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Simultaneous Measurement of Oscillations in Oxygen Evolution and Chlorophyll *a* Fluorescence in Leaf Pieces[1]

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DAVID A. WALKER, MIRTA N. SIVAK, ROSLYN T. PRINSLEY, AND JOHN K. CHEESBROUGH
ARC Research Group on Photosynthesis, Department of Botany, University of Sheffield, Sheffield, S10 2TN, United Kingdom

ABSTRACT

In spinach (*Spinacia oleracea*) and barley (*Hordeum vulgare*) leaves, chlorophyll *a* fluorescence and O_2 evolution have been measured simultaneously following re-illumination after a dark interval or when steady state photosynthesis has been perturbed by changes in the gas phase. In high CO_2 concentrations, both O_2 and fluorescence can display marked dampening oscillations that are antiparallel but slightly out of phase (a rise or fall in fluorescence anticipating a corresponding fall or rise in O_2 by about 10 to 15 seconds). Infrared gas analysis measurements showed that CO_2 uptake behaved like O_2 evolution both in the period of oscillation (about 1 minute) and in its relation to fluorescence. In the steady state, oscillations were initiated by increases in CO_2 or by increases or decreases in O_2. Oscillations in O_2 or CO_2 did not occur without associated oscillations in fluorescence and the latter were a sensitive indicator of the former. The relationship between such oscillations in photosynthetic carbon assimilation and chlorophyl *a* fluorescence is discussed in the context of the effect of ATP or NADPH consumption on known quenching mechanisms.

In 1949, Van der Veen (23) described what he called 'irregularities in photosynthesis' during the period when a leaf is suddenly illuminated after a period in darkness. These irregularities during the induction period, *i.e.* the lag before maximal photosynthesis is achieved following re-illumination (28), included 'secondary peaks' (or increases and decreases in rate which finally gave way to a more or less steady rate of CO_2 uptake). Similar but somewhat simpler irregularities associated with re-illumination had already been observed by McAlister and Myers (16) and by Aufdemgarten (2). They were immediately apparent when renewed attempts were made to follow O_2 evolution from leaf discs by polarographic methods (26). More recently (5, 10, 14, 17, 19), oscillations have been induced by changing the O_2 or CO_2 content in the atmosphere surrounding an illuminated leaf. The fact that oscillations have been detected by such disparate methods as diaferometry (23, 24), IRGA[2] (5, 10, 14, 17), polarography (26, 28, 29), and ^{14}C incorporation (30) suggests that they are a genuine manifestation of changes in photosynthetic rate and not artifacts of measurement. It has been suggested that they might be brought about by over-reaction of regulatory mechanisms (21, 22, 27, 28).

Chl *a* fluorescence kinetics during induction have also been studied for many years, and many attempts have been made to relate these relatively slow changes (that normally occur after the first few s of illumination), with changes in O_2 evolution and/or CO_2 uptake (see *e.g.* Ref. 16). Surprisingly, Van der Veen (24) did not follow the fluorescence changes associated with the more dramatic secondary peaks in CO_2 fixation which he had observed (*cf.* Refs 23 and 24) but nevertheless concluded that 'nearly always increase of photosynthesis during adaptation (induction) is correlated with a decrease of fluorescence.' In algae, the relationship seems less clear and Chl fluorescence has been found to rise in parallel with O_2 evolution in some circumstances (3) but not in others (18) and Ogawa (19) has recently reported that fluorescence yield and O_2 evolution oscillate in phase (in parallel) in *Vicia faba* leaves under anaerobic conditions. In preliminary experiments with spinach, antiparallel, dampening oscillations in O_2 and fluorescence were observed (29). These observations have now been confirmed and further characterization of these phenomena is reported.

MATERIALS AND METHODS

Plant Material. Spinach (*Spinacia oleracea* L. U. S. Hybrid 424, Ferry Morse Seed Co., Mountain View, CA) was grown for 6 weeks in water culture in a glasshouse as described previously (26) and discs were cut from leaves with a sharp cork borer. Barley (*Hordeum vulgare* L. var. Sonja, Nickerson RPB Ltd., Rothwell, Lincoln, U.K.) seedlings were grown for 8–9 d in vermiculite in a glasshouse under sunlight and supplementary incandescent lamps. The plant material was harvested when the leaves were between 13 and 15 cm long. Leaf pieces of 2-cm length were cut (at about 9 cm from the leaf base) using the more mature part of the first leaf but discarding the first 1.5 to 2 cm of the leaf tops.

Oxygen Evolution and Fluorescence. Methods were essentially the same as those used by Delieu and Walker (7). Temperature was kept constant at 20°C. Spinach leaf discs (10 cm^2) or 2-cm length barley leaf pieces (5 to 8 pieces, about 7 cm^2) were placed in a leaf disc O_2 electrode that had been modified to allow simultaneous detection of Chl *a* fluorescence.

This apparatus (Hansatech Ltd., Norfolk, U.K.) was constructed of anodized aluminum to give improved temperature control and to minimize stray light (7). Two fiber optics were used. The first was a 13-mm-diameter bundle which brought the actinic light into the top of the chamber. A second fiber optic, inserted into the upper section of the apparatus at an angle of 40°, was used to guide light emission to a photodiode for fluorescence measurement. The exciting light was either a 150 w tungsten/halogen source (Volpi Intralux 5000) or a light source designed by Delieu and Walker (7) powered by a Coutant regulated DC power supply and filtered through heat-absorbing glass, a heat-reflecting interference filter, and a Corning 4-96 glass filter. Emission was defined by a Corning 2-64 filter and meas-

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[2] Abbreviations: IRGA, infrared-gas analysis; PGA, 3-phosphoglycerate.

ured using an UDT Phot-op photodiode. The fluorescence signal, O_2 signal, and the differential of the O_2 signals were fed to a chart recorder. To follow O_2 evolution by polarography, the leaf chamber has to be used as a closed system (the relatively low sensitivity of the O_2 electrode systems currently available precludes open system use). Two alternative methods were employed to permit high rates of photosynthesis in a small closed chamber for relatively long periods without undue depletion of CO_2: (a) capillary matting moistened with carbonate/bicarbonate buffer (7); (b) flushing the chamber with a gas mixture containing 5% CO_2 and 20% O_2 (balance N_2) immediately before the chamber was closed. The second method was preferred when the gas phase was to be changed during the course of the experiment.

Absolute rates of O_2 evolution were only occasionally determined because experience showed that they, within the limits employed, were largely irrelevant. When measured accurately, they were found to be close to 150 μmol mg^{-1} Chl h^{-1} which is about half the rate displayed by the same leaf material in near-saturating white light (actinic blue light was used to facilitate the measurement of weak red fluorescence). The light source and filters provided 70 $w \cdot m^{-2}$ blue light at the leaf surface and 210 $w \cdot m^{-1}$ of PAR when the blue filters were removed.

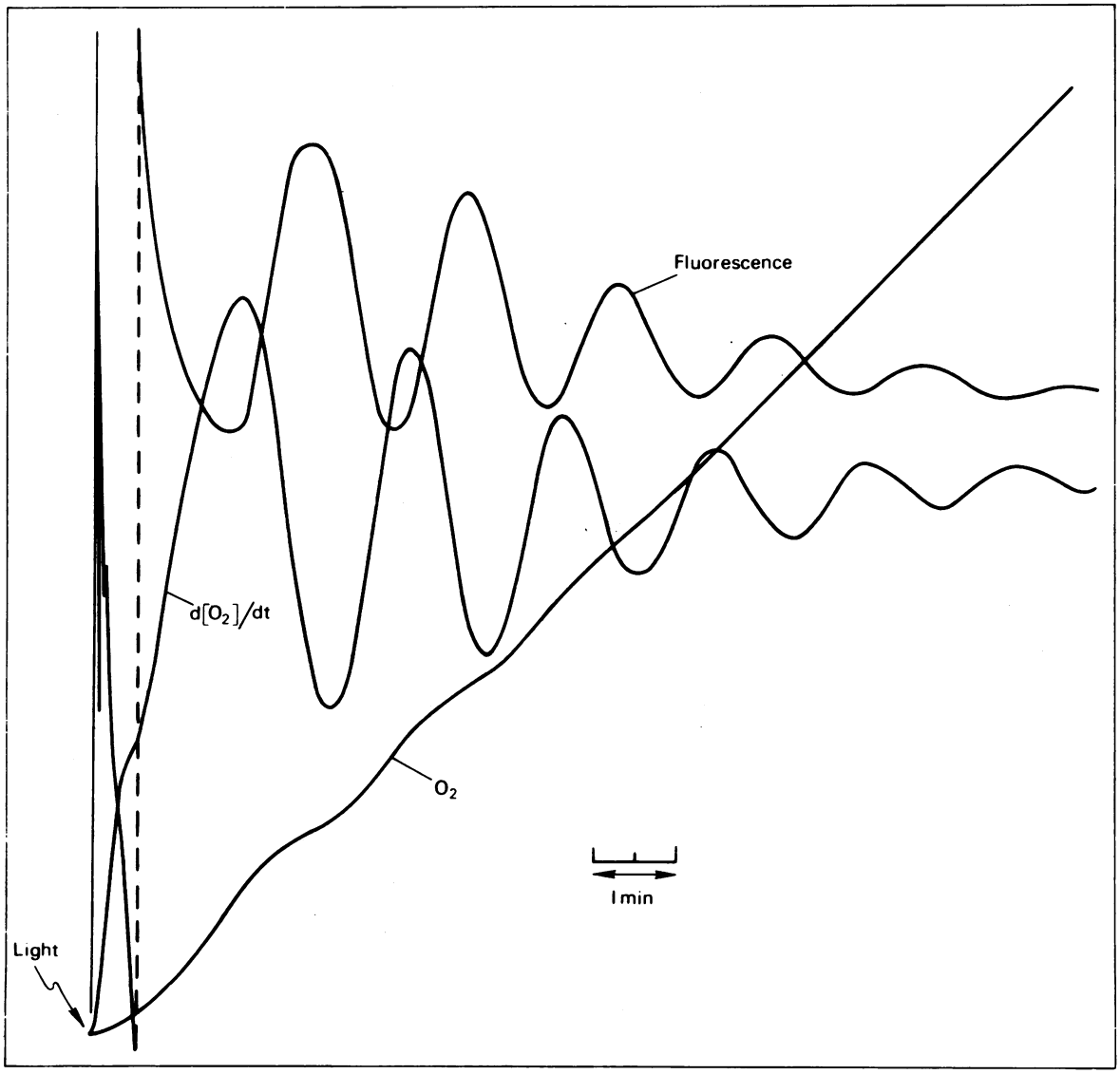

FIG. 1. Antiparallel relationship between Chl *a* fluorescence and rate of O_2 evolution. A spinach leaf disc was re-illuminated after 2-min darkness during which time the O_2 content of the atmosphere was lowered to 2%. CO_2 concentration, 5%. Temperature, 20°C. Strong oscillations in O_2 evolution and fluorescence were observed. The peaks occurred at approximately 2-min intervals and were antiparallel except that the fluorescence signal anticipated the O_2 change by about 15 s. (The electrode response is about 2 s, *e.g.* if illumination is restarted after a very short dark interval, a response is seen in just under 2 s.)

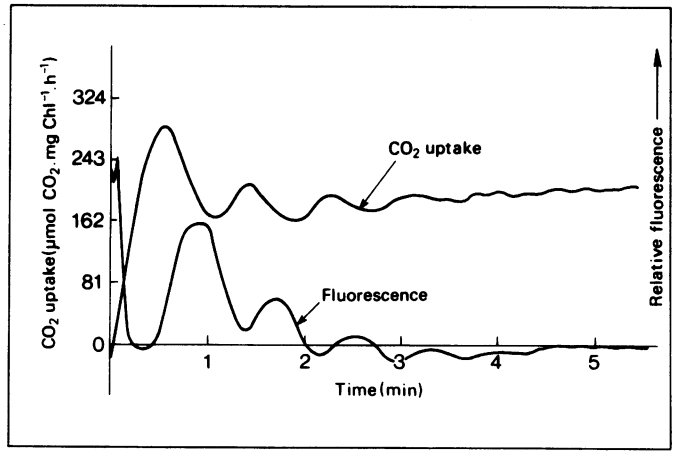

FIG. 2. Antiparallel relationship between Chl *a* fluorescence and CO_2 uptake. A spinach leaf disc was re-illuminated after 2-min darkness during which time the O_2 content of the gas stream was lowered to 2% and the CO_2 concentration was raised from 340 to 960 μl/l. Both CO_2 uptake and fluorescence showed dampening oscillations; peaks occurred at approximately 1-min intervals with the two signals antiparallel except for the fluorescence signal displaced by a phase difference of the order of 15 s.

In the open mode (fluorescence measurements only), gas mixtures were passed through the leaf chamber at 400 ml·min^{-1} and their composition changed according to previously described procedures (22). Under these conditions, fluorescence approached a quasi-steady state after about 6-min illumination.

IRGA and Fluorescence. The IRGA system has been described previously (10) except that the top of the IRGA chamber was modified to accommodate a fluorescence probe (similar to the one used in conjunction with the leaf disc electrode chamber) and a blue filter (Corning 4-96).

RESULTS

Antiparallel but Phase-Shifted Relationship between Chl *a* Fluorescence and Rate of O_2 Evolution in Leaves. Figure 1 shows dampening oscillations in Chl *a* fluorescence and corresponding but antiparallel oscillations in the rate of O_2 evolution. These were initiated in a disc cut from a spinach leaf by illumination after a period of darkness. The leaf material was surrounded by an atmosphere which had been selected because it had been found to favor large oscillations and, indeed, oscillations can even be initiated in continuous light by changing the gas phase from air to one with the composition used in Figure 1 (Fig. 3). Nevertheless, these particular conditions (high CO_2, low O_2 and interrupted illumination) were only one of many combinations in which similar dampening oscillations were observed (see below). Figure 1 is included because the oscillations themselves were pronounced and a number of characteristic features can be readily discerned. The antiparallel relationship between the fluorescence signal and the differential of O_2 evolution (29) are immediately apparent but it may also be noted that the fluorescence signal anticipates the changes in the rate of O_2 evolution by about 15 s (*i.e.* a fall in fluorescence gives warning of increase in the rate of O_2 evolution and a rise in fluorescence indicates that a fall in O_2 is imminent). How much of the delay in the O_2 signal is real and how much is attributable to any delay by the O_2 electrode in sensing a change in O_2 (see Ref. 7) is still somewhat uncertain. Present experience suggests that the extent to which fluorescence anticipates O_2 is somewhat variable (12 ± 2–3 s) but the rapidity of the electrode response (*e.g.* injected O_2 is sensed in less than 2 s) implies that most of the discrepancy

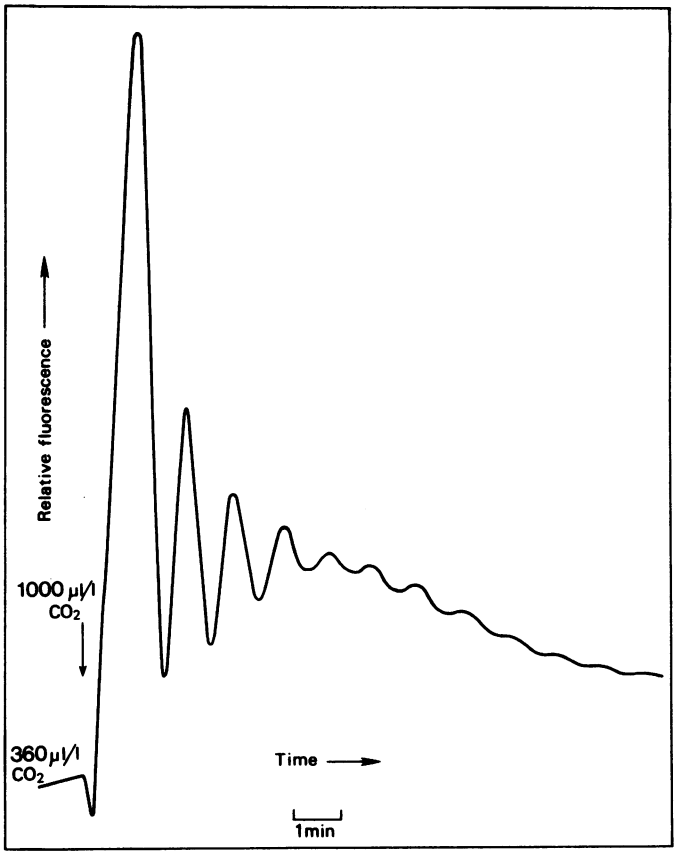

FIG. 3. Fluorescence oscillations initiated under unchanged illumination by increasing the CO_2 concentration of the gas phase, containing 2% O_2 from 360 to 1000 μl/l. Barley leaf pieces were placed in the leaf chamber and the gas mixture was passed at a rate of 400 ml min^{-1}. The leaf was then illuminated for at least 6 min to ensure that fluorescence approached a steady state level before the change in gas phase was made. The initial fall in fluorescence (see Ref. 21) is followed by a series of oscillations of declining amplitude until a new quasi-steady state level of fluorescence (higher than the level achieved in 360 μl/l of CO_2) is reached.

between fluorescence and O_2 is real (*i.e.* that the fluorescence signals are prompted by changes in metabolism which then bring about corresponding changes in net O_2 evolution). In some experiments (not illustrated), the extent to which the fluorescence signal anticipated the O_2 response clearly varied from oscillation to oscillation and the time difference between fluorescence peak and O_2 trough was greater than that between fluorescence trough and O_2 peak.

(It should be noted that we have taken the simplest view, that the signals are antiparallel but slightly out of phase. It could be argued, we believe with great difficulty, that the signals are parallel but substantially out of phase. This latter interpretation would mean that there is no obvious relationship between the size of the signals and that a large change in one could occur without a correspondingly large change in the other.)

Antiparallel and Phase-Shifted Relationship between Chl *a* Fluorescence and Rate of CO_2 Fixation in Leaves. Figure 2 shows simultaneous measurements of Chl *a* fluorescence and CO_2 depletion (IRGA). As in Figure 1, the oscillations were induced by re-illumination in an atmosphere containing low O_2 (2%) and high CO_2 (960 μl/l). Again it can be seen that a rise in fluorescence anticipates a fall in photosynthetic carbon assimilation (*i.e.* an increase in the CO_2 content of the gas stream). Although it has not yet proved practicable to measure CO_2, O_2, and Chl *a* fluorescence under precisely the same conditions, Figures 1 and

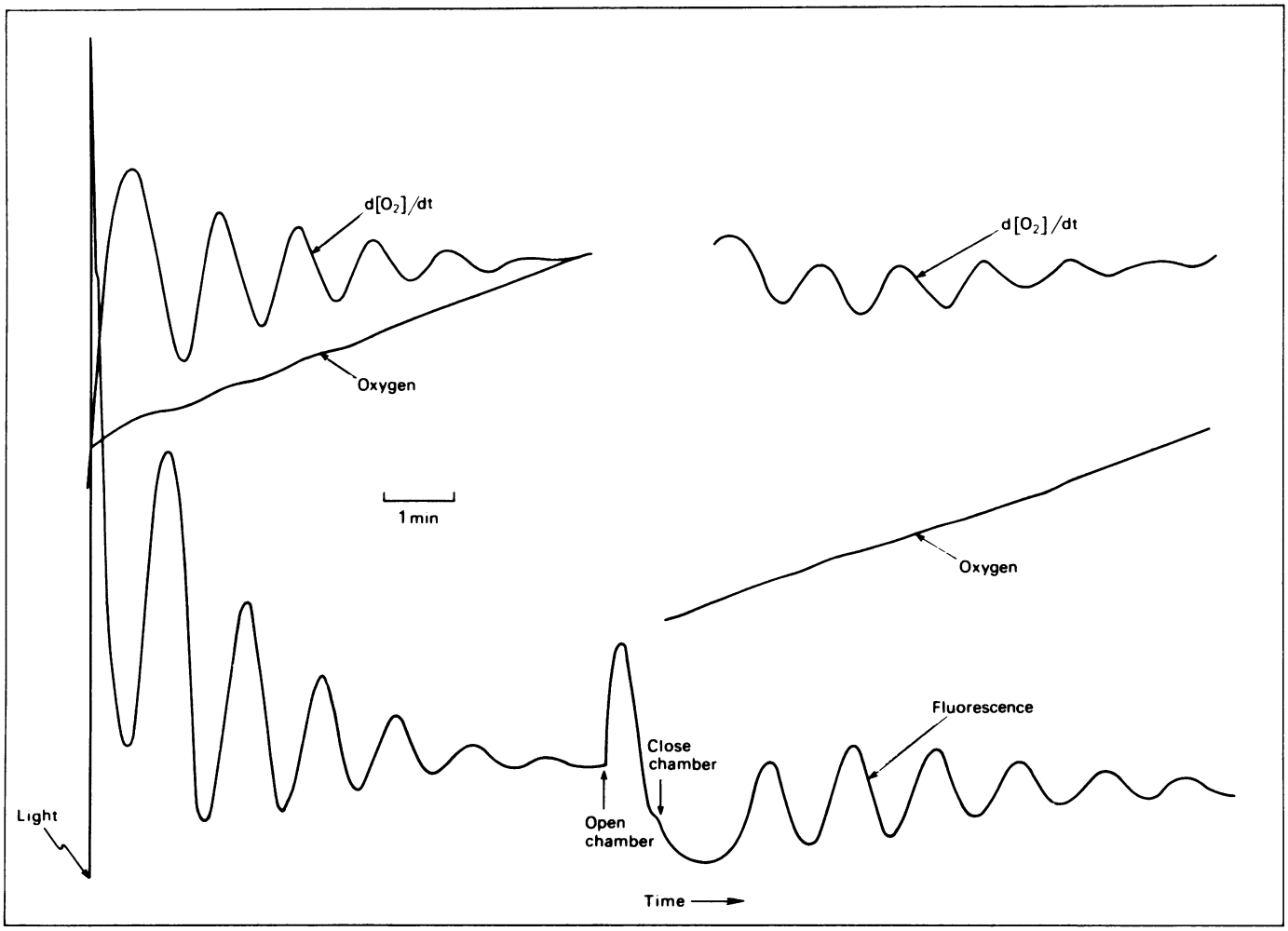

FIG. 4. Oscillations in Chl *a* fluorescence and rate of O_2 evolution under normal (20%) O_2 concentration induced by dark/light transition (left) or by augmenting CO_2 concentration (right). Oscillations were initiated by re-illumination (left) of barley leaf pieces after a dark interval of 2 min. Previously, the leaf pieces were illuminated in the closed chamber until steady state levels of fluorescence and photosynthesis were reached (not shown). Although at the start of the experiment the concentrations of CO_2 and O_2 were 5 and 20%, respectively, as photosynthesis progressed in the closed chamber, O_2 increased up to about 22% and we assume a commensurate decrease in CO_2. Oscillations were then re-initiated (right) when O_2 and CO_2 were returned to the starting levels, by briefly opening the chamber and flushing it with a gas phase containing 5% CO_2 and 20% O_2 (balance N_2). The chamber was then closed again.

2 nevertheless imply that fluorescence rises and falls slightly in advance of photosynthesis, and in an antiparallel manner and that it is largely immaterial whether photosynthesis is monitored by O_2 evolution or CO_2 fixation.

Initiation of Oscillations by Changes in Gas Phase under Continuous Illumination. *Changes in CO_2.* In Figures 1 and 2, oscillations were induced by re-illumination after a dark interval in atmospheres containing low O_2. Figure 3 illustrates an experiment in which O_2 was also low but in which the oscillations were initiated by increasing the CO_2 content from 360 to 1000 μl/l under constant illumination. Oscillations may also be initiated by either method (dark/light transition or increased CO_2 in continuous light) in 20% O_2 (Fig. 4). Data for spinach is not shown but Figure 4 (data obtained with barley) illustrates typical responses when spinach or barley leaves were subjected to these regimens. Although the external concentration of CO_2 used in Figure 4 (5%) is indeed very high, the actual concentration at the carboxylation site is not apparently deleterious for the leaf disc in the experimental conditions used, as shown by the high and constant O_2 evolution rates observed during experiments that extended over periods of hours (data not shown). It is not necessary to use such high CO_2 concentrations in order to elicit oscillations under 20% O_2 and, indeed, increasing the CO_2 content of the gas phase from 312 to 1000 μl/l produces the same sort of response (not shown).

In all of these experiments, oscillations were invariably seen when the CO_2 content was increased from about air levels of CO_2 to much higher concentrations. Oscillations could also, but with more difficulty, be initiated by increasing the CO_2 from very low concentrations to about air levels (Fig. 5) and fluctuations of a sort were even detected when the CO_2 concentration was dropped from a relatively high concentration to about air level (Fig. 6).

Changes in O_2. Although low O_2 favors oscillations (Figs. 1 and 2), it is not a prerequisite (Figs. 4 and 5). Indeed oscillations may be initiated, under constant illumination, either by decreasing or increasing the O_2 concentration. Thus, Figure 7 shows oscillations in fluorescence brought about by decreasing O_2 from 20 to 2% with CO_2 content held constant at 1000 μl/l. In Figure 7, the first cycle of oscillations was induced by increasing the CO_2 content from 384 to 1000 μl/l in 20% O_2, the second cycle by decreasing the O_2 to 2%, and the third cycle by increasing the

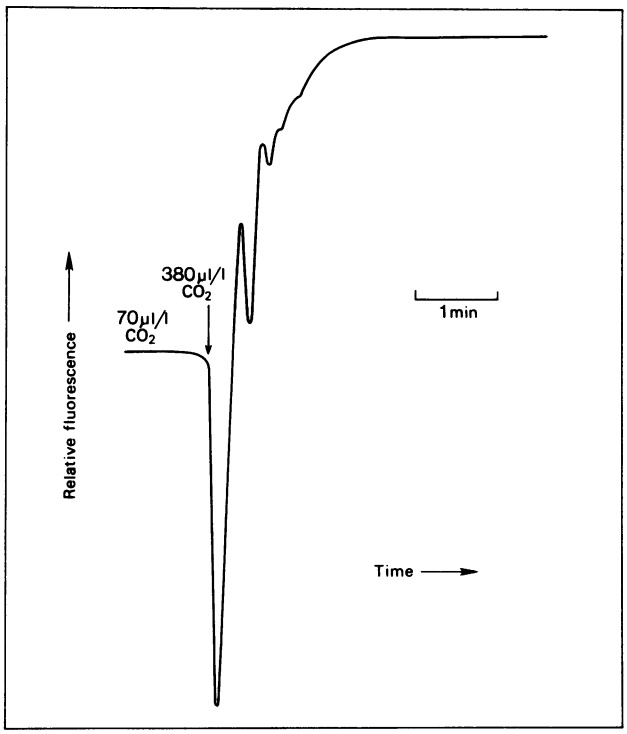

FIG. 5. Oscillations in Chl *a* fluorescence initiated, under constant illumination, by augmenting the CO_2 concentration. The increase of CO_2 concentration, under constant 20% O_2 from 70 µl/l to near the atmospheric level (380 µl/l) induced an initial fall (see Fig. 3 and also Refs. 21 and 22) followed by dampening oscillations superimposed on a relatively large increase in the steady state level of fluorescence.

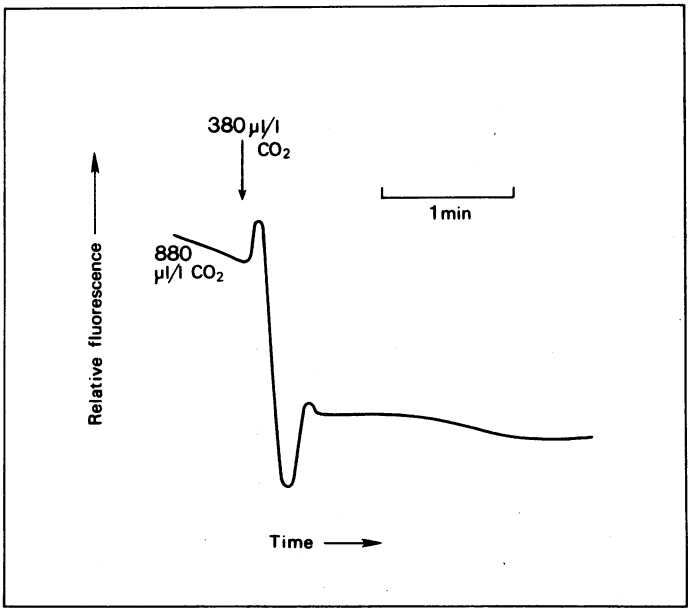

FIG. 6. Perturbations in steady state Chl *a* fluorescence level brought about by diminishing the CO_2 content of the gas phase (containing 2% O_2) from 880 to 380 µl/l. Conditions, otherwise, as in Figure 3.

O_2 from 2 to 20%. Again, high CO_2 concentrations favored oscillations initiated by decreasing the O_2 content of the gas phase, but were not a prerequisite. Oscillations can be initiated, albeit with some difficulty, by decreasing the O_2 concentration in constant air levels of CO_2 (not shown).

Further Interrelationships between O_2 and Fluorescence. In Figures 3, 5, 6, and 7 (above), fluorescence is shown without record of the corresponding changes in O_2 because (as indicated in "Materials and Methods") the appropriate gas mixtures were passed too rapidly through the leaf chamber in these particular experiments to permit accurate measurements of O_2. Nevertheless, in experiments in which the chamber was closed immediately after flushing with a new gas mixture (as in Fig. 4), O_2 oscillations could be followed and these seemed to bear approximately the same qualitative and quantitative relationships to the fluorescence signals as those initiated by re-illumination when the chamber was closed. Our general experience, derived from a large number (>100) of observations, suggests that in a given leaf sample, oscillations in O_2 and fluorescence can be equally as well initiated by any major perturbation (*e.g.* dark/light or low CO_2/high CO_2) and that when simultaneous O_2 or CO_2 measurements were precluded by practical considerations, the fluorescence signal could be regarded, with some confidence, as an indication of the changes in O_2 or CO_2 which would have been recorded had circumstances permitted.

The effect of lowered O_2 in the presence of high CO_2 was to extend the persistence of the signals and to increase the amplitude and the frequency of the oscillations. Thus, in Figure 8 the behavior of a single spinach leaf disc is followed first in 20% and then in 2% O_2 with the CO_2 constant at 5%. The fluorescence signals are compared in Figure 8A and the corresponding changes in the rate of O_2 in Figure 8B. The fluorescence transients which are observed during the first 5 s or so after re-illumination have features which are not the subject of this report. For ease of comparison, the fluorescence signals under 2% and 20% O_2 are normalized in Figure 8 so that the major secondary peaks coincide. The corresponding troughs in O_2 (*i.e.* the first troughs after the initial O_2 bursts) are similarly normalized in Figure 8B. It will be seen that the exaggeration of the oscillatory behavior in low O_2 (*i.e.* greater amplitude, greater frequency and greater extent) is, in this instance, as apparent in the O_2 signal as it is in the fluorescence signal. With the existing apparatus, however, the fluorescence signal is more readily amplified than the O_2 signal without the introduction of excessive electrical 'noise' and will then continue to indicate weak oscillations when these are not discernible in the O_2 trace.

Effect of Diminished Light. All of the preceding experiments were carried out at the same light intensity. When this was halved (Fig. 9), both the amplitude and the frequency of the oscillations were decreased. High light, as such, was not an essential requirement for the demonstration of oscillations. A change from air to a gas phase containing 5% CO_2 and 20% O_2 could still initiate oscillations in barley under light intensities as low as 10 $w \cdot m^{-2}$ (not shown).

DISCUSSION

It was our objective here to make two principal points: that the oscillations in photosynthesis which have been repeatedly observed over the last 50 years (5, 10, 14, 17, 19, 23, 24, 26, 29, 30) are readily reproducible phenomena and that, at least in certain circumstances, there is a clear antiparallel but phase-shifted relationship between Chl *a* fluorescence and photosynthetic carbon assimilation (29).

It is now widely accepted that there are at least two quenching mechanisms (q_Q and q_e; see Refs. 4, 8, 12, 13) which could be concerned in relating rapid changes in carbon assimilation to changes in variable fluorescence (21, 22, 27). An electron acceptor Q (8) was so called in the first instance because it quenches fluorescence (q_Q quenching) when it is in the oxidized state and therefore free to accept electrons. In this way, it connects with the consumption of NADPH in CO_2 reduction and, if CO_2 assimilation is unimpaired, NADPH and Q will be continuously reoxidized. Fluorescence is also quenched (q_e quenching) when

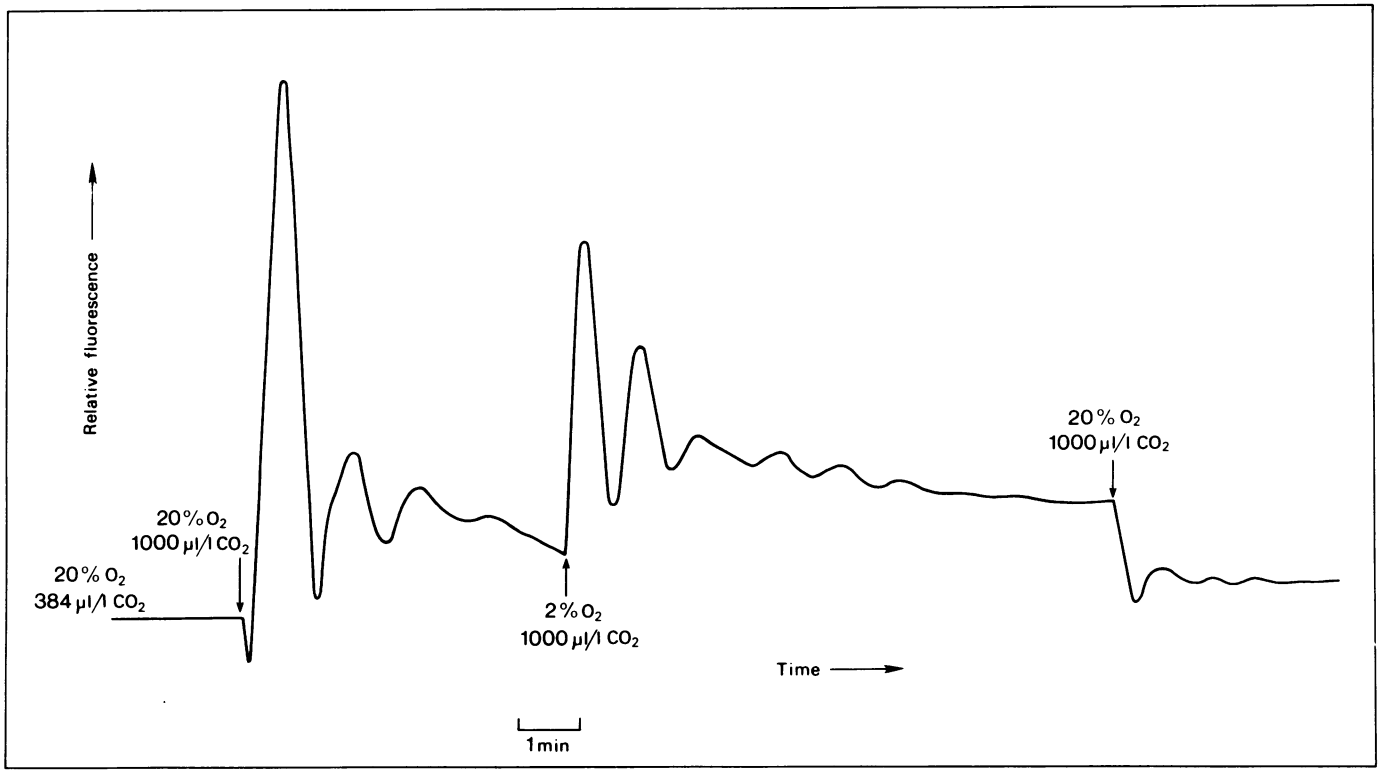

FIG. 7. Oscillations in Chl *a* fluorescence initiated, under constant illumination, by increasing CO_2 concentration or by diminishing or augmenting the O_2 concentration in the gas phase surrounding barley leaf pieces. When steady state fluorescence was achieved (see Fig. 3), cycles of dampening oscillations were induced by switching from the initial gas phase (20% O_2, 384 µl/l CO_2) to another with the same O_2 content but augmented (1000 µl/l) CO_2. Note the initial fluorescence fall, as in Figs. 3 and 5. Further cycles of dampening oscillations were induced under constant CO_2 concentration by diminishing (second cycle) or by augmenting (third cycle) the O_2 concentration as indicated.

the thylakoid membrane is energized and ΔpH is increased (4, 12, 13, 21, 22, 27). ATP consumption, which discharges the proton gradient, therefore tends to relax q_e quenching and, in its relation to carbon assimilation, this quenching mechanism normally acts in opposition to q_Q quenching (12, 21, 22, 27). Accordingly, when a leaf is transferred to CO_2-free air, fluorescence first rises as NADP and Q become reduced and subsequently falls to a value lower than the initial value because q_e quenching increases and predominates as shortage of ADP limits the discharge of the proton gradient (21). Inasmuch as all of the ATP and NADPH which is consumed in the reductive pentose phosphate pathway is utilized in three reactions, it is highly probable that these reactions are implicated in the oscillations and it has been proposed that the interrelationship between the two kinases (phosphoribulokinase and PGA kinase) may be crucial in this regard (6, 20–22, 27, 28).

The fact that the fluorescence signal often appears to anticipate the O_2 (or CO_2) signal by as much as 10 s (Figs. 1, 2, and 4 and Refs. 7 and 29) is of considerable interest. When illumination is restarted after steady state photosynthesis is interrupted by a very brief dark interval, O_2 evolution is detected, as in our system, within 2 s (7). This makes it most unlikely that diffusion of O_2 from the chloroplast to the electrode, or the electrode response itself, could account for more than about 25% of the observed delay between a change in fluorescence and a corresponding change in O_2 evolution. If, as we suspect, carbon assimilation is checked because a transient increase in ribulose-5-P opens an ATP sink and PGA phosphorylation cannot then proceed until the unfavorably low ATP/ADP ratio so created is corrected (6, 20–22, 27, 28), the two quenching mechanisms will eventually relax in concert. However, some time could elapse (depending on the size of the PGA pool) before the ATP/ADP ratio became sufficiently unfavorable to check di-PGA formation and NADPH reoxidation. Only then would the rate of O_2 evolution be diminished and only then would q_Q relaxation reinforce q_e relaxation. On the other hand, an increase in the degree of reduction of Q which causes q_Q to relax may not necessarily dictate a commensurate decrease in electron transport from water, via Q to P_{700} which, during unchanged excitation, would be more ready, if anything, to accept electrons from a more reduced plastoquinone pool. It may be assumed that some ferredoxin-catalyzed cyclic electron transport from PSI to plastoquinone (1) will occur in most circumstances and a fractional increase in this traffic might conceivably bring about some reduction of Q (and corresponding relaxation of q_Q) before electron transport from H_2O to Q is sensibly impaired. Thereafter, further decreases in the availability of NADP in its oxidized form would accelerate cyclic flow and diminish the flow of electrons from H_2O. Arnon and Chain (1) have reported a partial reduction of C-550 in the presence of ferredoxin and NADPH. (A bound plastoquinone has been identified as Q and its reduction gives rise to the C-550 absorption shift.)

Certainly if our measurements are what they seem, an explanation of the time lapse between the fluorescence and the O_2 signals is more likely to reside in steady state redox values and electron flow than in massive reduction of pools of electron acceptors (9). In most of our experiments, leaves displayed rates of O_2 evolution in excess of 100 µmol mg^{-1} Chl·h^{-1} (which is equivalent to about 100 nmol of electrons µmol^{-1} Chl·s^{-1}) and even the highest reported value for ferredoxin (15) would demand complete reduction of this pool in 0.25 s, at this rate. It is possible that the apparent phase shift of 10 to 15 s between the fluores-

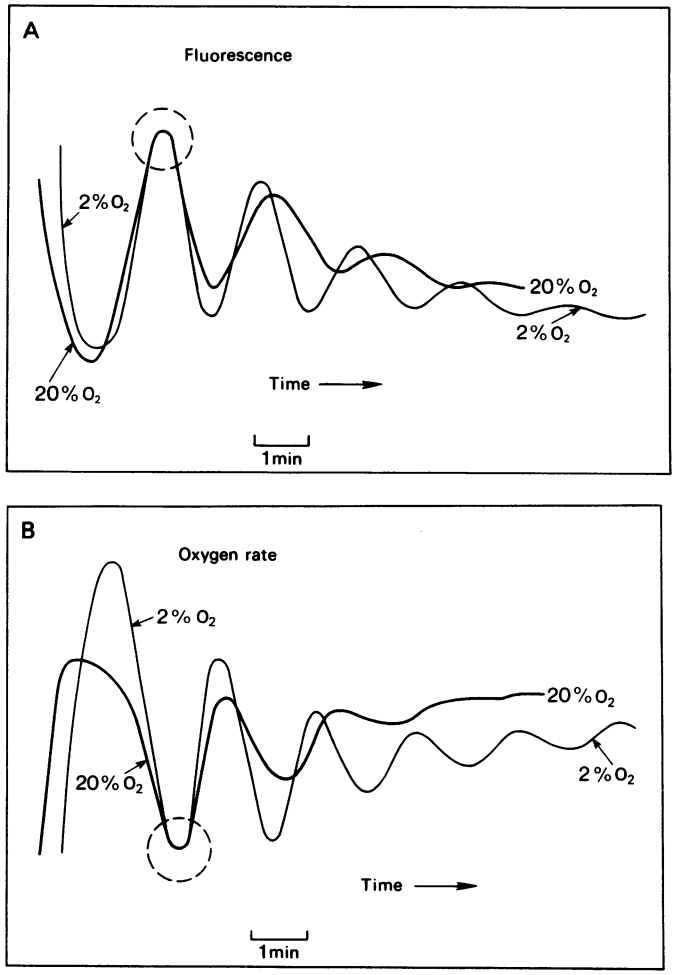

FIG. 8. Effect of the O_2 concentration on the characteristics of the oscillations in Chl *a* fluorescence and O_2 evolution rate. Oscillations were induced by re-illumination of a spinach leaf disc after a dark interval of 2 min during which the chamber was flushed with a gas mixture containing either 2% O_2 or 20% O_2 and 1000 μl/l of CO_2 to provide different starting O_2 concentrations, but the supply of CO_2 at a high and constant rate was ensured by using matting moistened with a carbonate/bicarbonate buffer (7). The fluorescence (a) and O_2 rate (b) signals are normalized so that the major secondary peaks coincide. This was done in order to show more clearly the effect of O_2 concentration on the oscillatory behavior without reference to the actual differences in fluorescence yield or in rate of O_2 evolution.

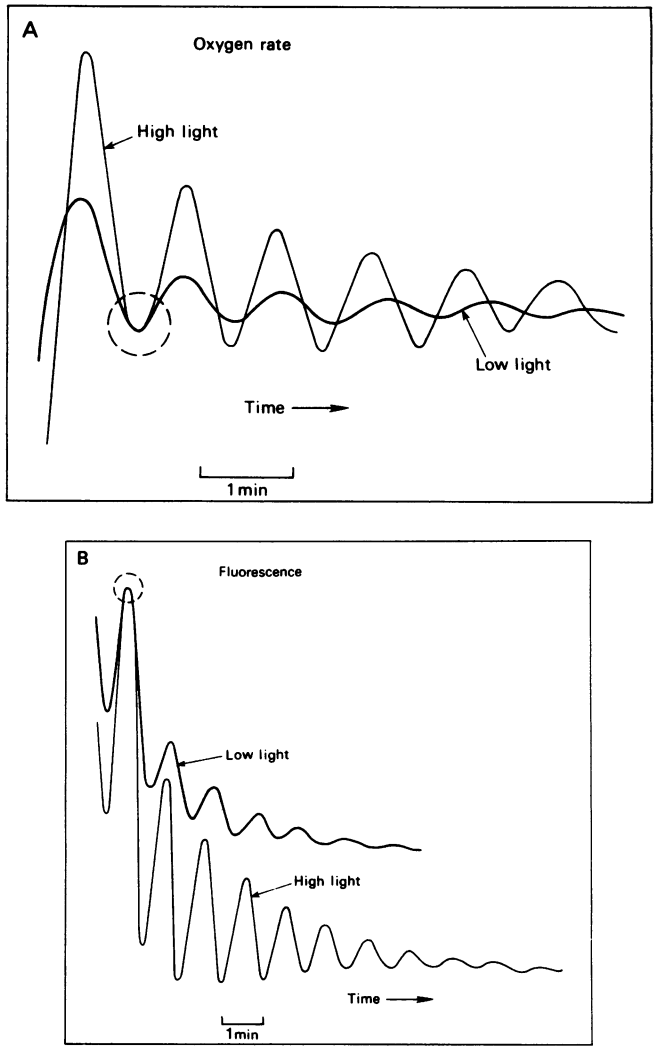

FIG. 9. Effect of light intensity on the oscillatory behavior of Chl *a* fluorescence and O_2 evolution rate. Oscillations were initiated by re-illumination of a spinach leaf disc at standard light intensity (high light) or 50% light intensity (low light) after a dark interval of 2 min under high CO_2 and 20% O_2. The O_2 evolution rate (a) and fluorescence (b) are normalized as in Figure 8.

cence and O_2 signals resides in the characteristics of the Clark type O_2 electrode which we have employed. We know that while the initial response of such electrodes is very rapid the speed of response can diminish significantly as the difference in partial pressure of O_2 between an initial and final value is approached (*e.g.* if the chamber is flushed with N_2, the O_2 signal declines very rapidly at first but much more slowly as the N_2 value is approached). Nevertheless, we are inclined to doubt that this electrode characteristic could account for all of the apparent phase shift because the oscillations in the rate of O_2 evolution can be very large initially and the frequency of the oscillations shows no tendency to decrease as the amplitude declines with time. If, however, the delays imposed by diffusion and the electrode characteristics were 4 to 5 times greater than our present estimate, the fluorescence and O_2 signals would be almost exactly reciprocal.

The initiation of oscillations by re-illumination and/or by changes in the gas phase is consistent, as is other evidence presented elsewhere (21, 22, 27, 29), with the view that many slow perturbations in fluorescence kinetics and/or O_2 evolution can derive from an imbalance introduced into the regulatory controls that govern carbon assimilation. An increase of CO_2 concentration (Figs. 3–5) would cause an increase in the availability of PGA and a consequent surge of ribulose-5-P. There is ample evidence that the reaction catalyzed by ribulose-5-P kinase can, by acting as an ATP sink, affect that catalyzed by 3-P-glycerate kinase (see *e.g.* Ref. 9). If ribulose-5-P increased, the ADP concentration would also increase. This, in turn, would be followed by a decrease in NADPH consumption and O_2 evolution as the increase in ADP slowed the PGA kinase reaction (6). Readjustment of ATP/ADP ratios, by cyclic or pseudocyclic electron transport during this period, would then reverse the entire sequence (6). Evidence in support of these proposals has recently been published by Horton (11) who showed, using the reconstituted chloroplasts system, that the addition of ribulose-5-P during PGA-dependent O_2 evolution caused an increase in fluorescence and a decline in ΔpH both of which anticipated the interruption of O_2 evolution brought about by the creation of an unfavorably low ATP/ADP ratio.

At limiting levels of CO_2, a decrease in O_2 to 2% (see "Results") would presumably be as effective in increasing the availability of PGA as an increase in CO_2 concentration but at 1000 μl/l of CO_2 and greater, photorespiration is unlikely to be rapid. The initiation of oscillations by changes in O_2 concentration under high CO_2 levels and the rapidity of this initiation is also of interest and points more to a role of O_2 as an alternative acceptor to CO_2 than to an effect on photorespiration. At air levels of CO_2, a decrease in O_2 to 2% would presumably be as effective in increasing the availability of PGA as an increase in $[CO_2]$ but at 1000 μl/l CO_2 and greater, photorespiration is unlikely to be rapid and the factors invoked by Canvin (5) to explain the inhibition of photosynthesis by decreased O_2 (see also Refs. 10, 17, and 25) may well be important. These include ATP production via pseudocyclic phosphorylation and promotion of cyclic electron transport by the maintenance of electron carriers at a suitable redox level. In the present experiments, oscillations were initiated not only by the procedures used by other workers (5, 10, 14, 17, 19, 23, 30) but also by making many other changes. In particular, we found that, although high light intensity, low O_2, and high CO_2 concentrations favored oscillations, they were not a prerequisite. Clearly, any explanation of the oscillatory behavior of photosynthesis and/or fluorescence has to accommodate this broad range of experimental conditions.

While some of our results are in accord with those of Ogawa (19), others do not support his inference that oscillations can only be observed in low O_2 nor are we in agreement with his views about the relationship between O_2 evolution and Chl *a* fluorescence which we believe, as stated above, to be antiparallel but slightly out of phase.

Moreover, Ogawa (19) states, "The absence of a complementary relationship between the rate of O_2 evolution and fluorescence yield indicates that the observed fluorescence cannot be regarded as an inefficiency index of photosynthesis ... " This, we believe, is too sweeping. It is true that there may be circumstances in which such an inverse relationship does not hold (3, 18) and, particularly in the first seconds of illumination (which did not concern us here), changes in fluorescence are determined more by the photochemistry than the reactions of carbon assimilation. Nevertheless, we have been impressed by the fact that there is often a very close, essentially reciprocal or antiparallel relationship between the slow fluorescence signals and photosynthetic carbon assimilation whether the latter is measured by O_2 evolution or CO_2 fixation. This, together with the fact that this reciprocal relationship appears to hold even when no oscillations are observed (or after oscillations have subsided) implies that, at least in well defined circumstances, it may well become possible to use fluorescence as a measure of photosynthetic performance.

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