followed by an even greater decline on 17 June. This larger decline corresponded with a higher PPFD during the morning. The ϕ on 17 June was *ca.* 60 % of ϕ on 11 June, this reduction being highly significant (t, $p < 0.001$). The ϕ remained significantly reduced on 18 June, but showed recovery on the next two days, to a value of ϕ similar to that on 11 June. The significant reductions in ϕ coincided with low dawn temperatures and bright morning sunshine.

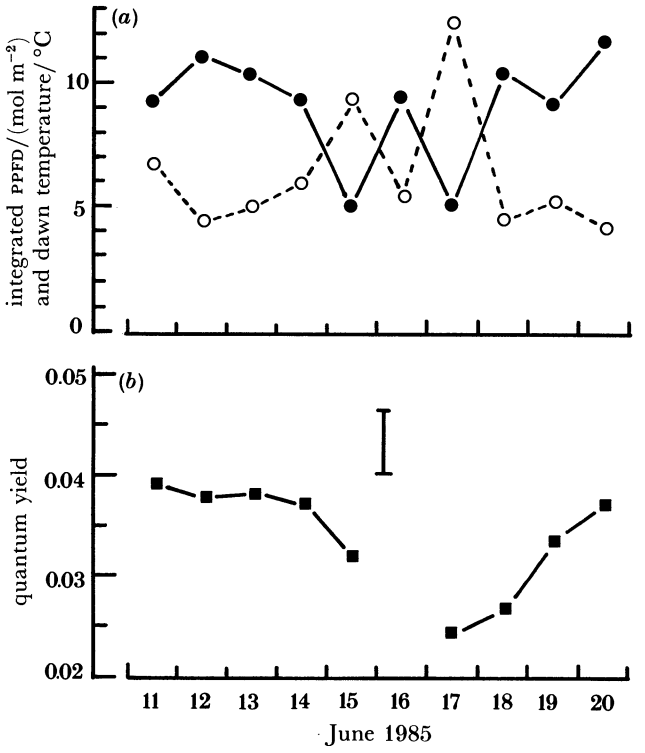

FIGURE 4. (*a*) Dawn canopy air temperatures (●) had integrated PPFD (○) incident above the maize canopy between dawn and 09h30 for the period 11–15 June 1985. (*b*) The quantum yield of CO_2 assimilation (ϕ) for mature maize leaves sampled at random from the canopy; the vertical bar indicates the least significant difference ($p < 0.05$).

A more detailed study of the microclimate associated with such reductions in ϕ was made for a maize crop during June 1986. The morning of 2 June was very cloudy with a dawn temperature of above 10 °C and a PPFD of only *ca.* 200 μmol quanta m^{-2} s^{-1} being reached at 09h00, whereas on 12 June the sky was clear, the dawn temperature was below 4 °C and the PPFD at 09h00 was *ca.* 1400 μmol quanta m^{-2} s^{-1} (figure 5). No significant decrease in ϕ was

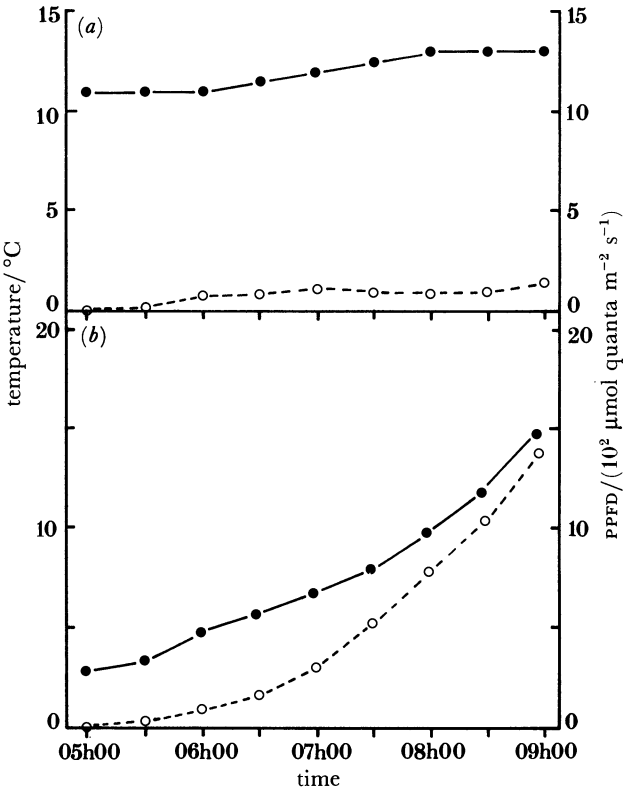

FIGURE 5. Canopy air temperatures (●) and PPFD (○) incident above the maize canopy from dawn until 09h00 on the mornings of 2 June (*a*) and 12 June (*b*) in 1986. No significant decrease in ϕ was observed for leaves measured on 2 June compared with 1 June. However, a 17% decrease in ϕ was found (t, $p < 0.05$) for leaves on 12 June compared with 11 June.

found for leaves measured during the mornings of 1 and 2 June. However, a significant 17% decrease in ϕ (t, $p < 0.05$) was observed for leaves measured on the morning of 12 June compared with 13 June. A significant difference in ϕ was found between leaves that had been shaded from direct sunlight by black plastic screens and unshaded leaves on the morning of 12 June, thus demonstrating the requirement of direct sunlight for the depression of ϕ. As in the laboratory studies on maize (Long *et al.* 1983) the combination of chilling temperatures and high light correlates with a reduction in ϕ implying that photoinhibitory damage to the thylakoid photochemical processes does occur in the field. Exposure of leaves in the laboratory to a PPFD of 1.5 mmol quanta m^{-2} s^{-1} for 6 h while the leaves were chilled at 5 °C reduced ϕ by *ca.* 45% (Long *et al.* 1983). In the field during June 1985 a 40% reduction follows the two days of low dawn temperatures (figure 4). On these days temperatures remained below 10 °C for *ca.* 4 h after dawn, when the PPFD would have risen slowly from 0 to *ca.* 1 mmol quanta m^{-2} s^{-1}. The value of ϕ on 11 June was just below 0.04. The low ϕ observed here suggests either that some damage occurred before 11 June or that development at low temperature has affected the photochemical efficiency. Following the reduction in ϕ to its minimum on 17 June, a slow recovery of ϕ occurred over 2 days, showing that the photoinhibitory damage is at least partially reversible in the field.

Under controlled laboratory conditions, chilling-dependent photoinhibitory reduction of ϕ

was always accompanied by characteristic changes in the chlorophyll fluorescence induction curve (Baker *et al.* 1983). The kinetics of chlorophyll fluorescence induction were monitored for leaves on 12 and 17 June 1985 to determine whether damage to the photosynthetic apparatus was similar to that observed previously in the controlled laboratory environment. Leaves measured on 12 June exhibited fluorescence induction kinetics containing PSMT transients typical of control, non-stressed leaves (figure 6). The fluorescence kinetics of leaves measured on 17 June were markedly different (figure 6). The fluorescence levels at both P and T on the induction curve were reduced and the S–M transient was lost; these characteristics are similar to those observed from maize leaves exposed to high light and low temperature in the laboratory (Baker *et al.* 1983). The fluorescence decrease from P to T on the induction curve is attributable to two types of quenching: photochemical, q_Q, and non-photochemical, q_N, quenching. Photochemical quenching is the result of oxidation of the primary quinone electron acceptor of photosystem II, Q_A, whereas under physiological conditions non-photochemical quenching is considered to be primarily caused by the energization of the thylakoids consequent upon the establishment of a proton electrochemical potential difference across the thylakoid membranes (Krause & Weis 1984). The amounts of photochemical and non-photochemical quenching occurring throughout the fluorescence induction curve from P to T were determined for leaves on 12 and 17 June (figure 6). The changes in both q_Q and q_N observed during induction of photosynthesis in the 11-June leaves are similar to those previously reported for unstressed leaf tissue (Bradbury & Baker 1984). Photochemical quenching at P is similar for 12-June and 17-June leaves, suggesting that the redox state of Q at this point on the

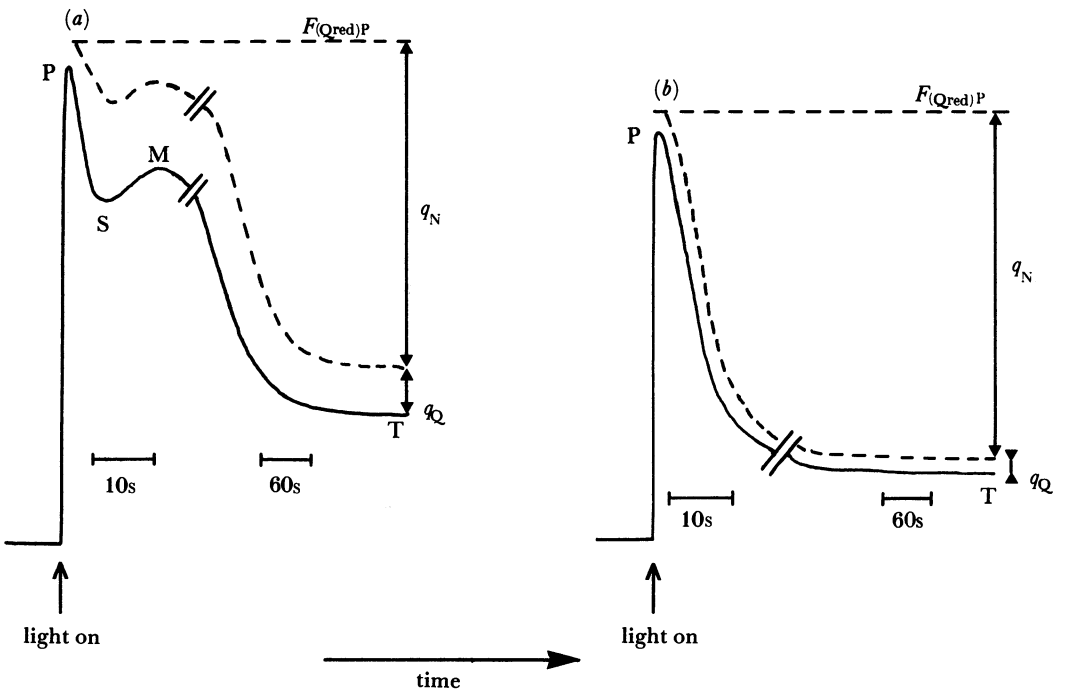

FIGURE 6. Modulated fluorescence induction curves generated from dark-adapted, field-grown maize leaves. Leaf (*a*) had experienced a dawn temperature of 11 °C and an integrated PPFD between dawn and 09h30 of 4.6 mol m^{-2} on 12 June 1985, whereas leaf (*b*) had experienced 5 °C and 12.5 mol m^{-2} for these parameters respectively on 17 June 1985. The photochemical (q_Q) and non-photochemical (q_N) components of the fluorescence quenching are shown. $F_{(Q_{red})P}$ represents the fluorescence emission that would be observed if Q_A was maximally reduced at P on the induction curve.

induction curve is similar in both leaves and unaffected by the environmental stress. The marked reduction in q_Q throughout the induction curve of the 17-June leaves compared with 12-June leaves demonstrates that Q_A is maintained in a more highly reduced state in 17-June leaves during quenching from P to T than in the 12-June leaves; this is also a characteristic change of chilling-dependent photoinhibition of photosynthesis (Baker *et al.* 1983).

With a view to developing a rapid, non-destructive technique for screening leaves in the field for photoinhibitory damage, modulated fluorescence transients induced on exposure of leaves of the maize crop growing during June 1985 to white light of PPFD saturating for photosynthesis were monitored. The modulated fluorescence level produced when the leaves were exposed to natural daylight at midday in the field was initially monitored and then the transients induced in the leaves by the addition and removal of the saturating, artificial white light of PPFD 1500 μmol quanta m^{-2} s^{-1} were recorded. The fluorescence transients observed from maize leaves measured on 12 and 17 June are shown in figure 7. For the 12-June leaf, which had not suffered a low temperature, light-induced depression of ϕ (see figure 4), the artificial white light induced an induction curve containing an initial rapid rise due to Q_A reduction followed by a slow quenching of fluorescence (figure 7). Removal of the artificial white light produced a rapid decrease in fluorescence, due to a rapid oxidation of Q_A, followed by a slow increase in the fluorescence, presumably attributable to a partial relaxation of the thylakoid high-energy state. These fluorescence transients are very similar, although not as large in magnitude, to those observed from maize leaves grown in a glasshouse at 23 °C. However, leaves measured in the field at midday on 17 June after they had experienced a dawn temperature of *ca.* 5 °C in conjunction with a high PPFD during the morning, exhibited very different fluorescence kinetics (figure 7). On addition of the artificial white light, fluorescence rapidly increased by a small amount but then decreased. The absence of a large, rapid rise in fluorescence on exposure to the artificial light is attributable to Q_A being highly reduced at steady-state photosynthesis in these leaves, as evidenced by the very small q_Q observed (see figure 6). The

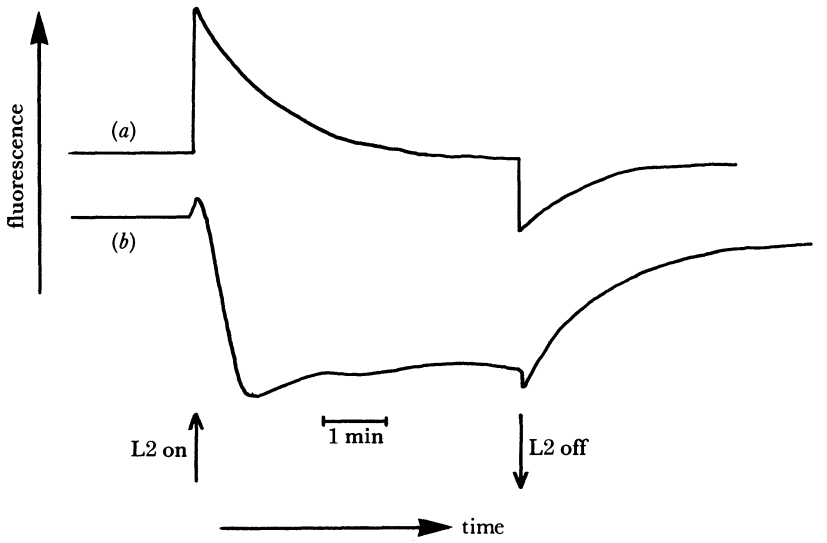

FIGURE 7. Modulated fluorescence transients generated on exposure of mature maize leaves excited by daylight in the field at 12h00 to a second excitation of 1500 μmol quanta m^{-2} s^{-1} of artificial white light produced from a quartz–halogen source (light 2, L2). Leaves (*a*) and (*b*) were sampled on 12 and 17 June 1985 respectively, and details of the dawn temperature and integrated PPFD that they experienced during the morning before measurement are given in the legend of figure 5.

large decrease in fluorescence on exposure to the artificial light is presumably the result of increasing non-photochemical quenching, which would occur if an increase in thylakoid energization occurred. This increase in non-photochemical fluorescence quenching is reversible on removing the excitation from the leaf (figure 7). It would appear that because the photoinhibited 17-June leaves are unable to oxidize Q_A significantly at steady state, whereas the 12-June leaves can, the fluorescence transients generated on addition and removal of a saturating, artificial light have the potential to be used as a rapid, routine screen for photoinhibitory damage in the field.

The data presented in this paper would suggest that changes in the fluorescence emission characteristics of leaves in the field relate directly to changes in the quantum yield of carbon assimilation. However, if fluorescence characteristics are to be used as a useful, rapid screen of plant photosynthetic productivity, the relation between the quantum yield of carbon assimilation and the efficiency of light-energy conversion to dry matter of the crop must be established. Unfortunately, such data for a maize crop are as yet unavailable. However, the relation between these parameters has been established for a winter rape crop growing in the field in northeast Essex during autumn and winter of 1986/7. Figure 8 shows that a linear correlation ($r^2 = 0.478$, $p < 0.001$) exists between the conversion efficiency of the crop and ϕ of individual leaves throughout the growing season. These data argue that changes in the quantum yield of carbon assimilation of leaves may be reflected directly in the productivity of the crop.

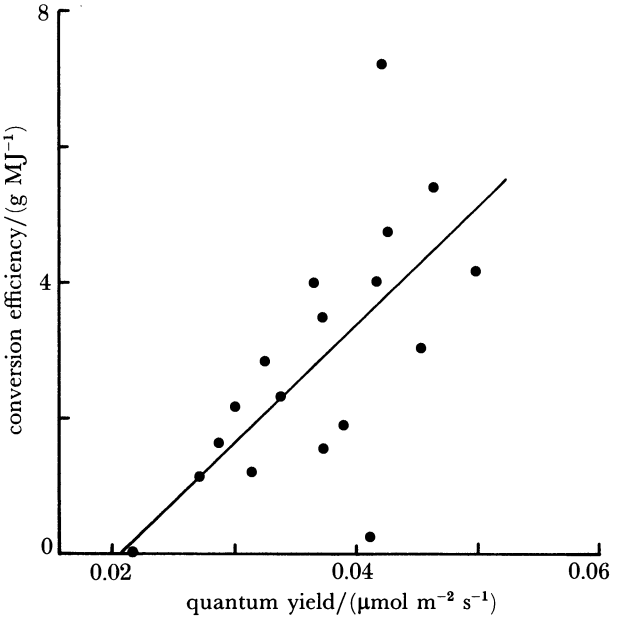

FIGURE 8. The relation between the weekly efficiency of the canopy to convert photosynthetically active radiation into dry matter and the absolute quantum yield of CO_2 assimilation for a winter rape crop between October 1986 and March 1987. Linear regression analysis gave $r^2 = 0.478$, $p < 0.001$.

CONCLUSIONS

In this paper we have described an apparatus and method for making routine, non-destructive measurements of the CO_2 exchange and chlorophyll fluorescence kinetic characteristics of leaves in the field. From the field studies of the photosynthetic performance

of the maize crops, it is evident that large, and significant, changes in the quantum yield of CO_2 assimilation, ϕ, of fully expanded leaves occur during the growing season (figures 2 and 3). The depression of ϕ at early stages of crop development during June can be attributed partly to low-temperature-induced, photoinhibitory damage. However, this stress alone cannot account for the reduced values of ϕ (figure 4). Other environmental stresses, which we have not identified specifically in this study, may reduce ϕ directly. Alternatively, such environmental stresses may modify detrimentally the development of the photosynthetic apparatus of leaves, and result in mature leaves having a lower ϕ than they would have later in the growing season when such stresses would be expected to be considerably less. Such environmental stress-induced modification of ϕ clearly have implications for crop productivity, as shown by the strong correlation observed between ϕ and the efficiency of the conversion of absorbed light energy to dry matter by the winter rape crop (figure 8).

These studies on maize crops have also demonstrated the potential of chlorophyll fluorescence parameters for screening rapidly the photosynthetic performance of leaves in the field. Somewhat surprisingly, a strong correlation was found to exist between F_V/F_M and ϕ of fully expanded leaves throughout the growing season, thus implying that F_V/F_M may be a useful rapid screen of the crop's potential photosynthetic performance. The obvious underlying basis of the relation between F_V/F_M and ϕ can be suggested to be that the quantum efficiency of photosystem II is the factor that limits carbon assimilation at limiting light levels. This concept is supported by the low-temperature-induced, photoinhibitory decreases of ϕ of maize leaves in the field. The molecular basis of such damage is considered to be a modification or loss of the reaction centre D_1 protein of photosystem II (Kyle 1987). As we have established that such damage to the D_1 protein occurs in maize leaves exposed to 5 °C and a photon flux density of 1500 μmol quanta m^{-2} s^{-1} (Percival *et al.* 1987), the decrease in ϕ of maize leaves exposed to chilling and high light in the field is not unexpected. As discussed above, correlations between F_V/F_M and the quantum yield of photosynthesis have been observed by other groups; however, this is not to argue that such a relation is definitive and will exist for all species under all environmental conditions. The observations made of the differences in the fluorescence transients generated on exposure of photoinhibited and non-photoinhibited leaves of the maize crop in the field to an intense white light (figure 7) would also support the contention that chlorophyll fluorescence measurements may offer a useful rapid, qualitative probe for determining stress-induced inhibition of photosynthesis. It should be emphasized that such probes do not offer a quantitative estimation of the effect of the stress. To determine whether such quantification could be achieved, careful analyses of the fluorescence transients and a calibration of their changes as a function of changes in ϕ and other photosynthetic parameters would be required.

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Discussion

G. D. Farquhar (*Department of Environmental Biology, Australian National University, Canberra, Australia*). Has Professor Baker taken into account the problem of water-vapour broadening of the CO_2 absorption lines? What caused the quantum yield of 0.02 mol CO_2 mol^{-1} quanta of the rape leaves?

N. R. Baker. In answer to Dr Farquhar's first question, this was tested by comparing the signal obtained with air of 345 μmol mol^{-1} CO_2 when dry and when humidified with water vapour to saturation at 20 °C. When the signal was corrected for the dilution of CO_2 by water vapour the remaining error was less than 0.3%. In our measurements of quantum yield both the reference and analysis streams were humidified and would differ by a maximum of 10%; at a differential of 20 μmol mol^{-1} this would therefore produce a maximum error of 0.5% in the estimate of the rate of CO_2 uptake. Given this small error it was considered unnecessary to add drying columns to a portable system. It would have the disadvantage of adding weight, increasing the time required for each measurement because of the greatly increased surface area over which the air stream must pass and increasing the complexity of the system. However, it should be appreciated that the error due to water-vapour broadening of the CO_2 absorption lines will be instrument dependent. It will vary with the properties of infrared source, filters and detector in non-dispersive infrared instruments, and because these properties may change with age they could differ even between analysers of the same model and make.

With regard to Dr Farquhar's second question, our short-term field shading experiments show that at least a part of this reduction in quantum yield results from photoinhibition. The remainder of the reduction correlates with the periods of severe frost, and it could be a result of longer-term photoinhibition coupled with failure of recovery at these low temperatures or a direct result of low-temperature damage.

J. V. Lake (*Agricultural and Food Research Council, London, U.K.*). I have two comments and a question.

The Ulbricht sphere has a long history in measurements of light absorption. Many early spheres were made from the ball floats used to operate the inlet valves of lavatory cisterns. Being made from copper, they were easily adapted to accommodate light sources and sensors and provided a nearly isothermal environment for the contained organism.

Turning to the relative importance of quantum yield and maximum photosynthetic rate, half the total solar radiation received at sites in southern England in any month of the year comes in a direct beam from the sun and half from diffuse sky radiation. So the maximum potential photosynthetic rate remains a quite important limiting factor.

Was soil temperature measured in the dawn gas-exchange experiments? Cooling the roots of maize can have a large effect on leaf function.

N. R. Baker. With respect to Dr Lake's second comment, we do not agree that the maximum potential photosynthetic rate is in itself a particularly important factor in determining the photosynthetic productivity of mature crop canopies. Field microclimatic studies, such as

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those of Inoue *et al.* (1968) on a maize canopy, have demonstrated that even during the brightest parts of cloudless days the uppermost leaves of the canopy are not light saturated.

In answer to the question, yes, soil temperatures were measured and were found to remain above 10 °C during both the night and early morning, whereas air and leaf temperatures fell well below 10 °C.

C. B. Osmond, F.R.S. (*Department of Environmental Biology, Australian National University, Canberra, Australia*). Does Professor Baker have any evidence that low night temperature, *per se*, predisposes leaves to subsequent light-dependent damage, or is the coincidence of light and low temperature required to produce the damage described in his paper?

N. R. Baker. The coincidence of light and low temperature is required for the damage we describe. In the field situation it is difficult to separate the effects of low night temperature in predisposing leaves to photoinhibition from the effects of low morning temperatures, as the two will obviously occur together. However, leaves exposed to low night temperatures and then shaded from direct sunlight in the early morning did not show a reduction in quantum yield compared with non-shaded leaves, and they also did not exhibit a reduction in quantum yield on exposure to full sunlight later in the day when the shade was removed.

H. W. Woolhouse (*John Innes Institute, Norwich, U.K.*). Professor Baker has provided good field data for chilling effects on quantum efficiency and has inferred from fluorescence measurements that this is closely correlated with photoinhibition damage to PSII. However, it is important to bear in mind that this is a correlation; has he made simultaneous measurements of the effects of chilling on the soluble protein content and maximum catalytic activities of Calvin cycle enzymes in the leaves? These enzymes may be affected by chilling, and may thereby contribute to the lowered quantum yields.

N. R. Baker. These factors have not been measured and may contribute to the damage to PSII and the reduction in quantum yield of carbon assimilation. A decrease in carboxylation capacity could lead to a reduction in the sinks available for excitation energy and thus produce a photoinhibition of PSII. A decrease in carboxylation capacity will not, however, directly reduce the quantum yield of carbon assimilation, because this parameter is defined by the maximum light-limited rate of carbon assimilation and estimated from the initial linear region of the light–response curve for carbon assimilation.

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