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A Laboratory Method for Monitoring Net Photosynthesis in Leaf Segments under Controlled Water Stress Experiments with Sorghum*

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Abstract

A method for monitoring net photosynthesis (P_N) in leaf segments by sensing oxygen evolution with a polarographic oxygen electrode in a closed system is described. Carbon dioxide is supplied by a carbonate-bicarbonate buffer as employed in Warburg manometry. The method incorporates an option for relative humidity (*RH*) control using saturated salt solutions and a *RH* sensing device for maintaining a standard atmospheric water stress on the leaf at the time of measurement. — Experiments revealed that sorghum (*Sorghum* sp.) varieties differed in P_N at both high and low leaf water stress as obtained by varying *RH*. Sorghum was found to have reduced P_N at leaf water potential below about − 5 to − 6 bar. The effect of *RH* on P_N rates was dependent on incident irradiance.

This study was initiated by interest in *in situ* measurement of the effect of controlled leaf water stress on net photosynthesis (P_N) and differences in this respect between sorghum (*Sorghum* sp.) varieties. The common methods for measuring P_N rate of a leaf in a chamber by monitoring CO_2 levels at the in- and outgoing air streams have some flaws. These systems do not maintain a constant CO_2 concentration in the leaf chamber, since air should be dried out prior to entering the infra-red gas analyser. *RH* control inside the leaf chamber is complicated, and these systems are bulky and expensive.

In pursuit of a simple, compact and inexpensive method for control and measurement, the polarographic oxygen electrode was used as part of a closed system to measure P_N rates by evolved oxygen at a constant CO_2 concentration. The development of this method was stimulated by previous experiments with the oxygen electrode in isolated chloroplast suspensions (SULLIVAN and LAVIN 1969) and the closed system principle underlying P_N measurement on leaf segments as employed in Warburg manometry*** (DUNNETT, BEARD and STAUFFER 1964, JEFFREY, COOPER and LAVIN 1968).

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*** BLUM, A. — Unpublished data with *Sorghum bicolor* and *S. sudanense*.

MATERIALS AND METHODS

Instrumental

The closed syste[illegible] 0·1 °C) plexiglass water bath, excluding the pump and flow meter. Air circulated by the pump passed through the following components: a carbonate-bicarbonate buffer solution provided a constant concentration of CO_2 (UMBREIT, BURRIS and STAUFFER 1964); a saturated salt solution (OBRIEN 1948; MEILES 1963) served to control humidity of air flowing into the leaf chamber; a trap cell served to trap any carry-over of salt solution; a relative humidity sensor connected to a *RH* indicator; leaf chamber; oxygen electrode; and back to pump. All tubings in the system were 1·56 mm I. D. copper tubes with tygon tube sections as connectors.

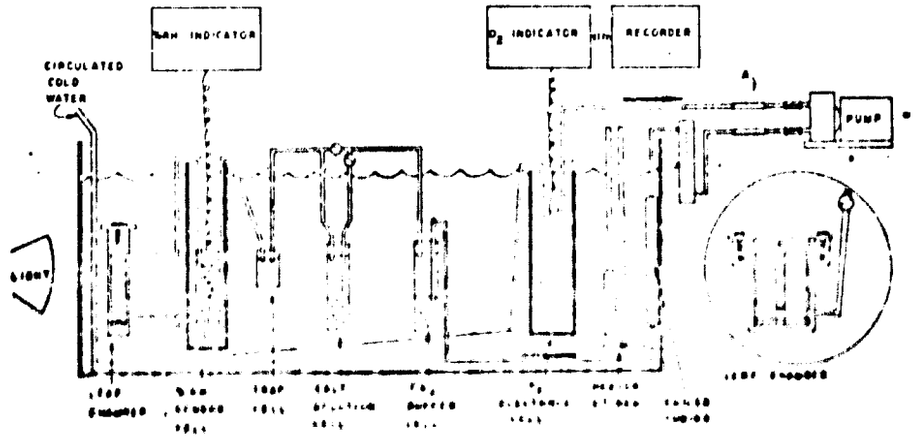

Fig. 1. System layout including water bath, pump, oxygen electrode cell and readout, CO_2 buffer cell, salt solution cell and *RH* readout, trap cell, leaf chamber and frontal view of leaf chamber (insert).

The saturated salt solution was $MgCl_2 \cdot 6H_2O$. Carbon dioxide content of air coming into and going out of the salt solution was checked by gas chromatography several times. It was thus found that for the ambient conditions of these experiments the salt solution did not have an appreciable effect on CO_2 concentration of the air. Tubing connections to the salt solution cell included two screw clamps which served to control the amount of air either passing or bypassing the solution. Manipulation of these screw clamps allowed regulation of the *RH* of the air entering the leaf chamber.

The *RH* sensor cell enclosed a *Hygrodynamics* wide range model [illegible] sensor with a nominal error of [illegible] *RH*. Since it consisted of several lithium chloride impregnated rods, each sensitive to a different humidity range, care was taken to assure a good distribution of the incoming air stream around the sensor. This was achieved by directing the incoming air stream towards the base plate of the sensor. In order to avoid possible spoilage of the sensor due to water leaks, this cell was placed inside a copper cylinder plugged with a glass wool ball. Temperature inside the cylinder did not vary from that of the water. The *RH* sensor's calibration curve, as supplied by the manufacturer, was checked in situ with various saturated salt solutions (Oubrien 1948, Meiles 1963) in the closed system, and found to hold also for the new configuration.

The leaf chamber was made of clear plexiglass (Fig. 1 — insert). Two leaf sections, measuring 2 × 8 cm each, were inserted vertically with their base (2 mm) dipped in deionized water. Deionized water was always injected through the stoppered side arm, thus avoiding wetting of the cell walls or upper parts of the leaves. The side arm served also to replenish water during a long experiment, without having the chamber and its tubings disassembled. Since sorghum leaf sections were slightly curved, they never adhered to the chamber's walls, and a free air passage was feasible over both leaf surfaces. A leaf holding frame may be necessary for some leaves in order to assure free air circulation.

The oxygen electrode cell had a relatively small volume (5.7 cm^3) and the incoming air stream was directed towards the electrode's tip. A *Beckman 39559* polarographic oxygen sensor was used in conjunction with the "*Tidlab*" oxygen analyser. The electrode uses a liquid salt bridge and is covered with a small teflon disc membrane. It was found that a layer of electrolyte gel (*Beckman No. 76438*) under the membrane, and use of a larger teflon membrane (*Beckman No. 77948*) facilitate the use of the electrode in gas. Analyser output was recorded with a *L & N "Azar"* strip chart potentiometer having an expanded range provision. Previously, the electrode tip had been immersed in water, and the incoming air stream was bubbled and mixed (magnetic stirrer) in the water. This method was less satisfactory since a constant watch was required, against bubbles adhering to the electrode tip. Furthermore, response time was slower.

The pump used was a *Cole Parmer Barton Diaphragm model 7064*, inspected and sealed against minute air leaks. Flow rate was reduced and stabilized by inserting glass capillaries into the pump's outlet and inlet tubes. The outlet was connected to a flow meter. Flow rate was constant, and air having on all liquids was given but not repeated as to cause overflow from one cell to another.

Air coming into the bath passed through a coiled copper tubing which allowed a fast temperature equilibration. Sensitivity of the assay to evolved oxygen was expected to decrease with an increase in the system's volume. Total gas volume of this system was 13[illegible] cm^3. The maintaining of a stable CO_2 level within the system depends on its relationship between the amount of CO_2 buffer, flow rate, the total system's volume and the rate of CO_2 fixation by the enclosed leaf tissue. With the specifications described here, and [illegible] of CO_2 buffer, a fairly stable CO_2 level was maintained, as checked by gas chromatography.

Incident irradiation was provided by one of two [illegible] halogen lamps. Irradiation of the leaf surface was regulated by the number and distance of lamps from the leaf chamber. Photosynthetically active radiation was measured with a photometer (Bruce et al. 1970) placed at the same leaf-to-lamp distance, with appropriate amounts of water and plexiglass interposed.

Procedure

All measurements were initiated with a level of 10% oxygen in the system. Any air leaks would therefore be recognized immediately by a very fast increase in oxygen reading, especially at the dark. After placing the leaf sample and sealing, the system was flushed with 10% oxygen gas by disconnecting the tube at point A in Fig. 1 and supplying the gas to pump inlet. Flushing took about 3 min and a constant oxygen reading was reached. At this point the pump was stopped, gas supply disconnected and tubing at point A connected. This procedure was monitored very carefully so as to avoid any changes in pressure relative to atmospheric pressure at the time inside the closed system.

The maintenance of any reduced level of CO_2 was achieved by manipulating the soda solution cell. Two ways were used to determine the proper volume of solution needed to maintain the solution. With some experiments, a comparison to the rate of [illegible] CO_2 of respiration was measured. If the evolution from high to low levels is required during an experiment, the appropriate soda solution has to be selected and the above manipulating procedure is unnecessary.

The modified experimental procedure was to equilibrate the system with the leaf sample for 15 min after closing and then measure oxygen exchange for 30–45 min. After switching

to a different *RH*, a 10 min equilibration period was allowed before measuring. A reduction in *RH* from 90 to 51% took about 5—10 min with a fresh salt solution.

It was found that CO_2-buffer solution lost activity in 1—2 d, so a new mixture from carbonate and bicarbonate stock solutions was made and changed on each experiment run.

Calibration to P_N rates as $\mu l\ h^{-1}\ cm^{-2}$ of oxygen involved calibration of the electrode with pure oxygen gases (0, 0·9, 1·9 and 10% oxygen) at a flow rate of 1·35 l min^{-1}, and the determination of system air volume and leaf segments area. This flow rate was maintained in all experiments.

Water potential of the leaf segments, after being subjected to a certain *RH* stress, was determined directly after completing the measurements. Four 1-cm^2 samples from each leaf segment were placed in a psychrometer cup. A "*Spanner*" type thermocouple psychrometer, patterned after Box (1965a, b), was used.

Plant material used in all experiments was sampled from plants, 30—50 d after emergence. The top fully expanded leaves were sampled, excluding the midrib. Plants sampled for experiments presented in Fig. 2 were grown in the growth chamber (20 °C day, 25 °C night, 70 ± 5% *RH* and 0·10 μeinstein $cm^{-2}\ s^{-1}$ incident irradiance). In all other experiments field grown irrigated plants were utilized.

RESULTS AND DISCUSSION

It was found that at 25 °C sorghum leaf sections from plants 35—50 d from emergence, maintained a constant oxygen evolution within the system for at least 2 h. After 3·5 h oxygen evolution was reduced to 6% of the initial rate. This reduction is not considered to result from insufficient sink for photosynthate since a 0·5 h period of dark did not promote an increase in oxygen evolution rates, thereafter.

The effect of various CO_2 levels (obtained with various carbonate/bicarbonate mixtures) and incident irradiance is presented in Fig. 2. A CO_2-light interaction effect on P_N was clearly in evidence. The combinations of 0·14 μeinstein $cm^{-2}\ s^{-1}$ and 550 ppm* CO_2 were employed in most sorghum experiments herein.

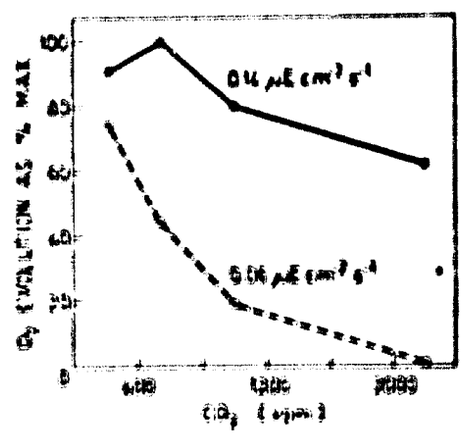

Fig. 2. The effect of CO_2 concentration [illegible] on the [illegible] of sorghum leaf segments at two incident irradiances.

The effects of low *RH* stress on P_N of two sorghum genotypes were very clear (Fig. 3). For the sake of differential elucidation, *RH* stress was estimated as the corresponding osmotic deficit values (0, [illegible] of 40, 50, 60, 70, 80 and 90 percentages with equivalent deficits of [illegible]). At high *RH* and high irradiance, [illegible] of P_N, while [illegible] the same P_N at low irradiance. At high irradiance [illegible] the approximately [illegible] P_N even [illegible] at all levels of stress. Although [illegible] decreased to [illegible], *RH* as [illegible] to [illegible] as compared to [illegible] at [illegible]

* ppm = volume per million = 10^{-6}

in RS-610, P_N was reduced to 26·3% and 8·4% of maximum rates, in M-35-1 and RS-610, respectively. At low irradiance, relative differences were also prominent, where P_N at 51% *RH* was reduced to 73·6% and 20·6% of maximum rates, in M-35-1 and RS-610, respectively. It was therefore evident that, irrespective of irradiance, M-35-1 variety was superior, both absolutely and relatively, over RS-610, in *NP* rates under water stress. When the percent reductions in *NP* with low *RH* stress are compared between the two light regimes, it can be seen that high incident irradiance intensifies the effect of *RH* stress on P_N reduction.

P_N was determined (at 30 °C) at two levels of *RH* (or saturation deficit) stress in several sorghum varieties, each representing a different varietal group, *i.e.* Durra, Shallu, Hegari, Feterita, Milo (Table 1). Although at least six determinations were made in each variety, error variance was still fairly high. Still, it was found that varieties differed significantly in P_N rates at high and low *RH*. Hegari stood out in high P_N at both high and low *RH*, and was least reduced relatively in P_N at low humidity in spite of an appreciable drop in leaf water potential. Feterita seemed to be fairly susceptible to water loss from the leaf segments, whereas Dwarf Yellow Milo appeared to maintain a high level of hydration in spite of the low *RH*. These are only indications which should be verified with further experiments.

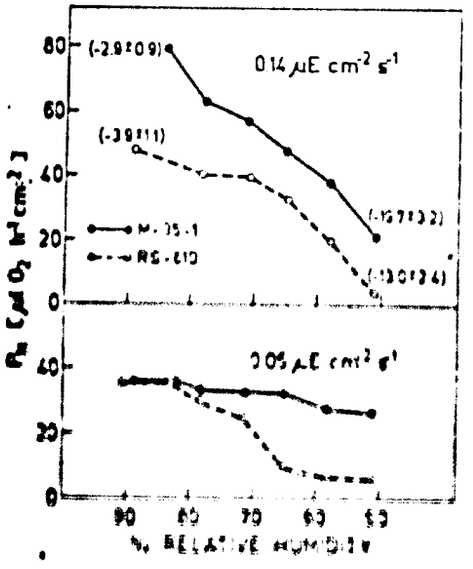

Fig. 3. Oxygen evolution in two sorghum varieties as affected by % *RH* at two incident irradiances (Numbers in parentheses indicate leaf water potential in bar at highest and lowest % *RH*).

[illegible]

[illegible]

Table 1

Effect of % *RH* (or saturation deficit) on oxygen evolution in five sorghum varieties (20 min in each *RH*, temperature of 30°C and irradiance of 0·14 μeinstein cm^{-2} s^{-1}).

Variety	Oxygen evolution [μl h^{-1} cm^{-2}] at *RH* [%] 90 ± 3 and air saturation deficit [mbar] 4·24 ± 0·14	38 ± 3 and air saturation deficit [mbar] 26·30 ± 3·34	38 as % of 90	Leaf water potential [bar ± S.E.]*
Dwarf White Durra	79·9	46·7	58	— 6·0 ± 2·5
Shallu	119·8	29·7	25	— 7·2 ± 2·6
Hegari	182·0	132·0	73	—11·3 ± 4·1
Feterita	121·2	44·8	37	—14·8 ± 3·0
Dwarf Yellow Milo	83·0	45·2	55	— 5·0 ± 1·1
L.S.D. (p = 0·05)	52·4	32·8		

* Determined after measurement at 38% *RH*.

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