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Automated Measurement of Leaf Photosynthetic O<latex>$_2$</latex> Evolution as a Function of Photon Flux Density [and Discussion]

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Automated measurement of leaf photosynthetic O_2 evolution as a function of photon flux density

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An automated procedure is described that allows the rate of photosynthesis, as a function of photon flux density (PFD), to be determined and plotted within 30 minutes. The method is based on polarographic measurement of O_2 evolution from a piece of leaf enclosed in a chamber and illuminated from above by an array of light-emitting diodes. The light emitted from these diodes is altered by a computer which also facilitates analyses of the data so derived. Applications of the procedure to leaves of shade and sun plants, to studies of photoinhibition and to analysis of the Kok effect, are described.

Introduction

There is still much to be learned from measurement of the rate of photosynthesis as a function of photon flux density (PFD). This simple relation can be used to quantify 'dark' respiration, the light compensation point and the photosynthetic capacity of a leaf under given conditions. The initial slope is a measure of maximum photosynthetic efficiency in terms of quantum yield and its reciprocal, quantum requirement (for a discussion see Emerson (1958)).

Graphically (figure 1), the rate–PFD relation approximates to a curve, often near-linear in parts, which is constrained by two limits: a 'roof' and a 'ceiling' (cf. Blackman's concept of limiting factors (Blackman 1895*a*, *b*, 1905; Rabinowitch 1951)). The first of these constraints, the 'roof', is thermodynamic. If it is accepted that the Z-scheme (Hill & Bendall 1960; Hill 1965) constitutes an accurate description of photosynthetic electron transport, eight photons are required to transport four electrons from H_2O to NADP (i.e. four photons to move four electrons through each of the two photosystems). Photosynthetic carbon assimilation embraces additional energy-requiring reactions (Edwards & Walker 1983), and contemporary measurement (Adams *et al.* 1986; Ben *et al.* 1987; Björkman & Demmig 1987; Evans 1987; Walker & Osmond 1986) suggests that a quantum (photon) requirement of about nine (a quantum yield of about 0.111 mol O_2 mol^{-1} photon) may be approached (cf. figure 1) but is unlikely to be bettered. The initial slope of the rate–PFD plot (the pitch of the 'roof') is therefore most unlikely to be much steeper than this value of 0.111 dictates.

The second constraint is the metabolic 'ceiling'. In given circumstances, there must be a finite limit to the rate at which ATP and NADPH, generated by photosynthetic electron transport, can be utilized in photosynthetic carbon assimilation. Similarly, there must be a limit to the rate at which electrons can be transported from water to NADP via the photosynthetic electron-transport chain. Indeed, when limitations that would otherwise be imposed by low temperature or inadequate CO_2 supply are removed, the maximum measurable rate of photosynthesis in spinach is close to the maximum rate of coupled electron transport from water to NADP in isolated chloroplasts (Lilley & Walker 1975). As measured,

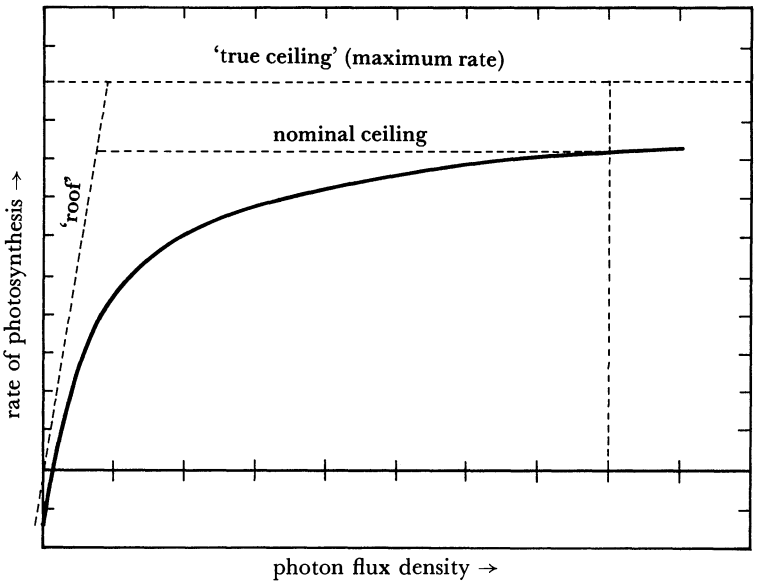

FIGURE 1. Roofs and ceilings. A typical rate–PFD plot (data from figure 3*c*). The intercept on the vertical axis is a measure of dark respiration, that on the horizontal axis the light compensation point. The initial slope is a measure of quantum yield and its reciprocal, quantum requirement. The curve lies within two constraints, a sloping 'roof' and a horizontal 'ceiling'. The roof is a thermodynamic constant pitched at an angle dictated by the maximal photosynthetic efficiency (in this instance a quantum yield value of 0.111). The ceiling represents the absolute maximum rate of carbon assimilation. For purposes of comparison (see figure 3) a lower ceiling, imposed by the rate of carbon assimilation at 800 μmol quanta m^{-2} s^{-1}, is taken.

this metabolic ceiling is not horizontal, as in figure 1, mostly because of leaf structure and differences in behaviour of chloroplasts situated in different cellular environments (Laisk 1977; Terashima & Saeki 1985; Terashima *et al.* 1986; Terashima & Takenaka 1986) nor invariable (because of regulation) but nevertheless, in the end, constitutes a second limit which cannot be exceeded. Photosynthetic efficiency is usually defined in terms of the quantum yield (or its reciprocal, the quantum requirement) but, in environmental terms, it also concerns the height of the ceiling and the extent to which the rate–PFD curve approaches the roof and the ceiling under defined conditions; in part what Terashima & Saeki (1985) have dubbed the 'convexity' of the curve and what has been referred to elsewhere as the 'nominal light utilization capacity' (Walker 1989).

What follows is an account of an automated procedure for the production of rate–PFD plots. It is clear that such determinations can elicit a wealth of information about photosynthetic performance (figure 1). The automated procedure (Walker 1987, 1989) moves this once difficult and tedious exercise into the realm of routine measurement. Automation also greatly enhances reproducibility and, perhaps most importantly, facilitates the application of analytical procedure to the data so derived.

EXPERIMENTAL

Measurement is based on the 'leaf-disc electrode' (Delieu & Walker 1981, 1983; Walker 1987), an array of light-emitting diodes (LEDs) and a computer. The leaf–disc electrode (figure 2) is a small, temperature-controlled chamber (now, like the LED array, manufactured by Hansatech Ltd, King's Lynn, U.K.) which accommodates 10 cm^2 discs of leaf (or smaller leaf

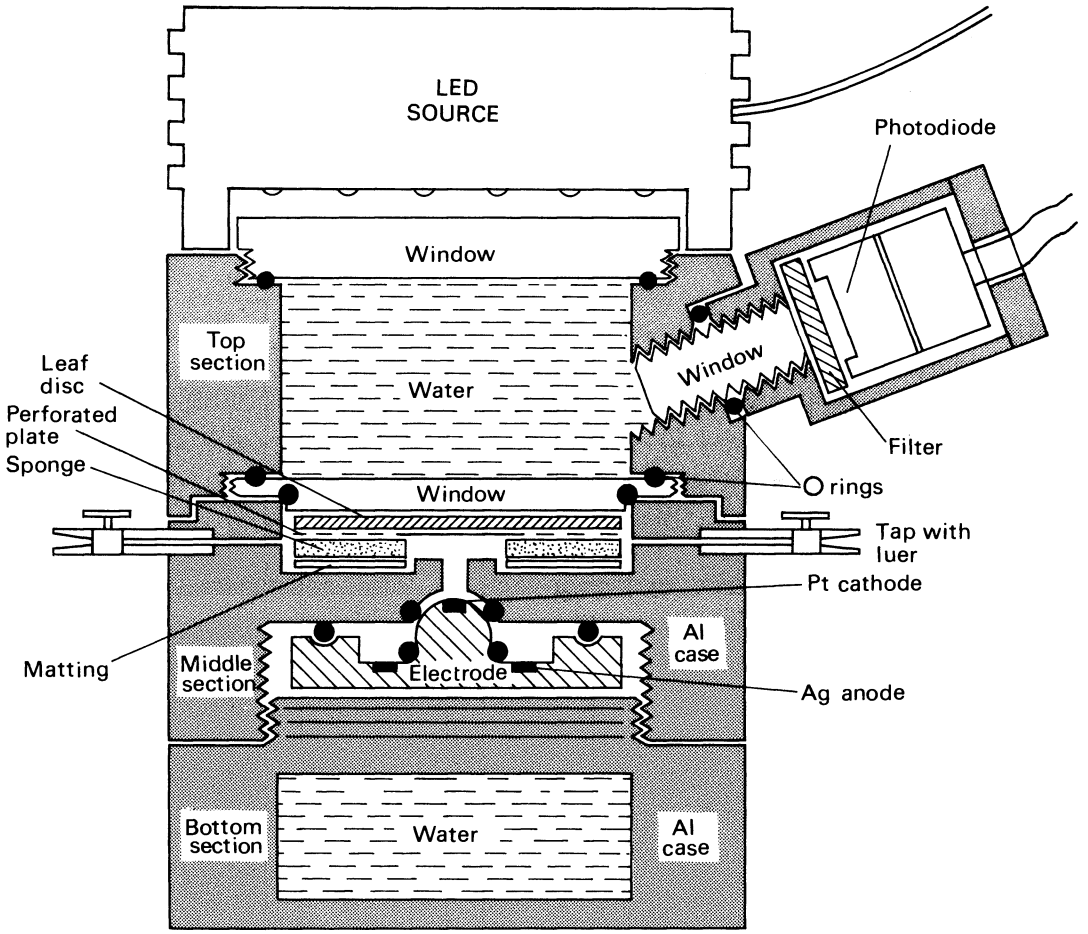

FIGURE 2. The leaf-disc electrode assembly. The apparatus is made of anodized aluminium. Above and below the leaf chamber, circulating water maintains a constant temperature. A disc or piece of leaf is accommodated immediately below a window in the top section and immediately above the Clark-type O_2 electrode. A photodiode (used to measure chlorophyll *a* fluorescence in experiments mot described here), looks down on to the leaf surface. Photon flux density is varied by changing the electrical supply to the array of light-emitting diodes which is located above the top window. The O_2 electrode measures the change in partial pressure of O_2 within the closed chamber.

pieces) and a Clark-type O_2 electrode for polarographic measurement of O_2 exchange (Delieu & Walker 1981, 1983). Carbon dioxide can be generated within the chamber from a bicarbonate buffer or supplied in the gas-phase or both (Walker 1987). All of the experiments described here were done in saturating CO_2 to avoid CO_2 (and diffusive) limitation and to suppress photorespiration. The light intensity was changed by changing the electrical current supplied to the LEDs. The relation between light emitted and current supplied to LEDs is almost linear, simplifying computer control. A feedback circuit diminishes changes in light output associated with heating. The spectral quality of the light from the LEDs used (peak emission at 650 nm) is not sensibly changed by this procedure. Almost all the light emitted is photosynthetically active (the absorption maximum of chlorophyll *a* fluorescence in the red is close to 680 nm), and the virtual absence of infrared and the low operating temperature of the LEDs greatly simplifies temperature control of the leaf chamber. Incident PFD at the leaf surface was measured with a quantum sensor specifically constructed and calibrated for this purpose

by Syke Instruments Ltd (Llandrindod Wells, U.K.). The LED source gives less uniform light than some tungsten sources that have been used for this purpose (Björkman & Demmig 1987) but as both O_2 exchange and PFD are effectively integrated this is largely immaterial in many circumstances. A diffuser can also be inserted between the LED array and the chamber and this ensures extremely uniform PFD over the 'quantum yield range' (0–125 μmol quanta m^{-2} s^{-1}). Without this diffuser, PFDs as high as 900 μmol quanta m^{-2} s^{-1} of red light can be achieved at the leaf surface. (Full sunlight is about 2000 μmol quanta m^{-2} s^{-1} white light and C_3 species usually approach light-saturation at about one fifth of full sunlight.)

The instrument is calibrated by injecting 1 ml of air into the chamber, thereby increasing the partial pressure of O_2 by a known amount (Walker 1987). The computer adjusts the oxygen scale accordingly. Oxygen can be measured over a range of up to 20 PFDs. The time at each PFD can also be varied within the limits imposed by computer memory but, in the experiments described here, PFDs were changed after 60 s and O_2 measured over the last 55 s of this time interval.

The computer program allows O_2 exchange rate (in μmol m^{-2} s^{-1}) to be plotted against PFD (in μmol quanta m^{-2} s^{-1}) on any desired scales and also permits the comparison of previously recorded data sets on the same scale. The initial slope can be determined by eye (the programme provides a moveable line for this purpose) or by least-squares regression analysis. 'Nominal light utilization capacity' (Walker 1989) can also be determined by expressing the area under the rate–PFD curve as a percentage of the area defined by the 'roof' and the 'ceiling' (see figure 1 and Introduction).

Results

Measurement and characterization of light–response curves

Figure 3*a* is typical of results obtained with the procedure described above. It compares 'sun leaves' and 'shade leaves' (i.e. leaves from the outside and inside of the canopy, respectively) of avocado. It is also typical of the well-known behaviour of many sun and shade leaves in that the latter very frequently display lower rates of 'dark' respiration, lower light compensation points (the 'light compensation point' is the PFD at which respiratory O_2 uptake and photosynthetic O_2 evolution are in balance, i.e. the intercept on the *x* axis) and lower rates of photosynthesis at higher PFDs than corresponding leaves that have grown in full light (see, for example, Rabinowitch 1951; Heath 1969; Björkman 1981). By the same token (figure 3*b*, *c*), the PFD required to give 50 % of the rate at 800 μmol quanta m^{-2} s^{-1} is lower (91 compared with 234 μmol quanta m^{-2} s^{-1}) and the '50 %' rate itself lower (4 compared with 9.6 μmol quanta m^{-2} s^{-1}) in shade compared with sun leaves. 'Nominal light utilization capacity' (Walker 1988) can be compared in several ways (figures 1 and 3) as follows. With the same 'roof' (defined by a nominal quantum yield of 0.111) and different ceilings (defined by the rates, at 800 μmol quanta m^{-2} s^{-1}, of the sun and shade leaves respectively) the nominal light utilization capacities of the sun and shade leaves (figure 3) are 76 % and 83 % respectively (figure 3*e*, *f*). If the same roof and ceiling are used for both (the roof passing through the origin and the ceiling defined by the rate displayed by the sun leaf at 800 μmol quanta m^{-2} s^{-1}) the nominal light utilization capacity of the sun leaf remains at 76 % whereas that of the shade leaf falls to 39 %, indicating the inability of the 'unacclimated' shade leaf to benefit from additional light (figure 3*d*). Finally, at 100 μmol quanta m^{-2} s^{-1} the sun leaf only utilizes 22 % of the

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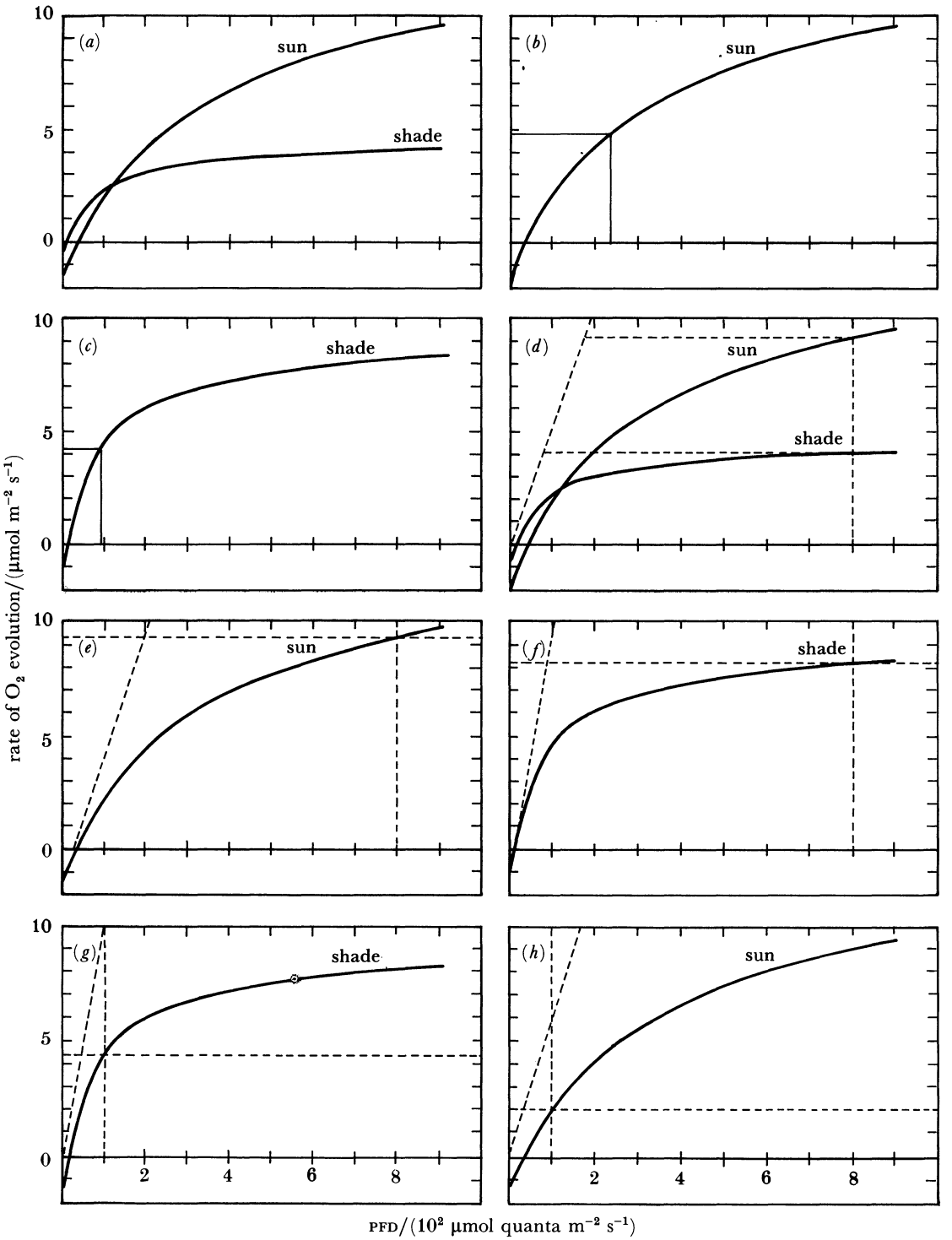

FIGURE 3. Rate–PFD curves for 'sun' and 'shade' avocado. (*a*) Direct comparison without further analysis; (*b*) PFD required to give 50% of rate at 800 μmol quanta m^{-2} s^{-1} by sun leaf; (*c*) PFD required to give 50% of rate at 800 μmol quanta m^{-2} s^{-1} by shade leaf; (*d*) comparison of nominal light utilization capacity, defined by the area under the curves expressed as a percentage of the area bounded by a roof (drawn through the origin) and a ceiling drawn through the rate at 800 μmol quanta m^{-2} s^{-1}; (*e*) nominal light utilization capacity of sun leaf, defined as the area under the curve as a percentage of an area bounded by a roof drawn through the light compensation point and a ceiling drawn through the rate at 800 μmol quanta m^{-2} s^{-1}; (*f*) shade leaf otherwise as for (*e*); (*g*) shade leaf, nominal light utilization capacity, defined as the area under the curve as a percentage of an area bounded by a roof drawn through the origin and a ceiling drawn through the maximum rate at 100 μmol quanta m^{-2} s^{-1}; (*h*) sun leaf (otherwise as for (*g*)).

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

available capacity whereas the shade leaf utilizes 63 % (cf. figures 3*g* and 3*h*). These differences would also be apparent without the benefit of computer-facilitated arithmetic, but the computer allows them to be quantified much more easily and more quickly.

Figure 3 illustrates the evaluation of a more or less static situation, but it should be remembered that measurements made over time intervals as short as 1 min are unlikely to record steady-state photosynthesis. (Whether or not it is ever possible to measure a true steady state is arguable.) Leaves taken from darkness often emerge from a short induction period in a matter of minutes but 'long induction', a gradual increase in rate that is at least partly attributable to the availability of cytosolic P_i (Sivak 1987; Sivak & Walker 1985, 1986; Walker & Sivak 1986), may continue for many hours. Similarly it is by no means certain that the quasi steady-states, then observed, are more than an interlude between activation and feedback inhibition of photosynthesis. As always, a compromise has to be reached between meaningful measurement and the impact of measurement on the system being measured.

Figure 4 illustrates the high degree of reproducibility that is achieved when the leaf itself is in a quasi steady-state by virtue of being harvested after several hours photosynthesis in a favourable environment. In addition, figure 5 shows how valuable this methodology can be in following a rapidly changing situation. Here, shade avocado (taken from subdued light in early morning and assayed repeatedly) displayed more or less unchanging quantum yield but progressively increasing rates at higher PFDs. It is in such circumstances that the automated procedure really comes into its own because of its speed and reproducibility. Few other currently available régimes would catch a leaf in this particular act of adjustment (which could, in this instance, be related to the lifting of a photosynthetic constraint imposed by the nocturnal inhibitor of ribulose bisphosphate carboxylase–oxygenase (Rubisco) (S. P. Robinson, personal communication).

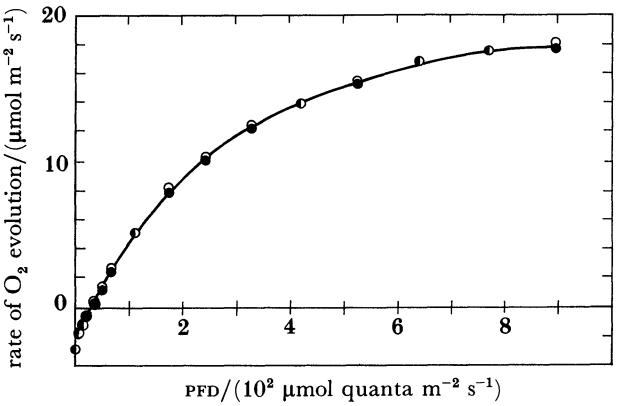

FIGURE 4. Reproducibility. Leaf disc *Vitis vinifera* (var. Rhine Reisling) growing in the field at the CSIRO, Division of Horticultural Research in Adelaide. Harvested at 1 p.m. on a cool (23 °C) and overcast day and assayed twice (○, ●) in quick succession at 25 °C.

Figure 6 illustrates another changing situation and also exemplifies the usefulness of a measuring procedure which permits repeated evaluation of rate against PFD within a reasonably short time. It shows the early stages of photoinhibition in *Helleborus niger*. Quantum yield has already fallen considerably after 2 h 'photoinhibitory' treatment but the rate at higher PFDs is as fast as or faster than before. (Different environmental stresses affect different

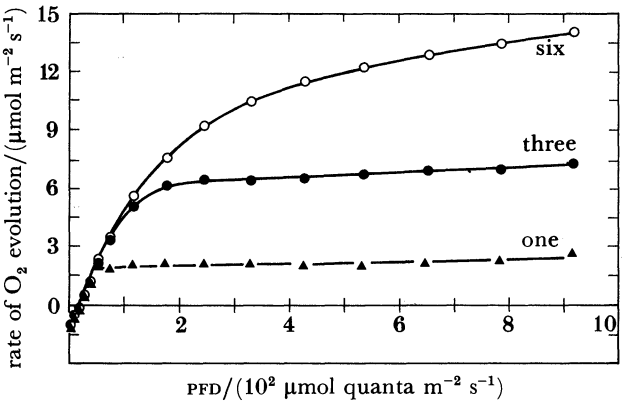

FIGURE 5. The usefulness of the automated approach in following a rapidly changing situation. Six measurements (three not shown for clarity) were made successively on a disc from a shade avocado leaf harvested the previous day and kept in darkness overnight. Before the first measurement, the disc was illuminated for 15 min in 90 μmol quanta m^{-2} s^{-1} light. The second, third and fourth measurements were consecutive. Between the fourth and fifth there was 6 min dark followed by 1 min 900 μmol quanta m^{-2} s^{-1} light, and between the fifth and sixth a further 10 min darkness and 2 min 900 μmol quanta m^{-2} s^{-1} light.

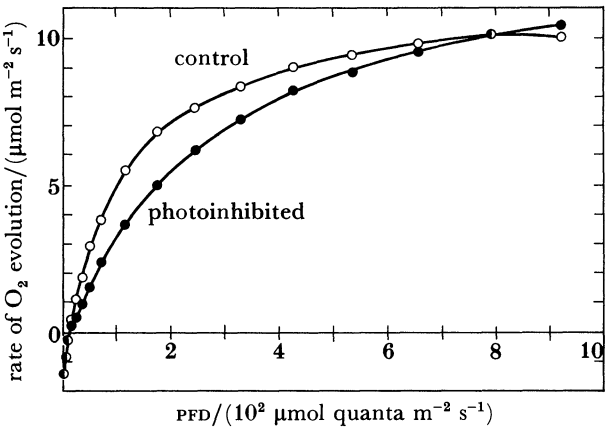

FIGURE 6. Photoinhibition in *Helleborus niger* without loss in maximum rate. A leaf disc from *Helleborus niger* growing in deep shade (2 μmol quanta m^{-2} s^{-1}) was assayed at 20 °C before and after 2.5 h in 10000 μmol quanta m^{-2} s^{-1} at 8 °C. The initial slope is affected (i.e. the quantum yield has fallen significantly from 10.5 in the control to 24 following photoinhibition), but there has been no decline in rate at the highest light intensities.

plants in different ways but many will lead to real or incipient photoinhibition (Anderson & Osmond 1987) and, here again, the automated measuring system offers a convenient form of assessment. Progress of photoinhibition (in the sense of diminished quantum yield) and recovery from photoinhibition (to rates higher than those displayed by the control) is also illustrated in a single leaf-disc experiment in figure 7. Similarly, figure 8 shows the difference in response of sun and shade leaves of *Helleborus niger* to a photoinhibitory treatment, implying a more effective repair mechanism in the former. Exposed to 1000 μmol quanta m^{-2} s^{-1} at 8 °C, the sun leaf actually increased its rate of subsequent photosynthesis whereas the shade leaf was photoinhibited as before (figure 7). The similarity in the rates of photosynthesis displayed by the sun and shade leaves of this species (figure 8) before photoinhibitory treatment

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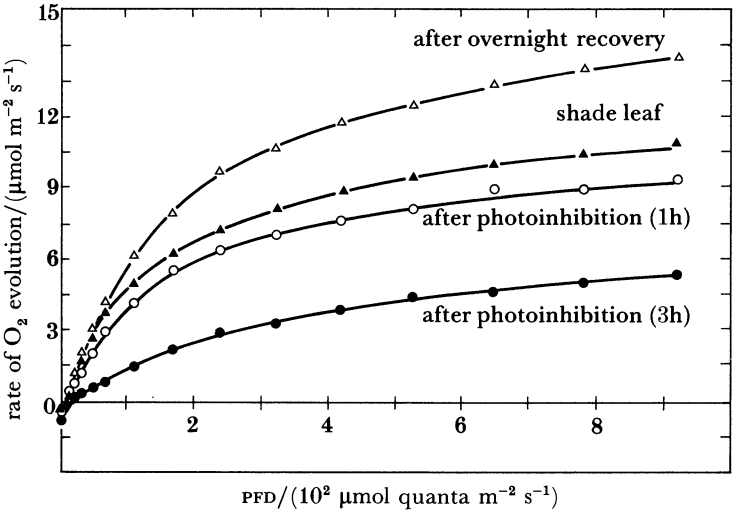

FIGURE 7. Recovery from photoinhibition in *Helleborus niger*. In this experiment photoinhibition of only 1 h caused some loss of rate (cf. figure 6) as well as a decrease in quantum yield. After 3 h photoinhibition was marked. Overnight recovery in the 2 μmol quanta m^{-2} s^{-1} at room temperature brought about a pronounced increase in rate (over and above that originally displayed by the control) and complete recovery of quantum yield.

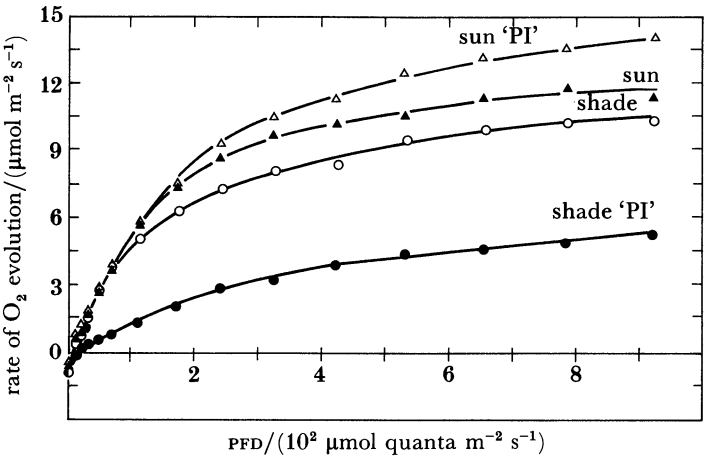

FIGURE 8. Photoinhibition in *Helleborus niger*. Sun leaf taken from full Australian summer light, shade leaf from 2 μmol quanta m^{-2} s^{-1}. Both leaf discs were assayed before and after the same photoinhibitory treatment (4.5 h at 100 μmol quanta m^{-2} s^{-1} and 8 °C). The shade leaf now showed loss of rate at high PFDs (not seen after shorter periods of photoinhibition; cf. figure 6) and even greater decline in quantum yield. The sun leaf showed not photoinhibition but rather an enhancement of rate.

underlines the fact (first demonstrated by Powles *et al.* (1984)) that dissipation of excitation energy via photosynthetic carbon assimilation cannot, in itself, account for the difference in behaviour of sun and shade leaves.

Measurements in the quantum-yield range (0–125 μmol *quanta* m^{-2} s^{-1})

For more accurate determination of quantum yield it is desirable to take as many readings as practicable in the 0–125 μmol quanta m^{-2} s^{-1} range (for many species the curve will depart from apparent linearity towards the higher end of this range). How to avoid the complications of induction on the one hand, and the effects of light on dark respiration on the other, remains a problem in such determinations. If respiratory uptake of O_2 remained constant in all circumstances it would not affect the slope of the rate–PFD relation. Precisely how

photosynthesis and 'dark' respiration interact is still uncertain (Graham 1980; Graham & Chapman 1979) but there is no doubt that they do. Kok (1948, 1949, 1951) interpreted a discontinuity (i.e. an abrupt change of slope) in the rate–PFD relation as an inhibitory effect of low light on dark respiration. This has often been reported since (Van der Veen 1949; Decker 1957; Sharp *et al.* 1984). It is also clear that there is increased O_2 uptake in the dark following strong illumination (see, for example, Delieu & Walker 1983). A fraction of this, as measured here, is an artefact (Delieu & Walker 1981). Some heating of the chamber and of the leaf is inevitable during bright illumination in an enclosed space, and if black matting or dead tissue is substituted for a living leaf there is a small apparent O_2 evolution in light and a corresponding apparent O_2 uptake in darkness which are both purely artefactual. When appropriate allowances are made, however, most leaves still display a much more rapid O_2 uptake as they go into darkness after bright illumination than they do even 5 min later. The rate of uptake then usually declines slowly and progressively in prolonged darkness.

If an investigator is solely concerned with the initial slope of the rate–PFD relation, there is much to be said for pre-illuminating the leaf at about 125 μmol quanta m^{-2} s^{-1} until a quasi steady-state rate has been established, and then decreasing the PFD in a relatively large number (15–20) of small steps to total darkness. At 125 μmol quanta m^{-2} s^{-1}, light enhancement of subsequent dark respiration will be low and will diminish progressively throughout the measuring procedure so that, at the lowest PFDs, distortion of apparent photosynthesis will be minimal (figure 9). (Clearly, when photosynthetic O_2 evolution is lower than respiratory uptake of O_2 even small increases in the dark respiration rate will have an appreciable effect on net gaseous exchange.) Figure 9 shows three consecutive measurements made on Cape Weed (*Arctotheca calendula* L.), in the 0–125 μmol quanta m^{-2} s^{-1} range, starting from darkness immediately after 5 min pre-illumination at three different light intensities. Following pre-illumination at the highest PFD (900 μmol quanta m^{-2} s^{-1}) the subsequent impact of enhanced dark respiration on the rate–PFD plot is considerable, and as this enhanced dark

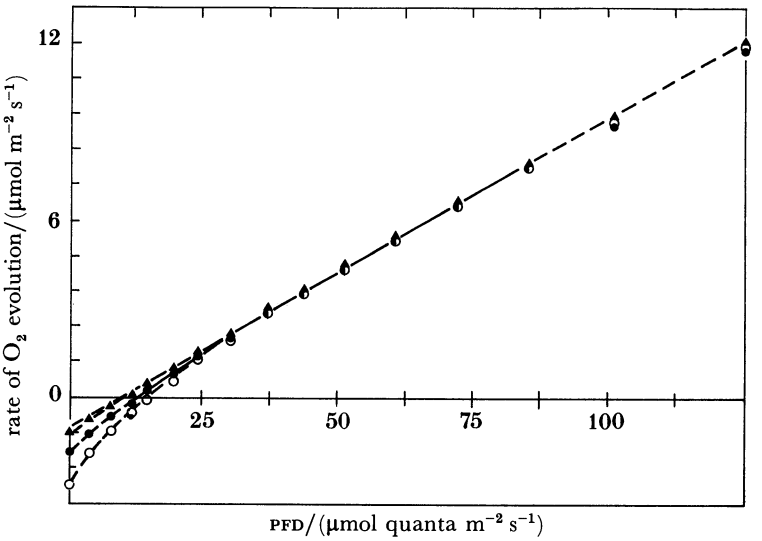

FIGURE 9. Rate against PFD in quantum-yield range (0–125 μmol quanta m^{-2} s^{-1}) for Cape Weed *Arctotheca calendula* (L.). Single leaf pre-illuminated for 5 min at high (900 μmol quanta m^{-2} s^{-1}) moderate (125 μmol quanta m^{-2} s^{-1}) and low light (12.5). Measurements were made consecutively in the order 'moderate' (●), 'high' (○), 'low' (▲). Note the departure from linearity at low PFDs (which is most marked when measurements are commenced immediately after pre-illumination at high PFDs), but otherwise the reproducibility of the data. See also table 1.

TABLE 1. LEAST-SQUARES REGRESSION ANALYSIS OF DATA IN FIGURE 9

	points 1–15	points to 15 above light compensation point
low light	9.37	9.36
moderate light	8.89	9.26
high light	8.53	9.28
mean	—	9.30

respiration gradually subsides during the period of measurement there is a similarly gradual approach to linearity in the rate–PFD plot. Even so, 'true' linearity is only reached above the light compensation point. When pre-illumination was done at 125 μmol quanta m^{-2} s^{-1} this effect was less pronounced. At 12.5 μmol quanta m^{-2} s^{-1} it was scarcely apparent and when measurements (not shown) were made from 125 μmol quanta m^{-2} s^{-1} to 0 (rather than 0–125) the relation was linear throughout. Table 1 (data from figure 9) shows the similarity of consecutive quantum-yield determinations based on values above the light compensation point and how these become distorted by the inclusion of *high* values of light-enhanced dark respiration.

It is difficult to say, at this time, whether or not the departure from linearity at low PFDs following pre-illumination at high PFDs (figure 9) is a 'Kok effect' *per se*. There is no doubt that it could be mistaken for a 'Kok effect', although this has most often been reported as a discontinuity in the rate–PFD plot below the light compensation point (Kok 1948, 1949, 1951; Van der Veen 1949; Decker 1957; Sharp *et al.* 1984) rather than a smooth curve. In turn, this immediately prompts other questions. Is the 'Kok effect' real or an artefact of measurement? Is it a universal phenomenon and, if not, why not? Is there only one effect or several? These questions cannot be addressed adequately here but some aspects can be touched upon. Firstly, it is by no means a universal phenomenon. Emerson (1958) was even sceptical about its existence, stating:

> The small amount of positive evidence for the 'Kok effect', together with negative results in several instances of search for confirmatory evidence, leads to the conclusion that a substantial difference in quantum yield above and below the compensation point is not a general phenomenon. If it is something associated with special conditions, these conditions have yet to be specified. It seems equally possible that the apparent positive evidence arises from changes in rate of respiration between the dark and light intervals chosen for calculation of rate of photosynthesis.

Decker (1957), like Van der Veen before him (1949), observed a discontinuity, but preferred the interpretation that dark respiration was constant below the light compensation point and thereafter *increased with increasing light intensity*. Sharp *et al.* (1984) found the discontinuity to be present *even when respiration was constant in the dark* (i.e. when effects such as those illustrated in figure 9 could not be advanced in support of an alternative explanation). Of course, it would be most unwise to seek to reinterpret changes in CO_2 exchange, made in very different circumstances, in terms of the changes in O_2 illustrated in figure 9. At the same time it must be noted that the experimental approach is central to this issue. Sharp *et al.* (1984) avoided the rapid decline in the rate of CO_2 efflux from sunflower leaves which occurred in the first 2 h darkness (after a 14 h photoperiod) and measured respiration in a 'relatively stable' subsequent period, reporting values of net photosynthesis (after as much as 45 min in PFDs up to 30 μmol quanta m^{-2} s^{-1}) only when 'the rate of respiration in the dark was similar at the beginning and the end of the experiment'. This was an admirably rigorous attempt to preclude the 'temporal changes in dark respiration' that caused Emerson (1958) and Heath (1969) to

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

question the reality of the Kok effect. Whether or not anything like normal photosynthesis would occur in such circumstances is another matter. Induction (Walker 1981) is still not fully understood, but it is clear that it involves light activation of enzymes, buildup of dark depleted substrates, and the supply of P_i from the cytosol to the chloroplast (Walker 1976; Sivak 1987; Sivak & Walker 1985, 1986; Walker & Sivak 1986). If a sunflower leaf grown in relatively high light (900 μmol quanta m^{-2} s^{-1} in the experiments of Sharp *et al.* (1984)) is illuminated at 30 μmol quanta m^{-2} s^{-1} (or below) following 2 h or more of preceding darkness, it is most unlikely that cytosolic pools will become filled during the period of measurement even if apparent photosynthesis has become constant. The changes in slope reported by Sharp *et al.* (1984) are too large to be explained in terms of a shift, at high PFDs, from Benson–Calvin cycle activity alone to Benson–Calvin cycle plus sucrose synthesis. Nevertheless the values of Sharp *et al.* (1984) for quantum yield of about 0.086 (measured in high CO_2 above the light compensation point) imply that photosynthesis in these circumstances was, for whatever reason, operating at less than maximum efficiency.

Notwithstanding the continuing uncertainty about the nature of the 'Kok effect', results of the sort reported here will have to be borne in mind in making quantum-yield measurements. As in the past, the investigator must face an inevitable dilemma. If 'dark' respiration is to be measured in the steady state, several minutes of darkness will be needed in order to avoid light-enhanced O_2 uptake and several hours, in many species, before the decline in dark respiration, following illumination, becomes imperceptible. However, even 1–3 min darkness will normally be enough to re-establish 'short induction' in photosynthetic O_2 evolution (Walker 1981). Moreover, re-illumination at high PFDs after a period of darkness often initiates a 'burst' of O_2 (and an associated 'gulp' of CO_2) within the induction period (Sivak & Walker 1985). Opinions about the origin of the O_2 gulp still differ but there is no doubt that 3-phosphoglycerate often persists in darkness in circumstances in which ribulose bisphosphate (RuBP) falls to near zero or that glycerate 3-phosphate (PGA) will serve as an oxidant in suspensions of isolated chloroplasts. If the Benson–Calvin cycle is 'switched off' and if the reduction of PGA exists, even as a possibility, there is an obvious danger in attributing gas exchange, during induction, to 'real' photosynthesis. Conversely, if induction is avoided by pre-illumination, the possibility of light enhancement of respiration cannot be disregarded.

No doubt these problems will continue to preoccupy those who wish to continue to refine the measurement of maximum photosynthetic efficiency. If, on the other hand, the initial slope of the rate–PFD plot is primarily of interest as an indicator of environmental stress or experimental intervention there is much to be said for a simple standard procedure (e.g. measuring from 125 to 0 after pre-illumination at 125 μmol quanta m^{-2} s^{-1}). In unstressed leaves, this approach can yield reproducible quantum-yield values close to those demanded by our present understanding of photosynthetic electron transport. The methods described here permit such determinations to be made, routinely, in less than half an hour.

Concluding discussion

Photosynthesis is driven by light. The efficiency of nominal light utilization by green leaves and the manner in which it may be modified by environment, development, genetic manipulation or experimental intervention is central to a fuller understanding of photosynthesis. It was the recognition of this that fired the long-lasting controversy between Warburg and Emerson (Franck 1953) and that has prompted the continuation of work in this

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

area to the present day. Warburg's measurements lasted many hours and involved many months of work. Despite the painstaking nature of his approach, a large body of subsequent work suggests that his values for quantum yield were incorrect by a factor of 2 or more. Recent measurements by Björkman & Demmig (1987) suggest that many C_3 species require about nine quanta to evolve one molecule of O_2. This value is close to that which would be mandatory if the Z-scheme (for which there is now massive evidence) is accepted as an adequate description of the central features of photosynthetic electron transport (Hill & Bendall 1960). The procedure described here is simple and its speed and reproducibility make it ideal for examining changes in the rate–PFD relation that may result from a plant's interaction with its environment or following intervention by man.

This work is affectionately dedicated to my dear friend and colleague Tom Delieu who died, aged 58, on 12 November 1987 after a short illness. Tom Delieu made the prototype electrodes, leaf chambers, etc., from which the present system was eventually developed.

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Discussion

P. G. Jarvis (*Department of Forestry and Natural Resources, University of Edinburgh, U.K.*).

(1) The concept of the 'convexity coefficient', θ, has been around quite a long time, certainly well before the work of Terashima, for the obvious reason that a non-rectangular hyperbola gives a better fit to the light–response curve. The non-rectangular curve also has the

advantage of dissociating mathematically the quantum yield from the light-saturated rate of assimilation (they are inversely correlated with a rectangular fit).

(2) There are many data for C_3 plants showing $\theta = 0.7$–0.9.

(3) θ is larger in leaves of low chlorophyll content, because, as shown by Terashima, there is less shading in leaves with a low chlorophyll content than in leaves with a high content.

(4) The chlorophyll content causing shading can be effectively changed by changing the method of illumination. Some years ago Jerry Leverenz showed at Edinburgh that illuminating spruce needles bilaterally, as opposed to unilaterally, approximately doubled θ (and he has subsequently shown this for a number of cases (Leverenz 1987)). More recently, by using the sphere assimilation chamber at Umeå with uniform, omnidirectional illumination, θ is further raised to 0.96.

D. A. Walker. I had not wished to imply that the concept of 'convexity' had originated with Terashima but simply to acknowledge the excellence of his work, which has done so much to confirm the underlying factors. It should be noted, however, that the shape of the curve is not entirely dictated by shading. Regulation is also involved and indeed the shape of the curve can change as a consequence of relatively short (30 min or so) constant illumination.

It is my hope that the concept 'nominal light utilization capacity' as defined in this paper will prove to be more useful than 'convexity' because this is determined in part by the light compensation point and, therefore, by the rate of 'dark' respiration.

The relation can also be modified by experimental intervention such as phosphate feeding (Walker & Sivak 1986) or by genetic factors (Sivak & Rowell 1988).

G. D. Farquhar (*Department of Plant Environmental Biology, Australian National University, Canberra, Australia*). The convexity of the relation between photosynthesis rate and light intensity could, in principle, be different at low and high CO_2 concentrations.

J. Leverenz (*Department of Plant Physiology, University of Umeå, Sweden*). With regard to the comment by Dr Farquhar, I have measured the convexity coefficient of the light–response curve for shade-acclimated conifer needles and found no change in θ when CO_2 partial pressure was changed from 34 to 200 Pa (Leverenz 1987). In subsequent experiments I have repeated this and in addition found no response to temperature between 5 °C and 32 °C. Moreover, these new experiments that utilized leaves grown at a higher photon flux density, strongly suggest that the cells within the leaves re-acclimate during measurements (the measurement environment was different from the growth environment). The convexity coefficient of the intact leaves increased towards a maximum value of 0.97, at a rate that averaged about 60% per day. The high convexity coefficient results in a noticeable increase in efficiency from very low photon flux densities (below 50 μmol quanta m^{-2} s^{-1}, Leverenz (1987), figure 9) up to full sunlight (Leverenz 1987, figure 10). Many people today use the term 'light-saturated photosynthesis' for systems that are not light saturated.

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