Trees (1990) 4: 1–8

Structure and Function
Trees

Use of simultaneous analysis of gas-exchange and chlorophyll fluorescence quenching for analysing the effects of water stress on photosynthesis in apple leaves

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Received July 7, 1989

Summary. A convenient system for the rapid simultaneous measurement of both chlorophyll fluorescence quenching using a modulated light system, and of CO_2, and water vapour exchange by leaves is described. The system was used in a study of the effects of water deficits on the photosynthesis by apple leaves (*Malus* × *domestica* Borkh.). Apple leaves were found to have low values of steady-state variable fluorescence, and the existence of significant fluorescence with open traps (F_o) quenching necessitated the measurement and use of a corrected F_o in the calculation of quenching components. Long-term water stress had a marked effect on both gas-exchange and chlorophyll fluorescence quenching. Non-photochemical quenching (q_N) in particular was increased in water-stressed leaves, and it was particularly sensitive to incident radiation in such leaves. In contrast, rapid dehydration only affected gas exchange. Relaxation of q_N quenching in the dark was slow, taking approximately 10 min for a 50% recovery, in well-watered and in droughted plants, and whether or not the plants had been exposed to high light.

Key words: Chlorophyll fluorescence quenching – *Malus* – Photosynthesis – Water stress

Introduction

With the recent introduction of portable modulated fluorescence systems (Schreiber et al. 1986; Ögren and Baker 1985) it is now becoming possible to study chlorophyll fluorescence of leaves growing in the field. The development of methods for resolving the different quenching processes into photochemical (q_Q) and non-photochemical (q_N) components, by the use of flashes of high-intensity light, was initiated by Bradbury and Baker (1981), and developed further by others (Quick and Horton 1984; Schreiber et al. 1986). Although much information can be obtained from such measurements, it is not usually possible unambiguously to determine the cause(s) of any observed changes in fluorescence quenching. For example, non-photochemical quenching when calculated according to Schreiber et al. (1986) can be related to energisation of the thylakoid, to state transitions, or to photoinhibition, among other processes (Baker and Horton 1987). Although it is possible, when studying isolated chloroplasts, at least partially to separate these components by the use of inhibitors (e.g. Krause et al. 1982; Oxborough and Horton 1987), it is more difficult when using intact leaves, though some information can be obtained from the time-course of recovery upon darkening (Horton and Hague 1988).

Our understanding of how environment or physiological factors affect assimilation can be improved by utilising a range of measurement techniques in combination. Most such multiple studies have combined analysis of chlorophyll fluorescence with O_2 exchange measured in an oxygen electrode (e.g. Walker et al. 1983; Walker and Osmond 1986). Other workers have combined chlorophyll fluorescence measurements with CO_2 exchange studies (e.g. Ireland et al. 1984) or with CO_2 and water vapour exchange (to allow estimation of stomatal conductance and hence of the intracellular partial pressure of CO_2, e.g. Wong and Woo 1986).

The purpose of the work described here was to study the potential use of combined fluorescence and gas-exchange analysis to investigate the effects of water deficits on photosynthesis of intact apple leaves and to develop protocols for obtaining maximal information on the relative contribution of different components of the photosynthetic system to any observed changes. Apple was chosen for this study because there is already considerable basic information on the photosynthetic responses in this species in response to drought (Fanjul et al. 1981; Jones and Fanjul 1983), and because preliminary studies using a modulated

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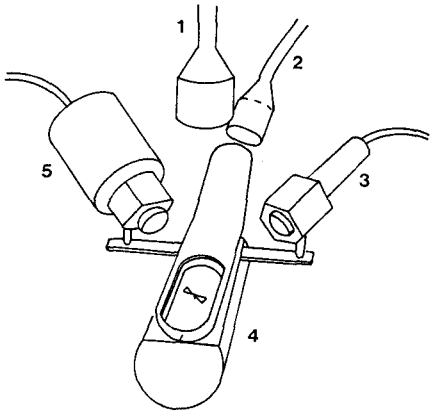

Fig. 1. Arrangement of the actinic (*1*), saturating (*2*), and modulated (*5*) light sources, and of the fluorescence detector (*3*) around the open-top leaf chamber (*4*). Because apple leaves are hypostomatous, the top surface of leaf chamber can be formed by the leaf

fluorescence system had indicated somewhat unusual fluorescence behaviour.

Materials and methods

Plant material

Three-year old apple (*Malus* × *domestica* Borkh.) mini-trees (cv. Greensleeves on M. 27 rootstock) were grown outdoors on an irrigated sandbed in 25-cm-diameter pots (Ford 1971). Water deficits were imposed by withholding water and enclosing the pots in transparent polyethylene bags that were loosely tied around the tree stem. The bags were perforated in appropriate places to allow adequate air circulation within the bags. The water status of young, fully expanded leaves was routinely monitored using a pressure chamber as described by Jones and Cumming (1984). Other experiments used 6-month-old M9 apple trees *ex* micropropagation, grown in a heated and lighted glasshouse.

Two types of drought experiment were conducted. In most experiments water deficits were induced slowly by regularly adding small amounts of water to the pots, but not enough to fully rehydrate the soil. The amount of water to be added each day was determined on the basis of pot weight. The precise time course of desiccation in each experiment depended on environmental conditions; details are given below. In the other type of experiment, water deficits were imposed rapidly by detaching leaves from well-watered control plants immediately after measurement, allowing them to dehydrate in the dark and then remeasuring the fluorescence induction curve 1.5 h later, after they had dried to a water potential lower than –4 MPa.

Gas-exchange and fluorescence measurements

Measurements were made in the laboratory using the porometer gas exchange system described by Fanjul et al. (1981), modified to make fluorescence measurements simultaneously with gas-exchange measurements. The arrangement of the actinic, saturating pulse and modulated light sources and of the fluorescence detector are illustrated in Fig. 1. White actinic light and saturating light pulses were provided at the leaf surface via fibre optic cables from Schott KG2500 light sources. Different irradiances were achieved by means of neutral density filters. Room-temperature chlorophyll-a fluorescence was monitored using a Hansatech (King's Lynn, UK) modulated fluorescence system (Ögren and Baker 1985).

Fluorescence parameters were calculated according to Schreiber et al. (1986). Fluorescence with open traps (F_o) was obtained for dark-adapted leaves using a low-irradiance pulsed light source adjusted as recommended by Ögren and Baker (1985). During photosynthesis, however, there was evidence for quenching of the F_o signal, so the quenched value F'_o (Bilger and Schreiber 1986) was estimated at intervals as the minimum fluorescence reached immediately after darkening the leaf for a few seconds (but continuing illumination by the weak modulated beam). In subsequent tests this method gave similar results to the use of a pulse of far-red light. This value was used, unless stated otherwise, in place of F_o in all calculations. The photochemical quenching (q_Q) was defined as $(F_{vs}-F_v)/F_{vs}$, where F_{vs} is the maximum variable fluorescence obtained with a saturation pulse (1–2 s at between 2800 and 4000 μmol m^{-2} s^{-1} depending on the experiment), and F_v is the variable fluorescence at any time. The saturating pulses were applied at 0.5- or 1-min intervals by moving a screen from under the appropriate fibreoptic or by moving the fibreoptic by hand, as trials with electronic timing systems gave unacceptable electrical noise at the time of switching. At the actinic light levels used, varying the rate of pulses up to these rates had no apparent effect on the timecourse of F_v during induction. The non-photochemical quenching at any time (q_N) was calculated as $(F_{vm}-F_{vs})/F_{vm}$, where F_{vm} is the maximum variable fluorescence ($=F_m-F_o$) obtained after the leaves had been dark adapted for at least 30 min. For each experiment the irradiance used was confirmed as saturating by studying the effect of varying the irradiance, and by varying system geometry. This latter test was used to eliminate the possibility that "saturation" was an artefact resulting from saturation of the lock-in amplifier.

The gas-exchange system was as described by Fanjul et al. (1981) and was used in an open-circuit mode, with the gas supply being provided from gas cylinders having either 1% or 21% O_2 in N_2 with either approximately 350 or approximately 1100 vpm of CO_2. Variation of the CO_2 concentration in the gas supply was achieved by use of an ADC (Hoddesden, UK) gas diluter. Carbon dioxide concentrations were determined using an ADC 225 Mark III infrared gas analyser, supplied with both the reference and analysis cells partitioned into 2%, 5% and 93% compartments, but using only the 5% cells for measurement, as described by Fanjul et al. (1981). The gas analyser was calibrated using Wösthoff pumps. The use of the 5% cells in the gas analyser maximises the rate of response of the system while retaining adequate sensitivity. Air humidity, and leaf and air temperatures were monitored and net assimilation (A), leaf conductance (g_l) and intracellular CO_2 concentration calculated as described by Fanjul et al. (1981), though molar units were used (Jones 1983).

In one series of experiments, detailed light and CO_2 response curves were obtained for stressed and control leaves in either 21% or 1% O_2. In these experiments, each change of conditions was made after between 5 an 10 min equilibration when the assimilation trace had stabilised. In addition to measurements of steady-state CO_2 and water vapour exchange, q_Q and q_N quenching were estimated at intervals by the use of standard saturating light pulses. Each flash induced a burst of photosynthetic CO_2 uptake, whose relative magnitude could be estimated from the height of the resulting peak on the recorder trace of CO_2 concentration in the outlet airstream.

Results

Figure 2 shows typical fluorescence traces during the initial period of photosynthetic induction for leaves from well-watered and severely droughted apple trees. These curves are characteristic and illustrate the general effect that fluorescence tended to be quenched more rapidly and more completely in the stressed plants than in the well-watered plants. In such severely stressed plants the variable fluorescence reached a value close to zero within 1.5 min from switching on the actinic light (145 μmol m^{-2} s^{-1}), and remained steady thereafter. In contrast, control leaves consistently showed slight oscillation, with variable fluores-

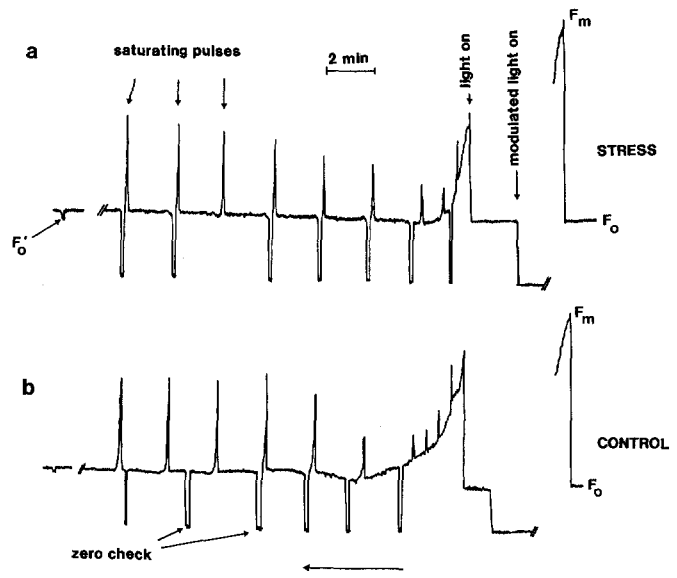

Fig. 2 a, b. Typical fluorescence induction curves measured at 145 μmol m^{-2} s^{-1} for (**a**) stressed and (**b**) control apple leaves during August 1987. The stressed plant had not been watered for 14 days and ψ_1 was –3.5 MPa, while ψ_1 for the control was –0.7 MPa. Also shown are the repeated electronic zero checks (to detect instrumental drift), F'_o and the changing values of F_s obtained with saturating pulses

cence reaching a minimum after about 2 min, and settling to a higher steady state value of approximately 0.15 F_{vm}.

Over the course of three long-term desiccation experiments during July, August and September, with four stress and four control plants in each, there was no evidence for a consistent effect of long-term stress (up to 14 days) on F_v/F_m, though the actual value observed was significantly lower for senescent leaves than for healthy young leaves (data not shown).

Typical responses of fluorescence parameters and of net assimilation rates for leaves on control and droughted plants as CO_2 concentration and irradiance were varied in a standard sequence, are shown in Fig. 3a and b. In all cases the responses for well-watered control plants were of a similar form, with q_N (at steady states) tending to increase from around 0.4 to 0.6 as CO_2 concentration was lowered from ambient to zero at low light (145 μmol m^{-2} s^{-1}). For droughted plants the basal level of q_N (after reaching the steady state) was generally substantially higher than in controls, but showed no further increase as CO_2 concentrations were decreased. Increasing the irradiance tended to raise q_N to a saturating value of between 0.8 and 0.9 for control plants, but often to 1.0 for droughted plants (e.g. Fig. 3b) where no increase in F was detectable on addition of the high-light pulse. The sharp rise in q_N with increasing light occurred at much lower light levels in the stressed plants as compared with the control plants.

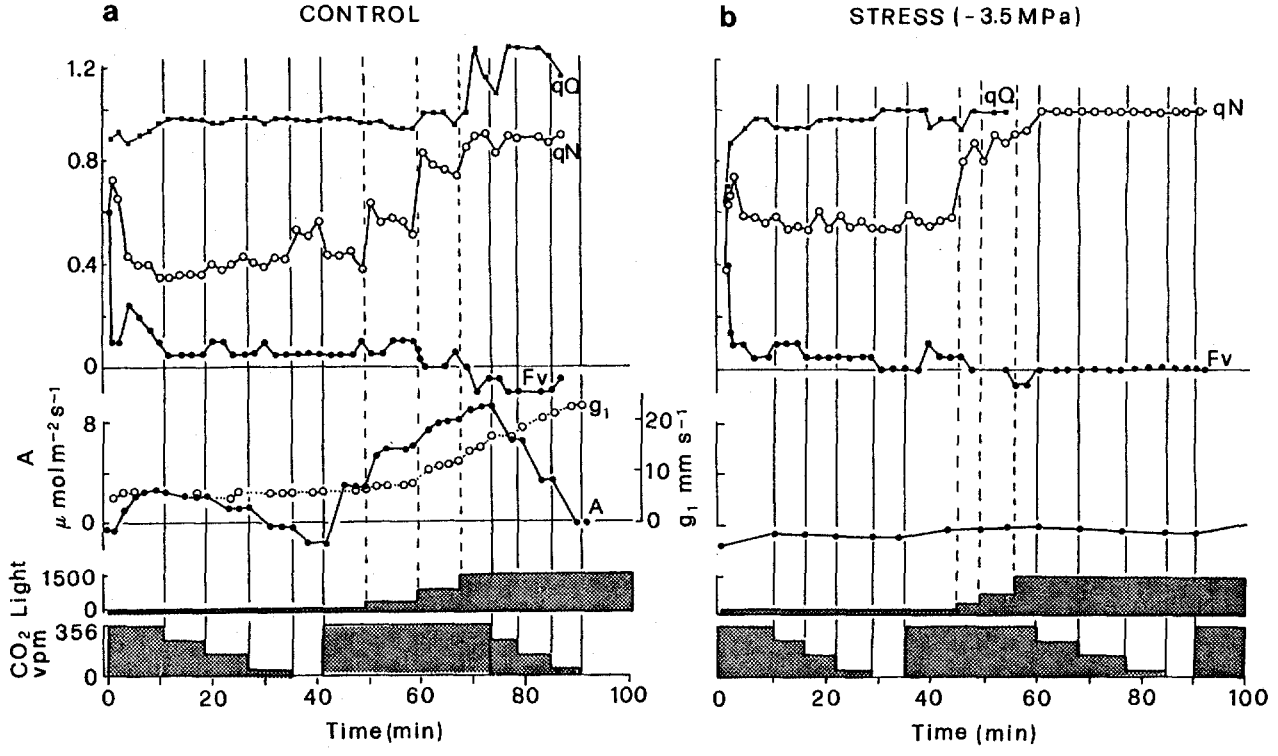

Fig. 3 a, b. Longer-term responses of F_v, q_Q,'q_N, stomatal conductance (g_1) and assimilation (A) for the control and stress leaves, respectively, from Fig. 2 as irradiance and ambient CO_2 concentrations were altered over a period of about 90 min

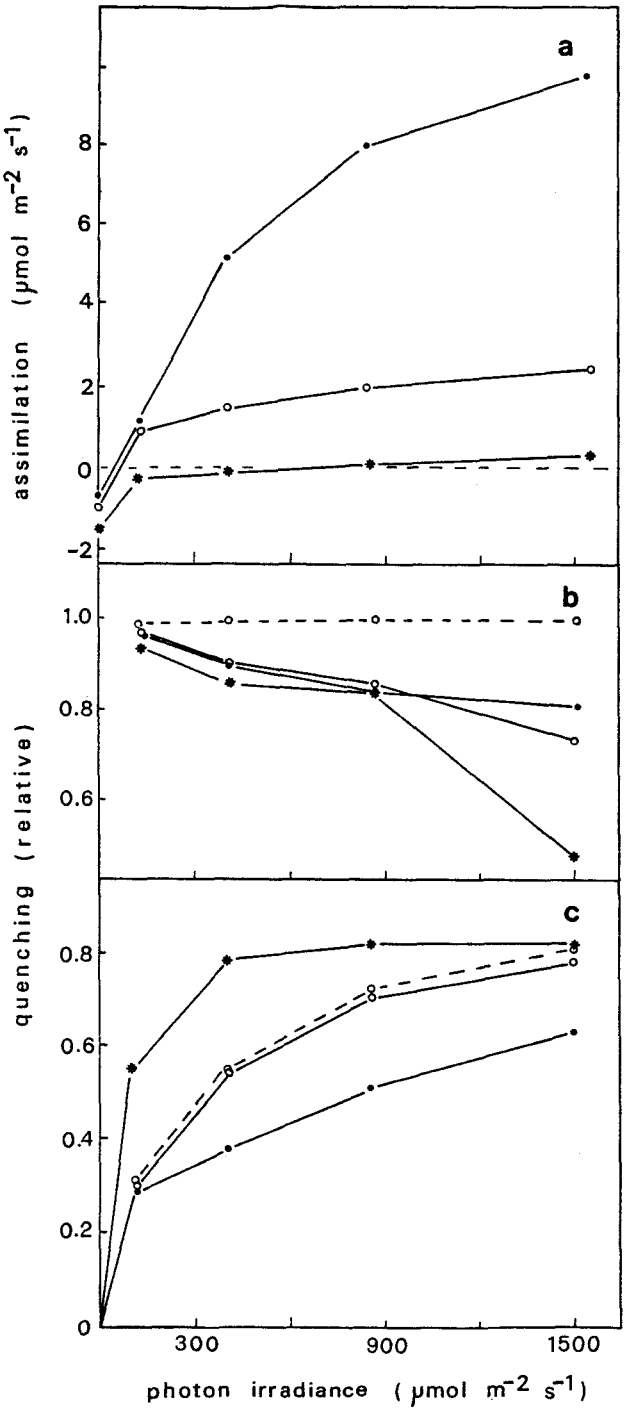

Fig. 4 a–c. Dependence of **a** net assimilation, **b** q_Q and **c** q_N, on irradiance for well-watered (●), moderately stressed (○, 9 days without water, ψ_l = –2.2 MPa) and severely stressed (*, 12 days without water, ψ_l = –3.5 MPa) apple leaves. Some values for q_Q and q_N were calculated used corrected F_o' values (———), or uncorrected (F_o) (– – –)

In all cases with apple the steady-state apparent variable fluorescence was found to be very small (see for example Fig. 3) with $(F_s-F_o)/(F_m-F_o)$ generally being less than 0.15, and often close to zero or even less than zero in many cases, especially for droughted leaves. This has the consequence that it is difficult to estimate q_Q accurately, since there was strong evidence that F_o could be quenched especially in water-stressed plants. To illustrate the necessity for correcting F_o for quenching, Fig. 4 includes a comparison of q_Q calculated either using the original F_o or the corrected value, F_o'. The effect of this correction on q_N was negligible.

Figures 3 and 4 both show the greater sensitivity of q_N to irradiance in the water-stressed plants than in the controls. In addition, it is also apparent that q_Q is rather less sensitive to irradiance (especially at low light levels) or to CO_2 concentration than is q_N, though the decline in q_Q as light increased was greater as the degree of water stress increased. Overall these figures also indicate that neither fluorescence quenching parameter was closely related to assimilation rate (compare, for example, values for stressed and control plants), though q_Q did tend to decline as the assimilation rate increased for any one plant.

When assimilation and fluorescence were studied in 1% O_2 rather than in 21% O_2, not only did net assimilation increase as expected (data not shown), but also the value of q_N was enhanced and that of q_Q depressed (Fig. 5).

Some typical time courses for the induction of q_N quenching during photosynthetic induction at an incident irradiance of 145 μmol m^{-2} s^{-1} are shown in Fig. 6a. The effect of long-term water stress on raising the steady-state value of q_N confirms other data (e.g. Fig. 3). The relaxation of q_N on darkening after a period at high light (1550 μmol m^{-2} s^{-1}) is shown for the same leaves in Fig. 6b. In all cases the decline of q_N was quite smooth with q_N still being between 0.4 and 0.6 after 10 min in the dark. Similar relaxation patterns were observed even when the flashing frequency was halved (data not shown). The time course of relaxation was similar in form whether or not the exposure to high light occurred at low or high O_2 levels. The differences between well-watered and droughted plants only appeared to be reflected in the mean level.

In other experiments with well watered apple leaves (data not shown) a similar incomplete recovery of q_N was observed even when the maximum irradiance had been only 240 μmol m^{-2} s^{-1} (with 21% O_2), conditions which are unlikely to have caused significant photoinhibition.

Typical examples of changes in fluorescence quenching during induction for a leaf before and after rapid dehydration to a leaf water potential of below –4 MPa are shown in Fig. 7. In no case was there a clear effect of such a treatment on the shape of the induction curve. In all cases stomatal closure after the dehydration was so complete that it was not possible to detect significant gas exchange.

In one series of experiments the ratio F_v/F_m was measured before and after a 1.5 h desiccation period for leaves of all ages on three *ex* micropropagation trees (Table 1). Although the average values of F_v/F_m obtained for this material (0.77) are slightly lower than, for example, corresponding values obtained using 77K fluorescence (Demmig and Björkman 1987), differences between simultaneous measurements on one leaf using the Hansatech system used here and the Walz modulated fluorescence system (H. G. Jones and W. P. Quick, unpublished data) can be as large as 15%. Use of polynomial regressions up to the fourth degree failed to provide any evidence for a

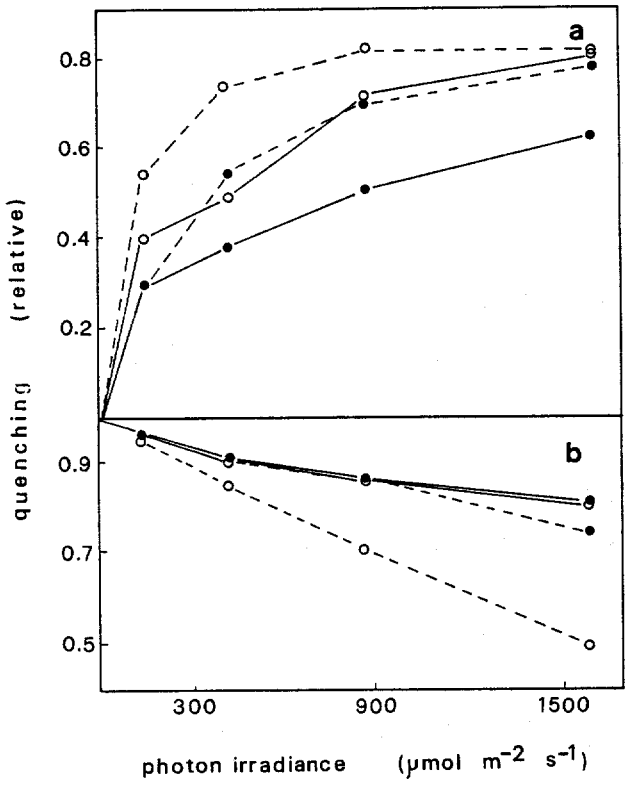

Fig. 5 a, b. Response of **a** q_N and **b** q_Q to irradiance for stress (– – –) and control (———) leaves when measured in 21% O_2 (●) or 1% O_2 (○) at 340 vpm ambient CO_2

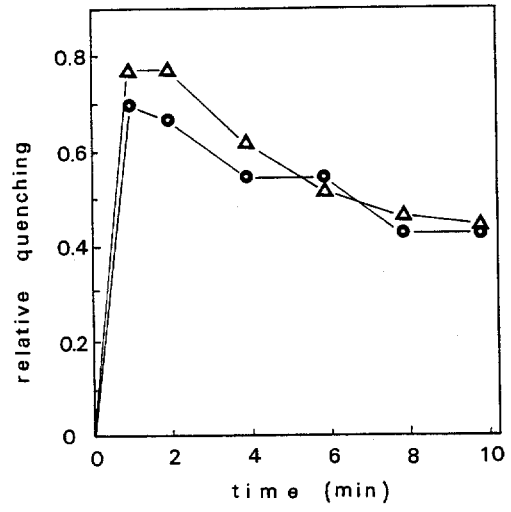

Fig. 7. Non-photochemical quenching (q_N) induction curve at 200 μmol m^{-2} s^{-1}, 350 vpm CO_2 and 20.8% O_2 for a control leaf (ψ_l = –0.8 MPa, △) and for the same leaf 75 min after detachment from the plant and desiccation to below –3.5 MPa (○)

gradient or other trend in F_v/F_m up the plant, nor was there a significant decrease in F_v/F_m after dehydration. There was, however, evidence that a large proportion of the total variance in F_v/F_m was a function of leaf-to-leaf variation rather than instrumental error, because of the pre- and

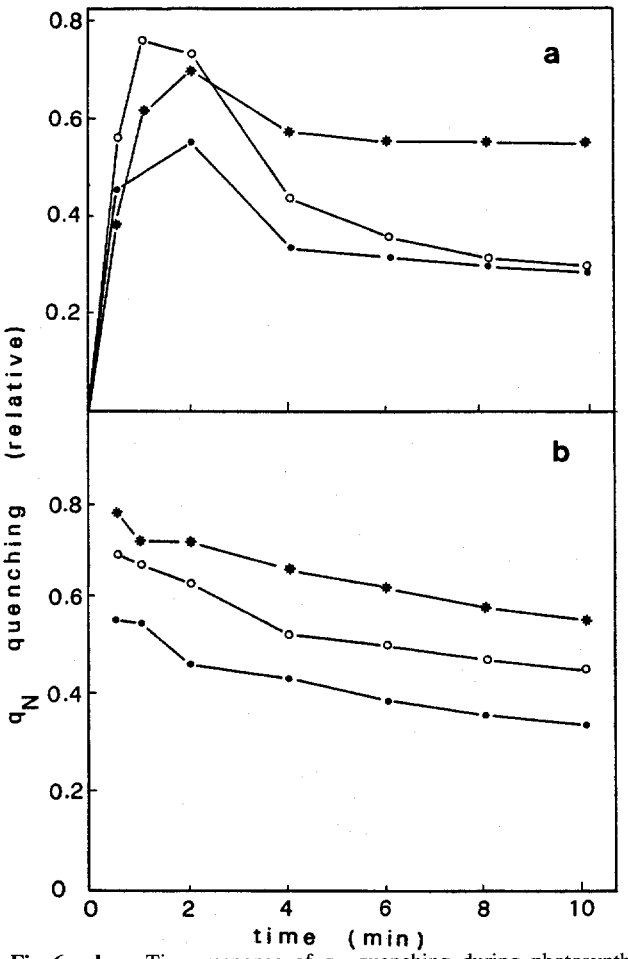

Fig. 6 a, b. **a** Time response of q_N quenching during photosynthetic induction at 145 μmol m^{-2} s^{-1} (same leaves and symbols as Fig. 4), **b** time course of relaxation of q_N quenching in the dark, after exposure to the light and CO_2 regime given in Fig. 2 (same leaves as Fig. 4)

post-stress measurements for different leaves were highly correlated, with 67% of the variance in F_v/F_m after stress being attributable to leaf-to-leaf variation before stress. The coefficient of variation for F_v/F_m for leaves on one plant varied between 2.4% and 12% for the different plants. This includes both leaf-to-leaf variation and experimental error. Similar results were obtained for a healthy plant (mean F_v/F_m = 0.77) and for a partially senescent plant (mean F_v/F_m = 0.59).

Discussion

The system used for the present study was designed to allow simultaneous measurement of chlorophyll fluorescence and CO_2 and water vapour exchange for attached leaves, if necessary on plants growing in the field. The use of a small leaf chamber and minimal system volume gives rapid equilibration and hence the potential either for measuring large numbers of leaves, or for following rapid changes in gas exchange (Fanjul et al. 1981). The short time constant obtainable is comparable to that reported by Peterson et al. (1987), who used a more complex system.

Table 1. Typical results when F_v/F_m measured for all leaves on a single micropropagated apple plant (after at least 90 min in the dark) then re-measured 75 min after detachment and desiccation to lower than −4 MPa

Leaf number	Control F_v/F_m	Stress F_v/F_m
1	0.76	0.76
2	0.77	0.77
3	0.75	0.75
6	0.76	0.77
8	0.74	0.74
9	0.70	0.71
10	0.78	0.78
14	0.76	0.78
15	0.77	0.78
16	0.79	0.77
18	0.78	0.76
19	0.79	0.79
20	0.77	0.76
22	0.75	0.75
23	0.77	0.76
24	0.77	0.76
25	0.77	0.77
26	0.76	0.77
27	0.77	0.78
28	0.77	0.78
Mean	0.766	0.764

The use of this facility for combining gas exchange and fluorescence for analysing the limitations to photosynthesis (Jones 1985) will be presented in a subsequent paper.

Variable fluorescence during photosynthetic induction with constant actinic light (145 μmol m^{-2} s^{-1}) tended to a steady-state value (F_{vt}) within about 2 min, at least in stressed leaves, though in well-watered control leaves some oscillation over the first 4–6 min of induction was common. Such oscillations are well known (Lavorel and Etienne 1977; Walker et al. 1983), though the secondary maximum is not always observed as late as in the present experiments (Sicher et al. 1988). Even in well-watered plants F_{vt} was normally less than 0.15 F_o, though it fell to near zero in stressed plants even after correcting for quenching of F_o. Similar effects of water stress on the form of the fluorescence induction curve, with both a damping of oscillations and a lowering of F_{vt}, have been reported for *Arbutus unedo* (Schreiber and Bilger 1987), though in that case the leaves had apparently been rapidly dried to 71% relative water content, which was probably a low water potential for that species. With further desiccation (to 36% relative water content) Schreiber and Bilger (1987) reported a decrease in the rate of quenching, an effect that has been observed with less severe stresses in other species (Havaux and Lannoye 1983; Conroy et al. 1988). No such delay in quenching was detected in the present experiments with either slow or rapid dehydration to –4 MPa. Variation of quenching rate with water stress can be modified by the ambient CO_2 concentration (Conroy et al. 1988).

The present experiments also gave no indication that variation of leaf water potential over the normal physiological range could lead to an increase of F_{vt}. This contrasts with some published data (e.g. Genty et al. 1987). The generally small values of F_v led to rather imprecise estimates of q_Q. This effect is compounded by any quenching of F_o, which has therefore to be estimated and corrected for, in order to get reliable estimates of q_Q (Bilger and Schreiber 1986). For apple we found that F_o quenching could be as much as 15%–20% of F_o and occurred in both well-watered and stressed plants, though the precise mechanism of this quenching is, as yet, uncertain.

The ratio F_v/F_m gives a direct estimate of the yield of photochemistry of photosystem II. Therefore the lack of any significant effect of water stress on F_v/F_m in the present experiments, whether in the long-term (days to weeks) or in the short-term (hours) dehydration tests implies that there was little direct effect of stress on the PSII donor side and the primary reactions. These results are in marked contrast to the large effects of dehydration on the peak to initial fluorescence ratio reported by Govindjee et al. (1981) for *Atriplex triangularis* and *Tolmiea menziesii* and by Havaux and Lannoye (1983) for maize. Genty et al. (1987), on the other hand, found only a minor effect of water stress on the related ratio of F_m/F_o in cotton or in oleander at low light, but they observed a significant effect at high light which may have been related to photoinhibition. Demmig et al. (1988) also found a decrease in F_m/F_o in oleander when subjected to progressive drought. The large differences between the results obtained by different experimenters probably arise from the differing amounts of photoinhibition occurring during the stress treatment, with the 10–30 min dark pretreatment generally not being enough to restore F_m (and F_o) to unphotoinhibited levels in all experiments.

Although F_v/F_m (measured on dark pre-adapted leaves) was insensitive to stress in these experiments, there were clear effects of stress on the light response of q_N measured after illumination. A clear effect of the long-term water stress was to increase the steady value of q_N achieved after about 10 min illumination at the moderately low irradiances (145 μmol m^{-2} s^{-1}) used for most experiments. In most such experiments the steady state q_N increased from about 0.3 in control plants to about 0.6 in severely stressed plants (e.g. Figs. 3, 6). There was also an indication that the relaxation of q_N after the initial peak during induction was slower in stressed leaves than in well-watered controls, though this effect was not entirely consistent. In contrast to the long-term stress results, when leaves were allowed to desiccate rapidly, no clear differences in relaxation of q_N or in the steady value of q_N were apparent (Fig. 7).

The lack of any effect of rapid water stress on q_N, even though stomatal closure had been so complete that net gas exchange had been almost completely inhibited (results not shown) is particularly important. Firstly, this suggests that the observed effects of the long-term stress on fluorescence behaviour were neither a direct result of altered assimilation (since this was affected similarly by short- and long-term stress) nor a direct result of leaf water status at the time of measurement, and that it involved some metabolic or structural response in the leaf. It is unlikely that increased internal recycling of photorespired CO_2 could have resulted in CO_2 fixation rate continuing unabated in stressed leaves. This conclusion is supported by the obser-

vation that results were similar whether measurements were made in 21% O_2 or in 1% O_2 (Fig. 5). The reason for rapid effects of water stress on q_N in some other systems (e.g. Schreiber and Bilger 1987) is not clear. A second interesting feature of the results is that the net assimilation rate could vary over a very large range with only small effects on fluorescence characters such as F_v/F_m, q_N or even q_Q, so these parameters cannot be used by themselves to obtain an indication of stress effects on net photosynthesis.

Another clear effect of the long-term water stress was to eliminate any sensitivity of q_N to ambient CO_2, and to increase greatly the sensitivity of q_N to increasing irradiance (Fig. 3). The CO_2 effect may relate to the fact that changing ambient CO_2 concentrations had only slight effects on net assimilation in the stressed plants, but a large effect in the controls. These responses of q_N to light and CO_2 at different levels of stress are what would be predicted if q_N is an indicator of the amount of excess energy not being used in photosynthesis. Because of the time required to do this experiment it was not possible to do a comparable experiment with rapidly desiccated leaves, as they would continue to lose water. It is also worth noting here that F_{vt} was almost completely insensitive to irradiance or to CO_2 in all experiments (cf. Sharkey 1985; Wong and Woo 1986).

Errors in the chlorophyll fluorescence parameters are hard to estimate, but some considerations have been referred to above. Although the signal to noise ratio achieved with the present set-up was less than is possible with optimal systems, analysis of variance of estimates of F_v/F_m on similar leaves indicated that the coefficient of variation was normally less than 12% and this included both environmental and instrumental components. Normalising results to F_o' (or to F_o in some early experiments) minimises problems arising from altered geometry (Ögren and Baker 1985).

The rate of relaxation of q_N can be used to give information on the mechanisms giving rise to the observed non-photochemical quenching (Baker and Horton 1987; Demmig and Björkman 1987; Horton and Hague 1987; Weis and Berry 1987; Genty et al. 1989). Although the "pH-dependent" or "energy-dependent" component of quenching relaxes within seconds in isolated chloroplasts, it appears to have a half-time ($t^1/_2$) $\cong$ 1 min in intact leaves. Quenching due to state transitions is thought to relax with $t^1/_2 \cong 5$ min, and that due to photoinhibition may be 30 min or longer, though Demmig and Björkman (1987) have proposed that longer-term relaxation may be related both to photoinhibitory processes and to a regulatory mechanism for dissipation of excess energy, possibly involving the zeaxanthin cycle. The present experiments, where recovery was measured only after approximately 10 min in the dark, cannot distinguish between photoinhibitory damage and any protective "down"-regulation (Weis and Berry 1987; Genty et al. 1989) of PSII quantum yield. Nevertheless, data in Fig. 6 indicate that less than 50% of q_N could be attributable to "energy-dependent" quenching because less than 50% relaxed within 1 min of darkening after a previous exposure to high light. The majority of q_N relaxed much more slowly, and at a similar rate in all treatments, with a $t^1/_2$ of more than 10 min. It is unlikely that the slow recovery can be attributable to re-energisation by the measuring flashes, as similar results were obtained even when the rate of flashing was decreased to once every 2 or 4 min.

The very different fluorescence characteristics observed in response to water stress in different laboratories and with different species suggests that the processes being observed are more likely to be secondary responses than direct responses to plant water status. Therefore they may not provide reliable indicators of stress sensitivity for use in breeding programmes.

Acknowledgements. We are very grateful to Professor N R Baker for advice and encouragement for this work and for commenting on a draft manuscript, to Keith Higgs for technical assistance and to Kathy Phelps for statistical advice.

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