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COMPLEX MEASUREMENTS OF PHOTOSYNTHESIS IN SUNFLOWER

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SUMMARY

The results are presented on the simultaneous measurements of photosynthetic oxygen evolution and chlorophyll fluorescence in the leaves of sunflower (Helianthus annuus L.) by the application of a modified oxygen electrode. Complex kinetics of oxygen evolution and chlorophyll fluorescence were measured in the presence of saturating CO_2 as a function of light intensity. Leaves were studied at different stages of plant development. The data on potential maximum rates and quantum yield of photosynthetic oxygen evolution for some wild varieties, parental lines and NS sunflower hybrids were obtained. The early effects of photoinhibition on photosynthetic reactions were detected. Complex measurements can give some important informations on photosynthesis in the course of plant development and yield formation.

INTRODUCTION

Complex measurements of photosynthesis have recently contributed to better understanding of regulatory mechanisms of photosynthesis and to an early detection of disorders in plant metabolism caused by stress, high or low temperature, drought, diseases and chemicals (Renger & Schreiber, 1986). By simultaneous measurements of gas exchange (CO_2 and O_2) and chlorophyll a fluorescence in the leaf, isolated protoplasts and chloroplasts, the data were obtained on the interaction of photochemical and biochemical processes and transport mechanisms in photosynthesis (Walker, 1987).
A fraction of the light absorbed by green plants, that has not been used in photosynthesis, is reemitted as chlorophyll fluorescence. Upon illumination chlorophyll fluorescence yield changes in a complementary way to the photosynthetic oxygen evolution rate. The rapid fluorescence transients reflect reactions close to the primary photoreactions, whereas the slower transients express the contribution of dark processes.
The purpose of this work is to describe experimental approach and to indicate what kind of information with practical relevance may be obtained. The basic principles of simple setup for simultaneous measurements of oxygen evolution and chlorophyll fluorescence in leaf discs will be briefly outlined.

MATERIALS AND METHODS

Plants: Helianthus annuus hybrid NS-H-43, H.annuus L-RHA-SNRF x L-13B, H.annuus (wild) and H.occidentalis were grown in the field (Summer) and in the greenhouse (Winter).

Figure 1. presents a schematic diagram of modified oxygen electrode (LD-2 Hansatech, King's Lynn,U.K.) for simultaneous measurement of O_2 exchange and chlorophyll fluorescence in leaf discs, designed by Delieu and Walker (1983). The chamber, in which we put the leaf disc and carbonate/bicarbonate buffer carried on capillary matting, is located in the middle section of the apparatus. Beneath the leaf chamber is the O_2 sensor with its Pt cathode exposed to the atmosphere within it. Actinic light is delivered to the top of the apparatus from the Björkman lamp (LS2 Hansatech). Fluorescence detector is the photodiode, inserted at an angle of 40°, which is protected from the actinic light by red filter. The apparatus, which is enclosed in an Al case, is kept at constant temperature (25°C) by circulating termostated water. The electrical signal from O_2 electrode and photodetector must be amplified and captured on a data recording system which is capable of resolving the fluorescence transient of interest (Figure 2.).

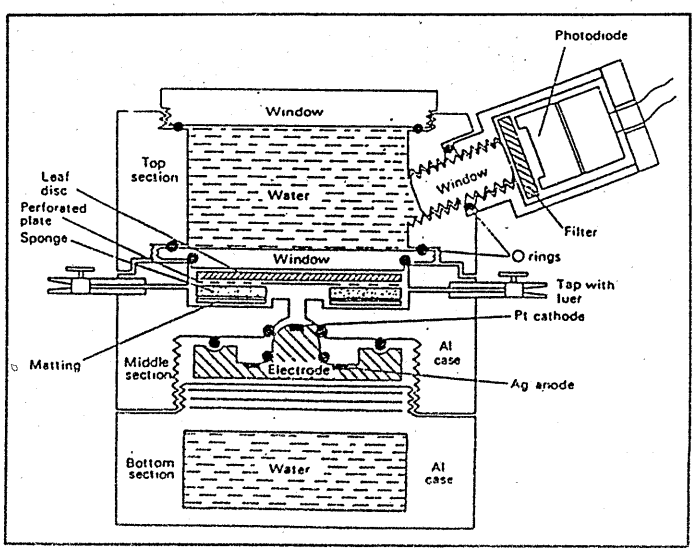


FIGURE 1. Schematic diagram of a leaf disc oxygen electrode and fluorescence probe.

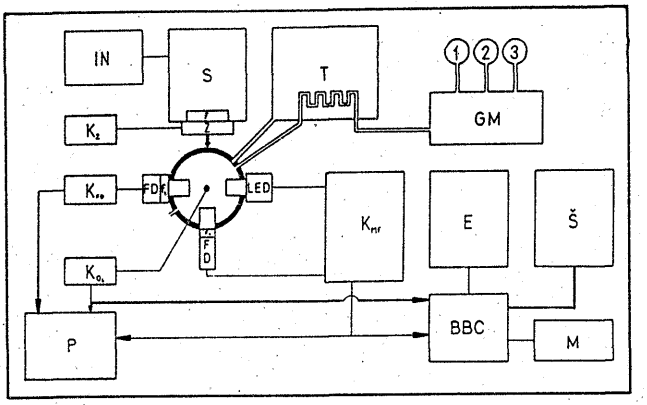


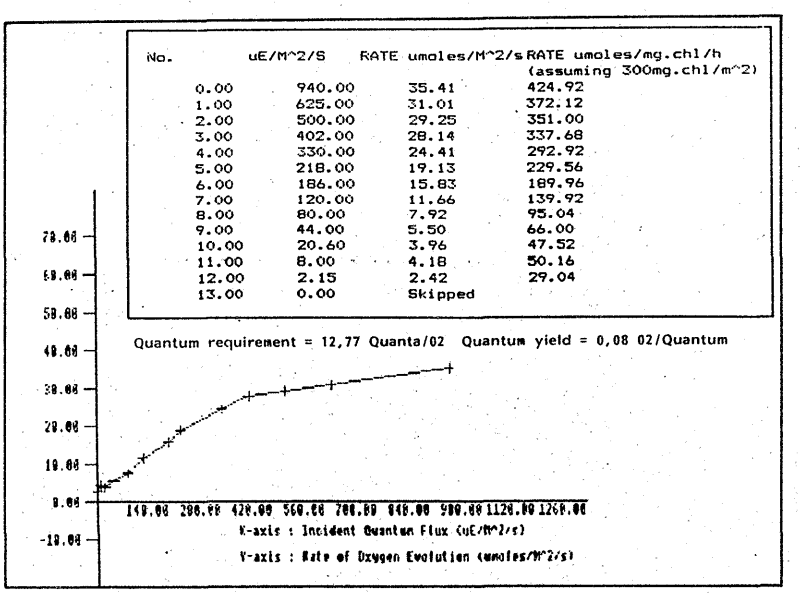
FIGURE 2. Block diagram of the setup for simultaneous measurement of O_2 evolution and chlorophyll a fluorescence kinetics.

S, actinic light source; F, blue filter; Z, shutter; K_z, shutter control unit; IN, power supply or 12V car battery; K_{02}, control box which provides a stable polarizing voltage of 700mV for the electrode and amplifies the output voltage from the electrode; FD, photodetector; F_2 red filter; K_{FD}, amplifier for the photodetector signal; P, recorder; LED, light emitting diodes, source of modulated light; K_{MF}, lock-inamplifier for modulated fluorescence signal; BBC, micro computer; E, ecran; S, printer; M, memory; GM, gas blender; T, termostat with pump.

Following experimental procedure was applied (Walker & Osmond, 1986): A leaf disc (10 cm^2) was placed in the chamber on a disc of a damp fibre matting, the chamber was closed and darkened. After the calibration (approx. 20 min in the dark) 5% CO_2 was introduced and fluorescence and O_2 evolution were measured during 5 min period of illumination with blue light. Illumination was interupted for 1 min (dark) and continued for further 5 min, to establish a steady rate of O_2 evolution and reproducible fluorescence oscillation pattern. After 2-5 min in the dark, the leaf disc was illuminated with white light of increasing intensities (previously calibrated photon flux density) in order to obtain the light response curve. Partially computerised measurements of quantum yield and maximum rates of O_2 evolution were performed by the application of BBC microcomputer and the computer programme made in the Research Institute for Photosynthesis in Sheffield, U.K.

RESULTS AND DISCUSSION

Light response curve for photosynthetic oxygen evolution by leaf disc of sunflower plant (H.annuus L-13B) grown in the greenhouse is shown in Figure 3. The examined leaf approached light saturation rate of net photosynthesis at about 400 µmol quanta $m^{-2}s^{-1}$. Quantum yield of photosynthesis was 0.08 µmols O_2 per µmol of incident photosynthetically active radiation. The results of simultaneous measurement of photosynthetic O_2 evolution and chlorophyll fluorescence on the same leaf disc indicate an inverse relationship (Fig. 4 & Fig. 5). Kinetics of fluorescence quenching depends on the incident photosynthetic flux density, on the light/dark pretreatment of the leaf and on the physiological status of the leaf. In our experimental conditions, under saturating CO_2, oscillations in chlorophyll fluorescence can only be obtained when sunflower leaves are illuminated with blue light of 830 µmol quanta $m^{-2}s^{-1}$, i.e. saturating light intensity (see Fig. 3). These oscillations are a sensitive indicator of oscillations in photosynthetic O_2 evolution rate, or CO_2 fixation rate (Walker et al, 1983) (Fig. 5).



No.	uE/M^2/S	RATE umoles/M^2/s	RATE umoles/mg.chl/h (assuming 300mg.chl/m^2)
0.00	940.00	35.41	424.92
1.00	625.00	31.01	372.12
2.00	500.00	29.25	351.00
3.00	402.00	28.14	337.68
4.00	330.00	24.41	292.92
5.00	218.00	19.13	229.56
6.00	186.00	15.83	189.96
7.00	120.00	11.66	139.92
8.00	80.00	7.92	95.04
9.00	44.00	5.50	66.00
10.00	20.60	3.96	47.52
11.00	8.00	4.18	50.16
12.00	2.15	2.42	29.04
13.00	0.00	Skipped	

FIGURE 3. Rate of O_2 evolution versus photon flux density. The initial slope of this plot provides an uncorrected measure of quantum yield.

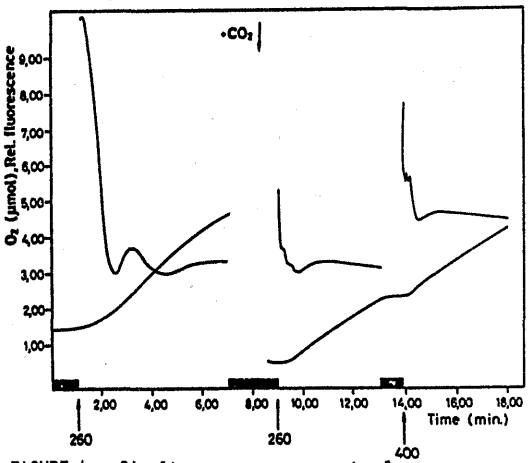


FIGURE 4. Simultaneous measurement of oxygen evolution and chlorophyll fluorescence in sunflower leaf. Excitation light (260 & 400 µmol quanta $m^{-2}s^{-1}$) was obtained with a blue filter.

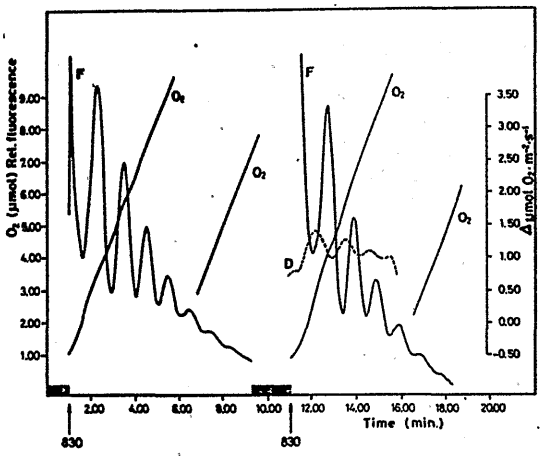


FIGURE 5. Dampening oscillations in the rate of O_2 evolution (D) and chlorophyll fluorescence upon illumination of leaf disc (830 µmol quanta $m^{-2}s^{-1}$) in the presence of 5% CO_2.

Photosynthetic acitivities and fluorescence quenching curves obtained under identical condition can differ appreciably, even if taken from the same leaf (Fig. 6) or the same plant (Fig. 7), depending on the developmental stage of the leaf and plant, and the local light intensity (Nesterenko & Sidko, 1986). Sample taken from the middle part of the leaf, with the highest maximal rate of photosynthesis, was used routinely for comparative analysis of different leaves and plants. When leaves 6, 11, and 18 of NS hybrid 43 were compared in the stage of flower bud, the highest potential rate of photosynthesis was detected with the leaf 11, representing central portion of the plant, the lowest with the leaf 6, which had the lowest light saturation point too (Figure 7). Leaf 18 had the highest light saturation point, which was due to its immaturity and to the relatively high light intensity at the level of the leaf in the field.

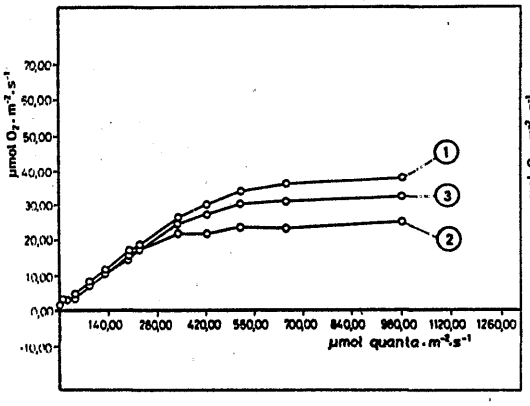


FIGURE 6 Light response curves of photosynthetic O_2 evolution at CO_2 saturation in different regions of the leaf: (1) middle, (2) tip, (3) basis.

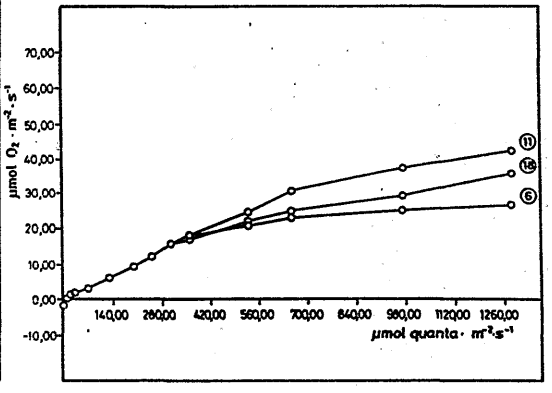


FIGURE 7. Light response curves for leaf 6, 11, and 18 of hybrid NS-H-43 in the stage of flower bud.

The rates of photosynthesis reported in this paper, obtained by O_2 evolution measurements under non-limiting conditions, support previous evidence obtained for sunflower leaves by infrared gas analysis (English et al, 1979; Rawson & Constable, 1980).

The effect of leaf position on fluorescence quenching curves was studied in sunflower line RHA-SNRF, in three stages of plant development (Figure 8). Fluorescence quenching was the fastest in the oldest

leaf (14) and the slowest in the youngest leaf (25). Leaves 16 and 19 were young and, therefore, they had slow fluorescence decay kinetics in the stage of flower bud, but they were older and had faster fluorescence decay kinetics in the stage of anthesis. Leaves 14, 16, and 19 in the stage of first anthesis show characteristic "SMT" kinetics (Lavorel & Etienne, 1977) before reaching steady state leavel of fluorescence. Leaves 23 and 25 exhibit very similar fluorescence decay kinetics characteristic of immature leaves.

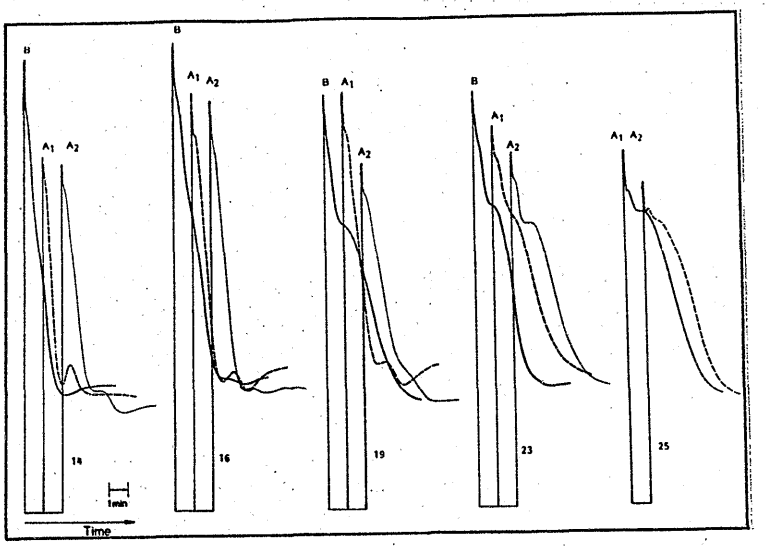


FIGURE 8.
The effect of leaf age and position on fluorescence quenching kinetics. Observations were made with the leaves 14,16,19,23, and 25 in the stage of flower bud (B), first anthesis (A_1) and last anthesis (A_2) of H.annuus L-RHA-SNRF.

Figure 9. presents the results on simultaneous measurements of photosynthetic O_2 evolution and modulated fluorescence quenching in three different genotypes: H.annuus NS hybrid 43, H.annuus (wild) and H. occidentalis. Modulated fluorescence is generated in the leaf by pulsed diodes emitting low-intensity yellow radiation and is detected with a photodiode whose output is fed to an amplifier locked in to the frequency of the light-emitting diodes (Fig. 2) /Ogren & Baker, 1985). The observed differences in light response curves and in fluorescence quenching kinetics are the result of both genotypic and phenotypic differences in the examined plants (see also the paper by Saftić et al, in this book).

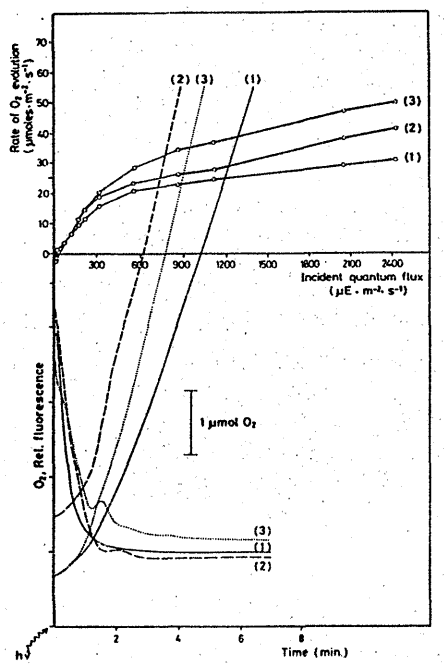


FIGURE 9. Kinetics of modulated fluorescence emission and oxygen evolution from a leaf disc exposed to weak, modulated yellow light and 1200 μmol quanta $m^{-2}s^{-1}$ of blue-green actinic light: (1) H.occidentalis, (2) H.annuus (wild), (3) H.annuus hybrid NS-H-43. Corresponding light response curves are presented in the upper part of the figure.

CONCLUSIONS

Fluorescence is shown to be a very sensitive probe of physiological status of the leaf and of variation in the plant environment. It is confirmed that there is a relationship between carbon assimilation (O_2 evolution) and chlorophyll *a* fluorescence. During oscillations in photosynthesis there is a broadly reciprocal relationship between fluorescence and O_2 evolution rate. Oscillations are an exaggerated expression of regulatory processes which act in photosynthesis. By studying oscillations in fluorescence, O_2 evolution rate and the level of some Calvin cycle metabolites and products we hope to learn more about limitations of photosynthesis and possibilities of its regulation.

A portable instrument is available, from Hansatech Ltd., U.K. for the generation and measurement of modulated chlorophyll fluorescence signals from leaves exposed to white light of high intensity. (Ogren & Baker, 1985). The information obtained from fluorescence measurement can be improved by defining its relationship to more direct assay of photosynthesis. After such calibration, the fluorescence method will be a reliable tool of great value because it is rapid, nondestructive, rather easily performed and of high sensitivity (Renger & Schreiber, 1986; Schreiber *et al*, 1986).

Simultaneous measurements of different aspects of photosynthesis will provide in the future more information on the potentials and limitations of photosynthesis and on possibilities of its regulation. This should help the plant breeder to choose a variety with higher potential productivity.

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