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AN ASSEMBLY FOR THE CONTINUOUS RECORDING OF CO_2 EXCHANGE AND TRANSPIRATION OF WHOLE PLANTS

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Summary. Photosynthesis, respiration and transpiration of whole plants are continuously recorded with the assembly described. An infra-red gas analyser is used for photosynthesis and respiration measurements, while for transpiration data psychrometers are employed. Air and leaf temperatures as well as light intensity are recorded. The assimilation chamber is constructed in such a way that whole plants of various plant species can be investigated under constant conditions of CO_2 content of the air and air humidity. Some experiments with simazine and monuron are described.

Un appareil pour enregistrer continuellement l'échange de CO_2 *et la transpiration des plantes entières*

Résumé. La photosynthèse, la respiration et la transpiration des plantes entières ont été enregistrées continuellement avec cet appareil. Un gazoanalyseur à l'infrarouge a été utilisé pour mesurer la photosynthèse et la respiration, pendant que la transpiration est mesurée avec des psychromètres. Les températures de l'air et des feuilles sont aussi notées. On peut faire usage d'espèces diverses dans les essais, qui sont praticables avec des conditions constantes de la teneur de l'air en CO_2 et vapeur d'eau. Quelques essais, par exemple avec de la simazine et du monuron ont été décrits.

Apparatur zur kontinuierlichen Registrierung des CO_2*-Gaswechsels und der Transpiration ganzer Pflanzen*

Zusammenfassung. Die Photosynthese, Respiration und Transpiration ganzer Pflanzen werden mit der beschriebenen Apparatur kontinuierlich registriert. Ein Ultrarotabsorptionsgerät wird für die Photosynthese- und Respirationsmessungen verwendet, während für Transpirationsmessungen Psychrometer benutzt werden, Luft- und Blatt-Temperaturen werden ebenfalls registriert. Die Assimilationskammer ist in solcher Weise konstruiert, dass ganze Pflanzen mehrerer Pflanzenarten untersucht werden können unter konstanten Bedingungen von CO_2-Gehalt und Luftfeuchtigkeit. Einige Versuche, z.B. mit Simazin und Monuron werden beschrieben.

INTRODUCTION

Photosynthesis and respiration are of primary importance in plant growth, and direct measurements of these processes are useful in studies on the influence of environmental factors on growth. The same holds for the measurement of transpiration of plants.

Herbicides may affect plants in several ways. Substituted ureas and *s*-triazines influence photosynthesis (e.g. Wessels & Van der Veen 1954; Gysin and Knüsli 1960). Also other new herbicides are supposed to influence plant growth in this way, and therefore direct measurements of the effects of these herbicides on this process may elucidate their mode of action and may give indications of the factors important for practical use.

The apparatus permits a continuous recording of photosynthesis, respiration and transpiration of whole plants. In experiments on photosynthesis of higher plants it is generally single leaves, either attached to the plant or excised, which have been used. This procedure facilitates the estimation of the light intensity

on the leaf surface (Gaastra 1959), but complicates the evaluation of the photosynthetic activity of whole plants, particularly as the rate of photosynthesis depends on the age of the leaves (Koch & Keller 1961). Furthermore, results obtained from single leaves are markedly affected by their orientation in the assimilation chamber (Gaastra 1959) and by the rate of air supply (Egle 1960). To evaluate the photosynthetic capacity of various plant species it is useful to construct assimilation chambers in which whole plants can be accommodated. Sufficient stirring of the air is necessary to prevent reduction in CO_2 concentration near the leaves and the large differences between air and leaf temperatures which may occur at low rates of air supply (cf. Decker 1947; Egle 1960; Gaastra 1959; Tranquillini 1954).

Infra-red gas analysis was used in this assembly to measure photosynthesis and respiration. Since the principles involved in this method have been described (e.g. Egle 1960; Gaastra 1959; Belikov *et al.* 1960) only a few details which are of importance in connection with the description of the complete assembly will be given. The transpiration of plants can be measured and recorded in several ways (e.g. Anderson *et al.* 1954; Brun 1961; Decker & Wien 1960; Gaastra 1959; Huber & Miller 1954; Johnston 1959; Koch 1957). The present method makes use of wet and dry thermocouples.

In experiments on photosynthesis and transpiration, control of temperature and humidity is desirable. Gaastra (1959) introduced air of constant temperature and humidity in the assimilation chamber, whereas Bosian (1959) and Egle (1960) conditioned the air inside the chamber. Since transpiration measurements had to be carried out in the present assembly, only the first-mentioned system could be used.

A description of the assembly is given in the present paper, in which also some experiments are reported. Further results obtained with the apparatus concerning the influence of herbicides upon photosynthesis, respiration and transpiration will be published in subsequent papers.

CONSTRUCTION OF THE ASSIMILATION CHAMBER AND IRRADIATION OF THE PLANTS

The design of the assimilation chamber is shown in Fig. 1. The inside dimensions of the chamber are $40 \times 22 \times 22$ cm. The bottom part has double, water-cooled walls (W_3) of stainless steel painted dull black on the upper side to reduce upward light reflection, and covered by a roughened plexiglass plate which prevents condensation on the cooled bottom of the chamber. Five holes (diam. 5 cm) in the bottom serve to accommodate the plants, the roots of which are immersed in the culture solution (C). The plants are sealed into the holes with perforated rubber stoppers (R). Two additional holes (I, diam. 1 cm) for the inlet of the air stream are fitted in the bottom. The upper part of the chamber is constructed of plexiglass and only the top is water-cooled (W_2). After mounting the plants the upper part is sealed to the bottom of the chamber with rubber strips and screws with butterfly nuts (S). The outlet for the air (O) is in a side wall of the chamber.

Two fans (F) are sealed into opposite walls with their motors mounted out-

side the assimilation chamber. Sufficient stirring is obtained to prevent a reduction of the CO_2 concentration near the leaves. The wind velocity in the chamber, measured with a Lambrecht flow meter, varied from 0·4-2·8 m/sec. The CO_2 concentration was found to be the same at all locations inside the chamber, between the leaves as well as in the space not occupied by plants. Due to the air turbulence there is little difference between air and leaf temperature (see Fig. 7, 9). Generally the air flows through the assimilation chamber at rates between 300 and 1000 l./hr, depending on the size of the plants.

The assimilation chamber is mounted on a movable frame suspended on iron

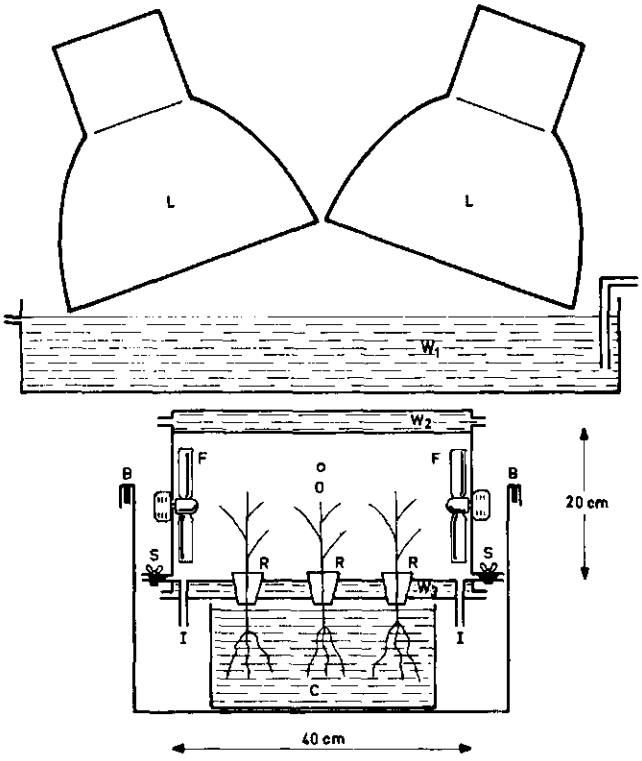

FIG. 1. Diagram of the assimilation chamber and the irradiation of the plants. B = iron bars; C = culture solution; F = fans; I = air inlet; L = lamps; O = air outlet; R = rubber stoppers; S = screws with butterfly nuts; W_1, W_2, W_3 = water layers.

bars (B), which can be pushed into a fixed position under the lamps (L.) Two high pressure mercury vapour lamps with fluorescent bulbs (HPL-700 Watt, Philips) with reflectors are mounted in an inclined position above the chamber. The light passes through 10 cm of streaming water (W_1) to remove the greater part of the infra-red radiation. The maximum light intensity at 10 cm from the bottom of the assimilation chamber is 0·4 cal cm^{-2} min^{-1}($\lambda < 0{\cdot}7\mu$; the infra-red radiation is 10% of this value). This corresponds to $2{\cdot}8 \times 10^5$ ergs cm^{-2} sec^{-1}, or (according to Gaastra 1959) to about 8×10^4 lux, and about $7{\cdot}4 \times 10^3$ foot-candles. The light intensity in the assimilation chamber can be varied by means of iron screens of known transmission, which are inserted under the

reflectors. The light intensity is recorded every 216 seconds by means of a photocell attached to the frame. The photocell is calibrated with a thermopile placed in the assimilation chamber.

The air temperature in the chamber is measured with a copper-constantan thermocouple shielded by a wick against direct radiation. In addition, leaf temperatures are recorded by thermocouples inserted into the veins of the leaves. The reference junctions are kept in a water bath of constant temperature. Although the upper and lower walls are cooled with water, a temperature rise in the assimilation chamber is observed. If dry air is used, or if condensation is not objectionable because transpiration is not measured, cooling may be increased to obtain lower temperatures. The temperature control can be improved by increasing the cooled wall surface of the chamber.

MEASUREMENT OF PHOTOSYNTHESIS AND RESPIRATION

The CO_2 content of the air in the assimilation chamber is measured with a Liston-Becker infra-red gas analyser, Model 15A. One of the tubes of the analyser contains nitrogen and is completely closed. The sample air, of which the CO_2 content has to be determined, flows through the other tube. The difference in CO_2 content between the gases in both tubes induces a signal in a variable condenser which, after amplification and rectification, is measured and recorded with a potentiometric recorder. For this, a Brown recorder, Model 153 × 64 with twelve different records, (yielding 2 millivolts for full-scale deflection) was used.

From the twelve records four were used for CO_2 registration. As a complete series of twelve records takes 216 seconds, CO_2 records were obtained at intervals of 54 seconds. During the experiments the CO_2 content of two air samples was recorded alternately (every 216 seconds) by means of a microswitch in the recorder, which operated an impulse relay after each twelve records. The relay operated a three-way valve, by which either one or the other air sample was made to flow through the analyser tube.

The thermostat in the analyser unit was not used since too large fluctuations of the CO_2 records were obtained in this way. Some gradual drift of these records then caused by temperature changes in the room did not interfere with the results.

The CO_2 in the outside air being variable, an artificial mixture with constant CO_2 concentration is prepared. Air is freed from carbon dioxide by passing it through a 40% potassium hydroxide solution. The apparatus used is shown in Fig. 2. A glass tube (diam. 10 cm, length 150 cm) is partly filled with 3 l. of a potassium hydroxide solution (C). Through tube A the air is conducted into an inner tube (B), which has an open connection with the solution. Some solution will be carried along with the upward-flowing air stream. Air as well as solution descends through ceramic beads (D). The solution drips back and CO_2-free air flows through tube E into vessel F, in which small droplets of the solution settle, and flow back to C through tube G. In this way up to 3000 l. CO_2-free air per hour can be prepared.

The CO_2-free air is mixed with pure CO_2 from a cylinder by means of two flow meters. Each flow meter (Fig. 3) consists of a capillary tube (C), and a closed manometer (F), filled with a coloured liquid for measuring the pressure difference between both ends of the capillary tube. At constant height of the column in the water overflow (O), a constant flow rate through the capillary tube is obtained. The flow meters are calibrated by comparing them with calibrated flow meters (e.g. a 'Rotameter', or a gas meter). The apparatus used for calibration at very low flow rates, as needed for the CO_2 flow meters (e.g. 300 ml per hour if 1000 l. of air with 300 ppm CO_2 has to be prepared) is also depicted in Fig. 3. It consists of a burette T of 50 ml capacity, in which a soap film (SF)

is produced from a soap solution (S). The device is connected with the flow meter, and the displacement of the soap film is measured with a stop watch. To obtain a homogeneous mixing of CO_2-free air and pure CO_2 (up to 1 ppm) the joint stream of CO_2-free air and pure CO_2 is passed through a large mixing vessel of 50 l. capacity.

For calibration of the infra-red gas analyser two standard mixtures (of e.g. 300 and 200 ppm CO_2) are passed alternately (every 216 seconds) through the analyser tube of the apparatus. By means of a shutter in front of the nitrogen-containing tube in the apparatus the recorder deflection can be adjusted to zero for air with 300 ppm CO_2. When air with 200 ppm CO_2 flows through the analyser, the recorder deflection is adjusted to 100 scale units by attenuating the input signal to the amplifier. Since over the range of 300 to 200 ppm CO_2

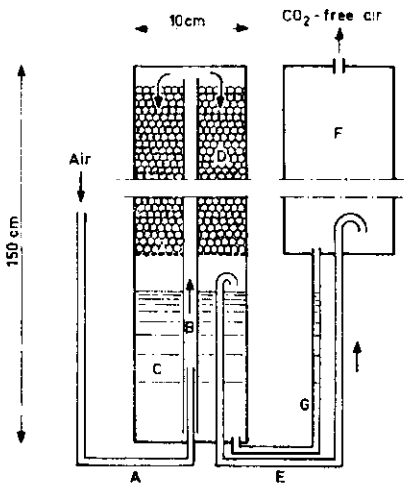

FIG. 2. Diagram of the apparatus for the removal of CO_2 from the air. A = inlet tube for air; B = inner tube; C = potassium hydroxide solution; D = ceramic beads; E = outlet tube for air; F = collecting-vessel; G = tube for flow-back of potassium hydroxide solution to C.

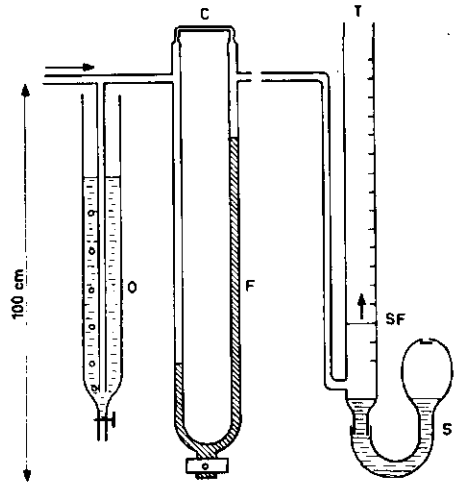

FIG. 3. Diagram of the arrangement with a flow meter. C = capillary tube; F = flow meter with coloured liquid; O = overflow. On the right side the calibration device for low flow rates. S = soap solution; SF = soap film; T = burette.

the recorder deflection is proportional to the CO_2 concentration, one scale unit corresponds to one ppm CO_2. So during calibration two interrupted series of points at a mutual distance of 100 scale units are obtained. Both mixtures are continuously prepared and calibrations can be made during each experiment at adequate intervals.

To measure photosynthesis and/or respiration, air with 300 ppm CO_2 is pumped into the assimilation chamber. By means of a three-way tap (S in Fig. 6) the alternate flow of the standard air samples through the analyser tube is changed into a flow in which air with 300 ppm CO_2 alternates with air from the assimilation chamber. The two interrupted series of points on the recorder chart now represent the CO_2 content of the air at the inlet, and at the outlet side of the assimilation chamber. If the constant flow rate of the air is known,

the amount of CO_2 exchanged in the assimilation chamber can easily be computed.

Water vapour in the air flowing through the analyser tube may slightly interfere with the CO_2 measurements. Air should be dried previously, or alternatively its moisture content should be held constant. Drying with silica

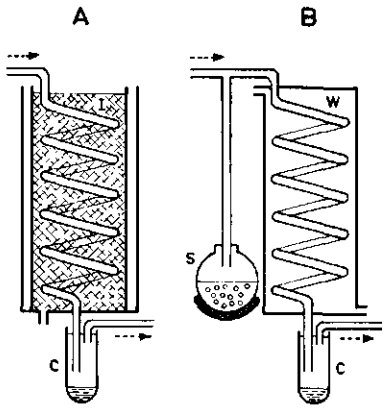

FIG. 4A. Drying condenser. I = ice; C = vessel for condensed water. The air stream is in the direction of the arrows.
FIG. 4B. Humidifier. S = steam vessel; W = water; C = vessel for condensed water. The air stream is in the direction of the arrows.

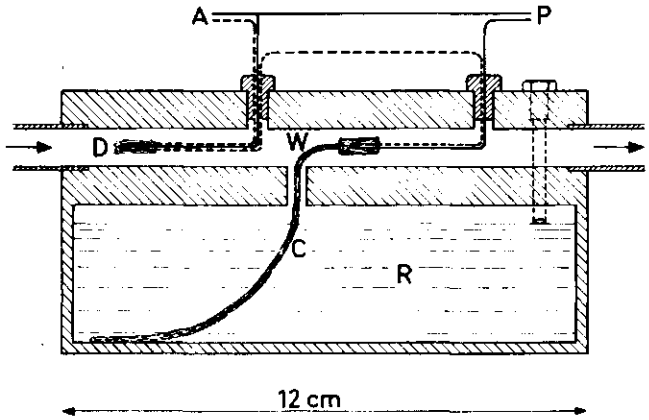

FIG. 5. Diagram of the psychrometer assembly. ——— = Constantan wires; - - - - - - = copper wires; D = dry thermocouple; W = wet thermocouple; C = cotton thread; R = reservoir with distilled water. The air stream is in the direction of the arrows. The air temperature is measured between the wires at A, and the psychrometer value between the wires at P.

gel was unsuccessful owing to slow equilibration between the CO_2 in the air stream and the silica gel causing a delay in the response to changes in CO_2. Therefore dry air (<2 mg H_2O/l. air at a flow rate of 1 l./min) is prepared by passing it through a spiral copper tube surrounded by melting ice (I) in a double-walled, temperature-insulated cylinder. Condensed water drips into vessel C (Fig. 4A).

MEASUREMENT OF TRANSPIRATION

To record transpiration, psychrometers consisting of wet and dry thermocouples (copper-constantan) are used. To compute transpiration the humidity of the air before and after passage through the assimilation chamber should be measured. The psychrometer assembly at the inlet and outlet side of the assimilation chamber is shown in Fig. 5. The assembly consists of a plexiglass block with a duct for the passage of the air sample, along which the dry (D) and wet (W) thermocouples have been placed after each other. Both junctions are covered with a cotton wick and the wet junction is kept wet by immersing a cotton thread (C) in the reservoir (R) containing distilled water. According to De Wit (1954) the psychrometer value (P) betweeen the wet and dry junction may be measured directly, because they have one wire in common, while the air temperature (A) is measured separately. The diameter of the duct (0·8 cm) is such that velocity of 2 m/sec is reached at a flow rate of 360 l./hr, which is below that mostly used in our experiments. The psychro-

meter values of this meter were the same as those obtained with an Assman psychrometer with a wet and a dry bulb.

The direct measurement of the psychrometer value with the Brown recorder yields only 3·2 scale units (40 μ) per degree centigrade. A D.C. microvolt amplifier (Type 30 B, Peekel) used in conjunction with the Brown recorder resulted in larger recorder deflections, e.g. 15·3 scale units per degree centigrade in Fig. 9. However, zero instability of the amplifier may result in somewhat unstable recordings.

As both humidities before and after the passage through the assimilation chamber should be recorded, and since only one psychrometer difference can be amplified at the same time, these values are recorded alternately (every 216 seconds) in a way similar to that described for the CO_2 records. The same impulse relay operates a mercury switch, by which either one or other psychrometer is connected with the amplifier. Two of the records are connected with this amplifier, so that psychrometer values are obtained at intervals of 108 seconds. The air temperature near the psychrometers (A in Fig. 5) is recorded directly (at intervals of 216 seconds; c. 3·2 scale units per degree centigrade). The reference junctions of the thermocouples are kept at constant temperature in a water bath. The moisture content of the air at the inlet and outlet side of the assimilation chamber is computed from the psychrometer records and the air temperatures indicated by the dry thermocouples.

DESCRIPTION OF EXPERIMENTAL PROCEDURE

A simplified diagram of the complete assembly is represented in Fig. 6. All air conduits are made of glass tubing (diam. 1 cm), since rubber and polyvinyl-

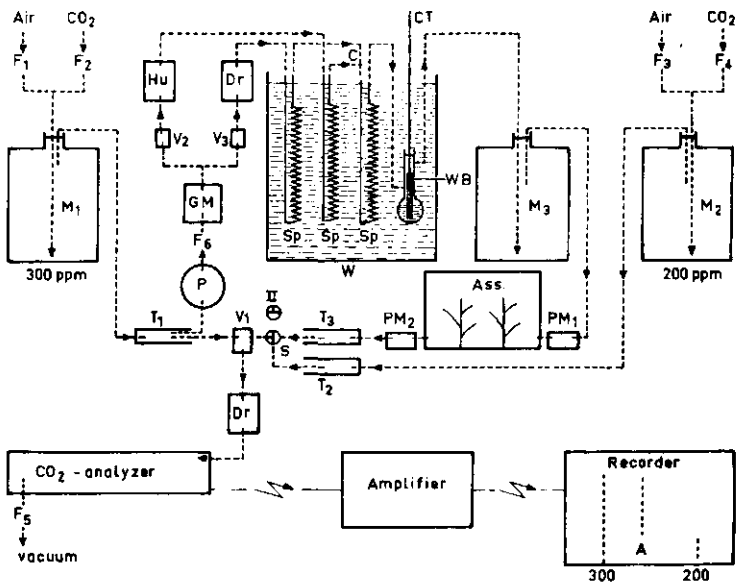

FIG. 6. Diagram of the complete assembly. A = CO_2 concentration on the recorder during photosynthesis; Ass = assimilation chamber; C = connection between drying and humidifying air circuits; CT = contact-thermometer; Dr = drying condenser; F_1-F_6 = flow meters; GM = gas meter; Hu = humidifier; M_1 = mixing vessel (300 ppm CO_2); M_2 = mixing vessel (200 ppm CO_2); M_3 = mixing vessel for dry and humid air; P = pump; PM_1 = psychrometer before, and PM_2 = psychrometer after the assimilation chamber; S = three-way tap; Sp = spirals; T_1, T_2, T_3 = open tubes: V_1 = three-way valve; V_2, V_3 = valves; W = thermostat-controlled water bath; WB = wet bulb of contact thermometer. The air stream given by ------ is in the direction of the arrows.

chloride are permeable to CO_2. Two standard mixtures of e.g. 300 and 200 ppm CO_2 are prepared continuously from CO_2-free air and pure CO_2 by means

of two sets of flow meters (F_1, F_2 and F_3, F_4). These air streams are passed through large mixing vessels (M_1 and M_2) which have connections open to the outside air in tubes T_1 and T_2 so as to prevent pressure changes in the further system. Via a three-way valve (V_1) air is sucked alternately from T_1 and T_2 through a drying condenser (Dr) (see also Fig. 4A) and the sample tube of the infra-red analyser by means of a vacuum conduit. The overflow provides a constant flow rate (measured with F_5) through the analyser, which is mostly kept at 1 l./min. In this way series of records are obtained for the two standard mixtures. One scale unit of the recorder chart is made to correspond to one ppm CO_2 by adjusting the analyser and amplifier.

After turning the three-way tap S in position II, air from T_1 (300 ppm CO_2) and T_3 (air from the assimilation chamber) are recorded alternately. A double-acting oil pump P (Edwards, Model RB 4) is made adjustable, and is used for pumping air from T_1 into the assimilation chamber, without changing the CO_2 concentration within the system. The rate of air flow is kept constant with an overflow, and is measured with a flow meter (F_6, 'Rotameter'). The integrating gas meter (GM), measures the total quantity of air during the experiment.

The humidity of the inlet air is regulated as follows. The water bath also accommodates a contact-thermometer (CT) with a bulb (WB) covered with a wick suspended in distilled water. The air stream passing the wet bulb operates the contact thermometer which, via a relay, directs this air flow either through a humidifier (Hu) or a drying condenser (Dr) by means of the valves V_2 and V_3. The system of air drying is as previously described (see also Fig. 4A); with a mixture of ice and sodium chloride lower temperatures, more effective condensation and correspondingly drier air are obtained. A diagram of the humidifier is given in Fig. 4B. The air is mixed with steam (Fig. 4B; S) and afterwards cooled in a spiral copper tube in water. Condensed water drips into vessel C (Fig. 4B).

After subsequent regulation of the temperature in the spiral tubes (Sp) in the water bath, the drying and humidifying circuits are connected (C), and conducted to the wet bulb of the contact-thermometer. Dry and humid air alternately passes the wet bulb, causing a rapid operation of the contact thermometer. A thorough mixing of dry and humid air is brought about in a large, temperature-insulated, mixing vessel of 50 l. capacity (M_3). The air humidity before the assimilation chamber is indicated by a psychrometer (PM_1). Via the assimilation chamber (Ass) and a second psychrometer (PM_2) which indicates the air humidity after passage through the chamber, the air stream is conducted into tube T_3, from which air can be sucked through the infra-red analyser. As mentioned before, the psychrometer values of PM_1 and PM_2 are recorded alternately.

SOME RESULTS OBTAINED WITH THE APPARATUS

Various species of plants e.g., maize, rye-grass, asparagus, pea, bean, cucumber, plantain, tomato, potato, chicory and carrot have been used. In the present paper only a few examples will be given in order to demonstrate the operation of the apparatus and the interpretation of the records obtained.

In order to determine the reproducibility of the results, the rates of photosynthesis of young maize plants were measured at different light intensities. This experiment was repeated with other maize plants on three different days. The plants were germinated in moist gravel, transferred to a Hoagland culture solution, and placed in a climate room at 20° C. High pressure mercury vapour

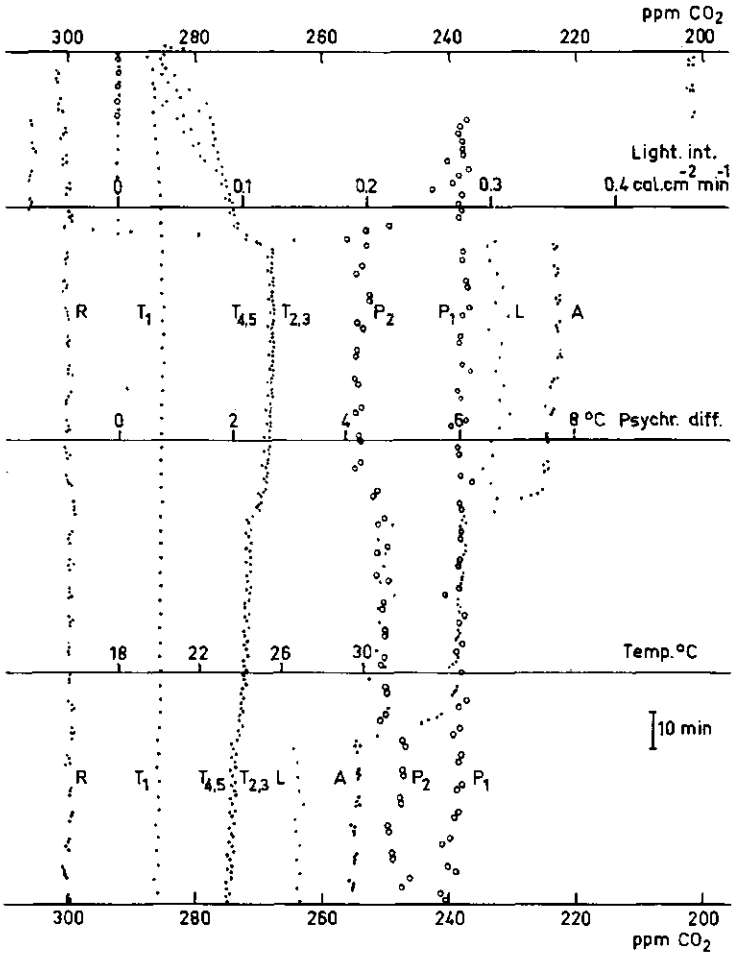

FIG. 7. Recorder chart ($^6/_{10}$ actual size) of an experiment with different light intensities. The scales for CO_2 concentration, light intensity, psychrometer value, and temperature are indicated in the figure. The different records are: L = light intensity; R = CO_2 concentration of the reference air at the inlet side, and A = that of the air at the outlet side of the assimilation chamber; P_1 = psychrometer value of the air at the inlet side, and P_2 = that of the air at the outlet side; T_1 = air temperature at the inlet side, and T_2 = that at the outlet side; T_3 = air temperature in the chamber; T_4, T_5 = leaf temperatures.

lamps with fluorescent bulbs (HPL—400 W, Philips) provided with reflectors, and yielding a light intensity of 0·1 cal.cm^{-2} sec^{-1} ($\lambda < 0{\cdot}7$ μ) at the level of the plants were used for irradiation. A photoperiod of 16 hours was alternated with 8 hours of darkness. After 12-16 days the plants were measured at the end of a dark period. In each experiment four plants were transferred to the

assimilation chamber. After calibration of the apparatus different light intensities were applied in a sequence from zero to full light intensity.

Part of the recorder chart of one of the experiments is shown in Fig. 7. R indicates the number of ppm CO_2 in the reference air at the inlet side of the assimilation chamber, and A represents the number of ppm CO_2 in the air after flowing through the chamber. The psychrometer value of the air at the inlet side is given by P_1, and that of the air at the outlet side by P_2. L represents the light intensity, while T_1 indicates the temperature of the air at the inlet and T_2 that at the outlet side of the assimilation chamber. The temperature in the chamber is given by T_3, and the leaf temperatures by T_4 and

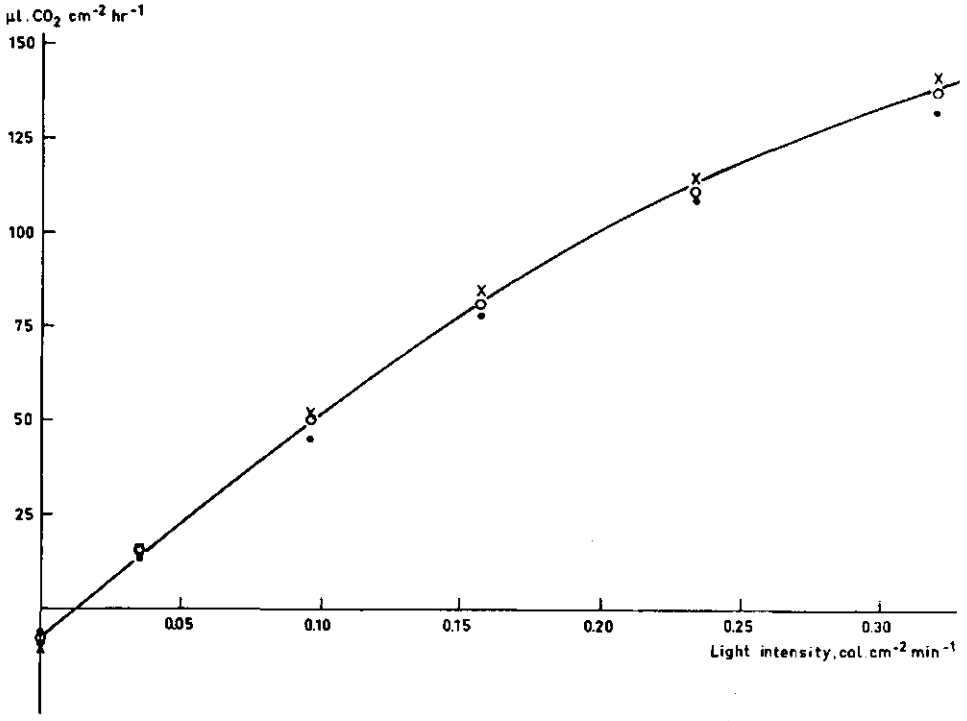

FIG 8. The rate of photosynthesis of maize plants in relation to light intensity, as measured in three different experiments (indicated by ●, ○, and ×).

T_5. The effect of light intensity upon the CO_2-consumption of the plants is evident. Constant consumption rates are reached 10-20 minutes after the change from one light intensity into another, which period, of course, also depends on the flow rate through the assimilation chamber (in this experiment 600 l./hr). The effect of light intensity upon transpiration is evident from the position of the psychrometer records P_2, and that of the temperature T_2, as compared with those of P_1 and T_1. Records P_1 and P_2 are somewhat unstable owing to zero instability of the amplifier. Apart from this, higher differences are obtainable (see Fig. 9). At the end of the experiment the plants were again placed in darkness, followed by a second calibration of the apparatus. Although some

drift (equal to 2 ppm) did occur during the experiment, the distance recorded between air with 300 ppm CO_2 (R) and 200 ppm CO_2 proved to be unaltered.

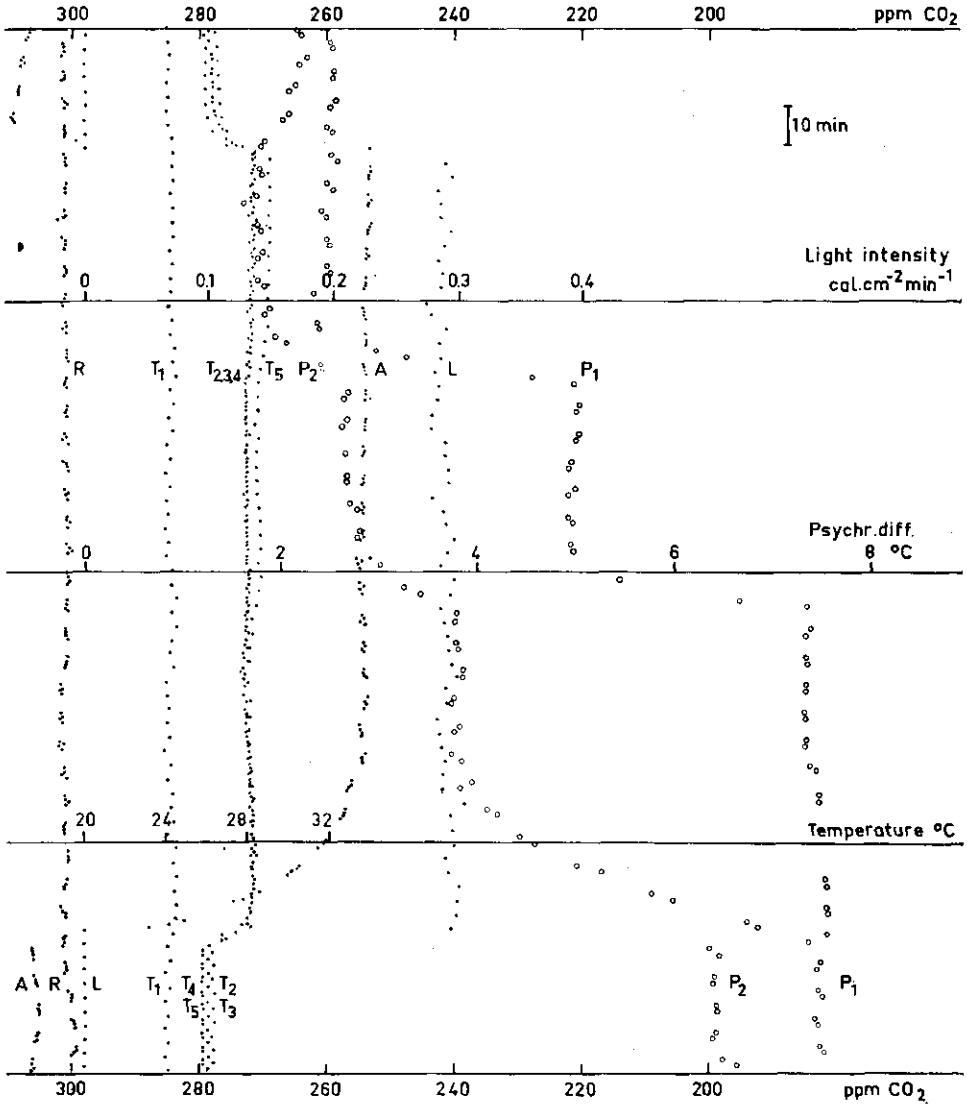

FIG. 9. Recorder chart ($^6/_{10}$ actual size) of an experiment with beans at different humidities. The scales for CO_2 concentration, light intensity, psychrometer value, and temperature are indicated in the figure. The symbols in the different records are the same as those in Fig. 7 (P_1 and P_2 being the psychrometer values of the air at the inlet and outlet sides of the assimilation chamber, respectively).

The rate of photosynthesis (in µl. CO_2 cm^{-2} hr^{-1}) is computed according to the formula:

$$F(R-A)/L$$

where F is the flow rate through the assimilation chamber (in l./hr), L the leaf area of the plants (in cm^2), R the number of ppm CO_2 in the air at the inlet side,

and A the number of ppm CO_2 in the air at the outlet side of the assimilation chamber. The results of the three experiments with maize as computed in this way are represented in Fig. 8. From this it can be concluded that the reproducibility of the experiments is fairly good. It is evident that with such whole plants light saturation is not reached.

Another recorder chart reading is given in Fig. 9. Four bean plants with two single leaves each, grown and measured as before, were used. The meaning of the symbols in the different records is the same as in Fig. 7. Three different humidities of the air (P_1) were successively established by adjusting the contact thermometer with the wet bulb to different values, to give relative humidities of 47, 62·5, or 80% at an air temperature of 24·1° C. Within 20 minutes after

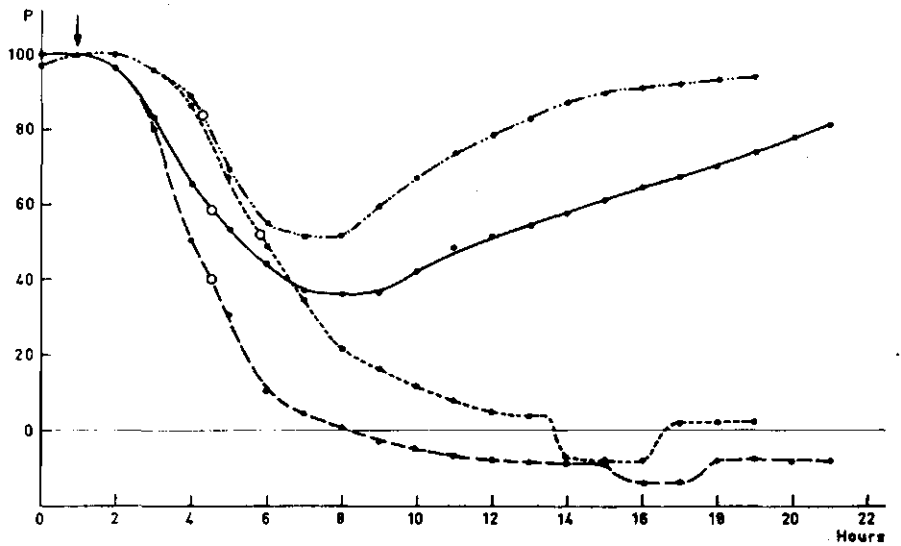

FIG. 10. The rate of photosynthesis (max. = 100) of maize, chicory and plantain, as influenced by simazine and/or monuron, and the effect of removing these herbicides from the culture solution. At ↓ 2×10^{-5} M simazine was added to maize (———) and to chicory (— — —), and 2×10^{-5} M monuron to plantain (——··——··——) and to maize) (------). Fresh culture solutions were given at ○, as indicated in the curves. (In the experiment with chicory and in that with maize treated with monuron a short dark period was intercalated).

a shift in humidity, constant records were obtained. The humidity of the air leaving the assimilation chamber (P_2) was also recorded. The transpiration rates of the plants were calculated from the differences between the water vapour content of the air at the outlet side (computed from P_2 and T_2) and that at the inlet side of the chamber (computed from P_1 and T_1), the flow rate of the air through the chamber, and the leaf area. For the three different humidities the transpiration values appeared to be 12·5, 10·9, and 8·4 mg H_2O cm^{-2} hr^{-1}, respectively. No changes in CO_2 assimilation were observed as is evident from the constancy of the difference between A and R. The temperature in the assimilation chamber increased to high values in this experiment.

In these experiments herbicides were added to the culture solution. A spraying device in the assimilation chamber has yet to be constructed. Most

of the inhibitors of photosynthesis are readily taken up through the root and translocated to the leaves. Applications of herbicides to the roots in culture solution may be used to demonstrate the rate of inactivation of herbicides in plants. Some experiments made in this connection are shown in Fig. 10. Young plants of maize, chicory and plantain, grown and measured as before, were treated with 2×10^{-5} M simazine or monuron in the culture solution. Some hours later the solutions were replaced by fresh solutions containing no herbicides, and the assimilation rate was recorded. In order to allow a comparison of the effects of herbicides on different plants, for each plant species the assimilation rate is expressed as a percentage of its value prior to treatment with the herbicides.

The rate of photosynthesis of maize and chicory treated with simazine was inhibited sooner than that of maize and plantain treated with monuron. After replacement of the culture solutions a recovery of the photosynthetic rate is observed for maize treated with simazine, and for plantain treated with monuron. Chicory treated with simazine, and maize treated with monuron showed no recovery of their photosynthetic rate upon replacement of the culture solution. This indicates an internal inactivation of simazine in maize and of monuron in plantain.

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REFERENCES

ANDERSSON, N. E., HERTZ, C. H. & RUFELT, H. (1954) A new fast recording hygrometer for plant transpiration measurements. *Physiol, Plant.*, **7**, 753-67.

BELIKOV, P. S., MOTORINA, M. V. & KURKOVA, E. B. (1960) Experience in the use of the infrared gas analyser (GIP-5) for the determination of photosynthesis intensity (Russian). *Proc. Timiryazev agric. Acad.*, **3**, (34), 30-39.

BOSIAN, G. (1959) Zum Problem des Küvettenklimas: Temperatur und Feuchteregulierung. *Ber dtsch. bot. Ges.*, **72**, 391-7.

BRUN, W. A. (1961) Photosynthesis and transpiration from upper and lower surfaces of intact banana leaves. *Pl. Physiol.*, **36**, 399-405.

DECKER, J. P. (1947) The effect of air supply on apparent photosynthesis. *Pl. Physiol.*, **22**, 561-71.

DECKER, J. P. & WIEN, J. D. (1960) Transpirational surges in *Tamarix* and *Eucalyptus* as measured with an infra-red gas analyser. *Pl. Physiol.*, **35**, 340-3.

DE WIT, C. T. (1954) An oscillating psychrometer for micro-meteorological purposes. *Appl. scient. Res.*, A iv, 120-6.

EGLE, K. (1960) Methoden der Photosynthesemessung, (a) Landpflanzen. In: *Handbuch der Pflanzenphysiologie*, ed. by W. Ruhland, V, (1), 115-63.

GAASTRA, P. (1959) Photosynthesis of crop plants as influenced by light, carbon dioxide, temperature, and stomatal diffusion resistance. *Meded. Landbouwhogesch., Wageningen*, **59** (13), 1-68.

GYSIN, H. & KNÜSLI, E. (1960) Chemistry and herbicidal properties of triazine derivates. *Advanc. Pest Control Res.*, **3**, 289-358.

HUBER, B. & MILLER, R. (1954) Methoden der Wasserdampf- und Transpirationsregistrierung im laufenden Luftstrom. *Ber. dtsch. bot. Ges.* **67**, 224-34.

JOHNSTON, R. D. (1959) Control of water movement by stem chilling. *Aust. J. Bot.*, **7**, 97-108.

KOCH, W. (1957) Der Tagesgang der 'Produktivität der Transpiration'. *Planta*, **48**, 418-52.

KOCH, W. & KELLER, TH. (1961). Der Einfluss von Alterung und Abschneiden auf den CO_2-Gaswechsel von Pappelblättern. *Ber. dtsch. bot. Ges.*, 74, 64-74.

TRANQUILLINI, W. (1954) Über den Einfluss von Übertemperaturen der Blätter bei Dauereinschluss in Küvetten auf die ökologische CO_2-Assimilationsmessung. *Ber. dtsch. bot. Ges.*, **67**, 191-204.

WESSELS, J. S. C., & VAN DER VEEN, R. (1956) The action of some derivates of phenylurethan and 3-phenyl-1,1-dimethylurea and the Hill reaction. *Biochim. biophys. Acta*, **19**, 548-9.

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