PHOTOSYNTHESIS OF CROP PLANTS AS INFLUENCED BY LIGHT, CARBON DIOXIDE, TEMPERATURE, AND STOMATAL DIFFUSION RESISTANCE

P. GAASTRA

PHOTOSYNTHESIS OF CROP PLANTS AS INFLUENCED BY LIGHT, CARBON DIOXIDE, TEMPERATURE, AND STOMATAL DIFFUSION RESISTANCE

(MET EEN SAMENVATTING IN HET NEDERLANDS)

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD
VAN DOCTOR IN DE LANDBOUWKUNDE
OP GEZAG VAN DE RECTOR MAGNIFICUS IR. W. DE JONG,
HOOGLERAAR IN DE VEETEELTWETENSCHAP,
TE VERDEDIGEN TEGEN DE BEDENKINGEN
VAN EEN COMMISSIE UIT DE SENAAT
VAN DE LANDBOUWHOGESCHOOL TE WAGENINGEN
OP VRIJDAG 4 DECEMBER 1959 TE 16 UUR

DOOR

P. GAASTRA

H. VEENMAN EN ZONEN N.V. – WAGENINGEN – 1959

STELLINGEN

I

Proeven over de invloed van verschillende spectrale gebieden op fysiologische processen in planten, kunnen niet met elkaar worden vergeleken wanneer het ingestraalde licht in fotometrische eenheden wordt uitgedrukt.

Dit proefschrift.

II

De invloed van de openingstoestand van de huidmondjes op de fotosynthesesnelheid kan slechts met zekerheid worden vastgesteld, wanneer de diffusieweerstand in de huidmondjes en de totale weerstand in de weg waarlangs het koolzuur naar de chloroplasten diffundeert, bekend zijn.

Dit proefschrift.

III

Hoewel de diffusieweerstand in de mesofylcellen de fotosynthesesnelheid in belangrijke mate kan bepalen, is de invloed van deze weerstand op de productie van onder natuurlijke omstandigheden groeiende gewassen veel geringer dan door PENMAN en SCHOFIELD wordt berekend.

H. L. PENMAN and R. K. SCHOFIELD, Symp. Soc. Exp. Biology V, 1951: 115–129.
Dit proefschrift.

IV

Wanneer bij proeven over de lichtafhankelijkheid van fysiologische processen in een blad, de factor licht wordt gekarakteriseerd door de hoeveelheid lichtenergie die het bladoppervlak treft, biedt het meestal geen voordelen om voor de meting van deze energie een overeenkomstig de cosinus-wet gecorrigeerde lichtmeter in plaats van een vlakke, ongecorrigeerde lichtmeter te gebruiken.

V

De directe invloed van de temperatuur op de fotosynthesesnelheid van onder natuurlijke omstandigheden of in kassen groeiende gewassen wordt vaak overschat.

K. V. THIMANN, Proc. World Symp. on Applied Solar Energy, Phoenix, Arizona, 1955: 256.
R. VAN DER VEEN und G. MEYER: Licht und Pflanzen. Philips' Technische Bibliothek, 1958: 17–18.

VI

De proeven van WENT met jonge tomateplanten houden geen voldoende rechtvaardiging in voor zijn conclusie dat groei en fotosynthese onafhankelijk van elkaar door licht worden beïnvloed.

F. W. WENT: The experimental control of plant growth. Chronica Botanica 17, 1957: 280–284.

Proefschrift P. Gaastra
Wageningen 1959

VII

Wanneer bij proeven over de invloed van licht op de groei en de ontwikkeling van planten, op het wisselspanningsnet aangesloten fluorescentie- of gasontladingslampen worden gebruikt, dient men er rekening mede te houden dat de groei en misschien ook de ontwikkeling, mede kunnen worden bepaald door de periodiek veranderende lichtemissie van de lampen.

VIII

De mogelijkheid om op een economisch verantwoorde wijze uit in ons land geteelde groenvoedergewassen en eventueel andere in aanmerking komende gewassen, een eiwitrijk maar vezelarm product te winnen, dient nader te worden onderzocht.

J. J. I. SPRENGER: Kunstmatig drogen in de landbouw. Staatsdrukkerij- en Uitgeverijbedrijf, Den Haag, 1958: 161–170.

IX

Het effect van de voedingsvoorlichting op het platteland zal worden vergroot, wanneer bij de voorlichting ook het mannelijke deel van de bevolking direct wordt bereikt.

X

Plantefysiologen kunnen een belangrijke bijdrage leveren tot het mogelijk maken van de bemande ruimtevaart, indien ze, in samenwerking met andere onderzoekers, er in slagen een gesloten ecologisch systeem mens-plant te verwerkelijken.

VOORWOORD

Graag gebruik ik deze gelegenheid om allen, die tot mijn vorming en tot de voltooiing van dit proefschrift hebben bijgedragen, hartelijk te danken.

Van mijn gevoelens van dankbaarheid jegens mijn Ouders en mijn Vrouw, wil ik op deze plaats getuigen door dit proefschrift aan hen op te dragen.

Hooggeleerde WASSINK, hooggeachte promotor, het was mij een voorrecht in Uw goed geöutilleerde laboratorium te kunnen werken. Ik heb het zeer gewaardeerd dat ik me niet alleen op het gebied van de plantenphysiologie, doch ook op dat van de meetmethodiek heb kunnen oriënteren. Uw suggesties en kritische opmerkingen waren voor mij van zeer groot belang.

U, hooggeleerde DEWEZ, hooggeleerde DORST, hooggeleerde SCHUFFELEN, ben ik oprecht dankbaar voor de wetenschappelijke vorming die ik tijdens mijn studie aan de Landbouwhogeschool van U heb ontvangen.

Hooggeleerde REINDERS, Uw kritische en systematische behandeling van de plantkunde en Uw technisch volmaakte demonstraties hebben een blijvende indruk op mij gemaakt.

Hooggeleerde OLIVIER, aan de boeiende wijze waarop U mij hebt ingeleid in de organische chemie, bewaar ik de beste herinneringen.

Zeergeleerde SPRUIT, zeergeleerde STOLWIJK, veel hulp heb ik van U ondervonden bij de ontwikkeling van de apparatuur. Deze hulp en Uw waardevolle suggesties gedurende het onderzoek, zijn voor mij van grote betekenis geweest. Ook de andere collegae van het Laboratorium voor Plantenphysiologisch Onderzoek ben ik erkentelijk voor de openhartige wijze waarop we hebben samengewerkt.

Een belangrijk deel van het experimentele werk is verricht door U, mevrouw PIETERS-HERTROYS. Aan de intelligente en nauwgezette uitvoering van Uw werk bewaar ik de beste herinneringen. In de laatste fase van het onderzoek werd ik op prettige wijze geassisteerd door Mej. M. F. F. OSSE. Het onderzoek werd eveneens zeer bevorderd door de accurate hulp die door het technische personeel van het Laboratorium werd verleend. Van hen wil ik de Heer D. STEDELAAR noemen, die de tekeningen keurig verzorgde.

Het onderzoek werd gesubsidiëerd door de Nationale Raad voor Landbouwkundig Onderzoek T.N.O. Ik heb het op hoge prijs gesteld dat een deel van de verkregen resultaten in dit proefschrift kon worden vastgelegd.

Overdruk

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PHOTOSYNTHESIS OF CROP PLANTS AS INFLUENCED BY LIGHT, CARBON DIOXIDE, TEMPERATURE, AND STOMATAL DIFFUSION RESISTANCE

(met een samenvatting in het Nederlands)

by

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CONTENTS

CHAPTER I

INTRODUCTION AND OUTLINE OF THE INVESTIGATION

I–1. INTRODUCTION

In higher plants, the organic matter and the energy required for the maintenance of the plant originate from photosynthesis, so that the latter is an important yield-determining process. Consequently, quantitative data relating to the photosynthetic activity of crop plants are important, *e.g.* for investigations into the maximum production capacity under normal outdoor conditions or under optimum conditions, for the establishment of the light-, CO_2-, and temperature requirements in glass house culture, and for investigations into the possibility of plant selection at an early stage of development.

In this connection, several aspects of photosynthesis are of interest:

1. Under normal outdoor conditions, the CO_2-concentration is relatively constant, but light and temperature vary considerably. Therefore, studies on the effect of such factors on photosynthesis in normal air may provide important data, *e.g.* on the light intensity at which respiration compensates photosynthesis, on the efficiency of light energy conversion, on the saturating light intensity and the corresponding rate of photosynthesis.

2. For determination of the absolute maximum rate, and in connection with CO_2-fertilization in glass house culture, the effects of light, temperature, and CO_2-concentration should be considered.

3. Systematic investigation of the influence of light, temperature, and CO_2 upon photosynthesis provide information about the nature of the rate-limiting process, because the photochemical processes, the diffusion of reactants, and the chemical processes are differently affected by these factors.

4. The rate of diffusion of CO_2 from the external air towards the chloroplasts, partly depends upon the dimensions of the diffusion path in the stomata, the intercellular spaces and the mesophyll cells. In this connection, the study of the stomatal regulation of photosynthesis is important, because the stomatal aperture is influenced by several factors, *e.g.* by the water content of the leaf and, consequently, the water supply to the leaf, and the transpiration rate (STÅLFELT, 1956), and by the light intensity and the CO_2-concentration (FREUDENBERGER, 1940, SCARTH and SHAW, 1951, HEATH and RUSSELL, 1954).

Qualitatively, much is known about photosynthesis, but the quantitative data which are available vary widely. This is clearly demonstrated in the survey by RABINOWITCH (1951, Table 28.VI) of the maximum rates by leaves of land plants under natural conditions: large differences occur, even for the same species under similar climatic conditions. Also, in experiments on the relation between CO_2-concentration and photosynthesis, the values given for saturating CO_2-concentrations vary widely. With *Asparagus*, MÜLLER (1958) required 10 % CO_2 to obtain saturation, whereas, in wheat, HOOVER, JOHNSTON, and BRACKETT (1933) observed saturation in air with 0.11 % CO_2.

We agree with RABINOWITCH that the differences observed have to be ascribed in part to experimental errors which, in experiments with higher plants enclosed in assimilation chambers, are easily introduced. Some possible sources of uncertainty are listed in the following paragraphs.

a. The physiological state of the leaf may change during an experiment. For example, in excised leaves or in plants with insufficient water supply to the roots, the water balance of the leaf may change, and this may result in a decreased photosynthetic rate, because the water balance affects the stomatal opening (STÅLFELT, 1956) as well as the activity of the photosynthetic process itself (SCHNEIDER and CHILDERS, 1941, LOUSTALOT, 1945, ASHTON, 1956). Furthermore, in excised leaves or in leaf discs, the transport of assimilates is hindered and, in experiments of extended duration, this may affect the photosynthetic activity.

b. The heat conductivity and the specific heat of the air are low, so that considerable differences between air temperature and leaf temperature may occur (TRANQUILLINI, 1954, RASCHKE, 1956). In many experiments, however, the temperature conditions are inadequately defined, because leaf temperatures have not been measured, while effective means of preventing excessive temperatures have been omitted.

c. In experiments with normal air, the CO_2-concentration limits photosynthesis at high light intensities, so that the observed rate depends upon the average CO_2-concentration in the assimilation chamber, and hence upon several experimental factors, *e.g.* the initial CO_2-concentration, the dimensions of the leaf, the rate of air supply, the dimensions of the chamber as compared with those of the leaf, and the position of the leaf in the chamber. In many experiments, however, no allowance has been made for the reduced CO_2-concentration in the chamber, so that the methodical features mentioned will have affected the observed rate of photosynthesis. To minimize such errors, high rates of air supply have been recommended (KOSTYTSCHEW *et al.*, 1927, HEINICKE and HOFFMAN, 1933, DECKER, 1947) but even so, fluctuations in the initial concentration (*cf.* CHAPMAN, GLEASON and LOOMIS, 1954) will still be reflected in the experimental results.

d. Even with sufficient control of the experimental conditions, several factors are interrelated, so that the effect of the factor under investigation may easily be obscured. Increased light intensity, *e.g.*, affects temperature, transpiration, and, in the event of increased photosynthesis, the average CO_2-concentration. Moreover, light and CO_2 affect the degree of stomatal opening, so that these factors may influence photosynthesis directly as well as indirectly.

e. In experiments in which the air is supplied to the chambers by the action of aspirators of limited volume (BOYSEN-JENSEN, 1934, HOLDHEIDE, HUBER, and STOCKER, 1936, STOCKER, REHM, and PAETZOLD, 1938, MICHAEL, 1954, LUNDEGÅRDH, 1954), the experiments are of short duration. Unless precautions are taken, the steady state values may not be obtained.

f. In experiments with entire plants and with non-collimated light beams from artificial sources, it is difficult to define the light intensity, because it is strongly influenced by the distance from the light source. Moreover, the comparison of the light intensities used in different experiments is rendered difficult because photometric units (lux or foot-candle) as well as energy values have been used.

In the present paper, experiments designed to analyse the photosynthetic activity of crop plants from the viewpoints outlined in the beginning of this chapter, are described. Several measures have been taken to exclude experimental errors as much as possible. The scope of the investigations is dealt with in the next section.

I–2. OUTLINE OF THE INVESTIGATION

For a successful attack on these problems, the rate of photosynthesis and the experimental conditions must be measured accurately, and independent variation of the conditions should be possible. Equipment designed to meet these requirements is described in Chapter II.

In fact, the experimental conditions actually measured (incident light energy, leaf temperature, CO_2-concentration of the air before and after passage of the leaf) only give an approximation of the physiologically important conditions, which are the temperature and the light intensity in the chloroplasts, and the CO_2-concentration close to the leaf surface. Obviously, the relation between both types of conditions, and, consequently, the reliability and reproducibility of the experimental results, is strongly affected by the experimental set-up. A discussion of this relationship is attempted in Chapter III.

Measurements of the influence of light, carbon dioxide, and temperature upon the rate of photosynthesis in cucumber, spinach, tomato, sugar beet, and turnip are presented in Chapter IV.

In the course of the experiments, it became desirable to investigate the stomatal control of photosynthesis. To this purpose photosynthesis, transpiration, and leaf temperature were measured simultaneously in the same leaf. From these data, the diffusion resistances in the external air, in the stomata, and in the mesophyll cells could be computed. The underlying principles, data on the effect of light intensity and carbon dioxide upon the stomatal diffusion resistance, and an analysis of the stomatal control of photosynthesis are presented in Chapter V.

CHAPTER II

MATERIAL AND METHODS

II–1. General

The plants used were grown under controlled conditions. For the measurement of photosynthesis, transpiration, and leaf temperature, plants were transferred to the experimental set-up, in which the light intensity, the CO_2-concentration, the leaf temperature, and the humidity of the air could be varied over a wide range. Most experiments were made with one leaf, attached to the plant, so that the water supply of the leaf and the transport of assimilates were not hampered. Moreover, as mentioned in the previous chapter, the light intensity can be better defined for a single leaf than for an entire plant. Air with a definite CO_2-concentration and water vapour content was supplied to the leaf, which was enclosed in a "Plexiglass" assimilation chamber. The CO_2-concentration in the air that had passed along the leaf was determined with the aid of an infrared gas analyzer and the water vapour content with a lithium chloride hygrometer. Leaf temperature was measured with thermocouples, applied to the under surface of the leaf.

II–2. Plant material and cultivation of the plants

So far, the majority of our experiments has been made with sugar beet, fodder beet, turnip, tomato, and cucumber. The plants were grown in a room at a temperature of 20° ± 1 °C for most of the year. In summer, however, higher temperatures occurred occasionally. The plant growth equipment has a battery of 40 "daylight" fluorescent tubes (*TL*-55, 40 W, Philips) on top, and is provided with light reflecting walls. A glass plate is installed 4 cm below the tubes, and the air space between plate and lamps is flushed by a fan, so that overheating of the plants is prevented. The light intensity at the top of the plants was 5×10^4 erg.sec^{-1}.cm^{-2}, in the spectral region between 400 and 700 mμ.

The plants were usually raised in fine gravel, in light-tight, two liter preserving jars. They were supplied with Hoagland's solution, with iron added as ferric potassium ethylenediamine tetra-acetate, *cf.* Jacobson (1951). By way of glass tubes, sealed in the bottom, sets of 10 containers were connected with a stock flask, containing 50 liters of nutrient solution. Two or three times a day the nutrient solution was aerated and pumped into the containers. As soon as the containers were completely filled, the solution was drained back into the flask. The amount of solution remaining in the gravel proved sufficient for healthy growth. The stock solution was renewed every week.

II–3. Apparatus for the continuous recording of the CO_2-concentration in the air

II–3–1. Principle of the method and technical details of the apparatus. The CO_2-concentration was measured with a recording infrared gas analyzer of the selective detector type. Except for the microphone, the apparatus was built

in our laboratory. In order to facilitate the description of technical details, a short explanation of the principle will follow. A more extensive exposition is given *e.g.* in EGLE and ERNST (1949), WEIGL (1950), STRUGGER and BAUMEISTER (1951), EGLE and SCHENK (1951), and KLUYVER (1952).

The method is based upon the infrared absorption of CO_2 (a strong band at 4.3μ and a weaker one at 2.7μ). The difference in infrared-transmission of two gas samples with known and unknown CO_2-content, is determined. The air samples are flushed through the analyzer tube *T*1 and the reference tube *T*2 (fig. 1). Both tubes are provided with infrared-transmitting mica windows *W*1, 2, 3, 4. Infrared radiation, generated in the radiators *R*1 and *R*2 is admitted to the tubes. Dependent on the CO_2-concentration of the air samples, part of the radiation in the CO_2-absorption bands, is absorbed. The difference between the intensities in these bands at the exit of *T*1 and *T*2 reflects the difference in CO_2-concentration of the air samples, and is measured.

The intensity differences are small, so that for adequate accuracy either the radiators or the detection method should be selective for the 4.3 and/or 2.7μ bands. Selective radiators, *e.g.* CO_2-producing flames, were applied by DINGLE and PRYCE (1940) and by SCARTH, LOEWY, and SHAW (1948). For reasons of stability, however, non-selective radiators, consisting of electrically heated metal wires are to be preferred, and are commonly used at present, in conjunction with a selective detection method. MCALISTER (1937) isolated the 4.3μ band by means

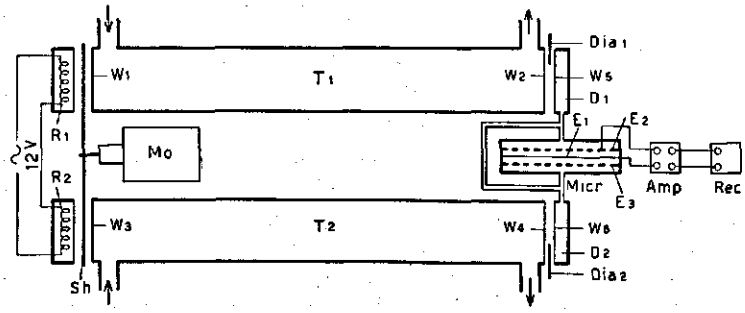


FIG. 1. Diagram, illustrating the infrared CO_2-analyzer. For explanation see text.

of a spectrograph and measured the intensity with a thermopile-galvanometer system. The apparatus required is rather delicate and expensive, so that at present non-dispersive, selective detectors are more commonly used. Essentially, they consist of two small cells, *D*1 and *D*2 (fig. 1), provided with infrared-transmitting rocksalt windows, *W*5 and *W*6, and separated by a thin metal membrane, *E*1. This membrane is one of the electrodes of a condenser microphone, *Micr*. The other electrode, *E*2, is rigid and perforated, whereas the perforated plate *E*3 is present for reasons of symmetry. *D*1 and *D*2 are filled with CO_2 or with air, with a high CO_2-content, so that the radiation in the 4.3 and 2.7μ bands still present at the exit of *T*1 and *T*2 is absorbed in these cells.

The beams of infrared radiation are interrupted synchronously by a rotating sector disc *Sh*, driven by the synchronous motor *Mo*. Thus, energy is intermittently absorbed in the detection cells, resulting in pressure oscillations of the gas in *D*1 and *D*2, and in vibration of the partition membrane *E*1, causing capacity oscillations in the condenser microphone. The size of these oscillations depends upon the difference in CO_2-concentration of the air samples in *T*1 and *T*2. The signal induced in the microphone is amplified, rectified, and measured with the recording mA-meter, *Rec*.

Our experiments were started with an apparatus, obtained from the Physical Laboratory of the Utrecht University, and designed by KLUYVER (1952) for the measurement of C^{13}-isotopes. In the course of the experiments it became desirable to adapt the apparatus more specifically to our problems, and, except for the microphone, a new apparatus was built. The greater part of it was designed by Dr. J. A. J. STOLWIJK, at that time a member of the staff of this laboratory. Several technical difficulties had to be overcome before our aim of an accuracy of 1 ppm was reached. Some technical details will be described.

The radiators comprise zigzagged nichrome wires, (diameter 0.4 mm, length 110 mm, resistance 1 Ohm), spotwelded onto steel pins, and protruding into the cylindrical cavity of a brass housing. The steel pins are led through small holes in the back of the brass housing, from which they are insulated by porcelain beads, clamped in the holes, while they are fixed to the back wall by a layer of cement (5 parts talcum and 1 part water glass). The radiators are connected in series, and fed from a stabilized 12 V AC-source. Thin mica windows protect them from draught.

An aluminum sector disc with four apertures, and driven by a synchronous motor at 750 rpm, interrupts the beams 50 times per second. A bicycle dynamo (PHILIPS Philidyne, 6 V, 0.5 A, connected with 6 V AC) is used as a synchronous motor. The motor housing is clamped in a brass jacket, so that the motor can be turned along its axis for phase adjustment of the signal. Moreover, the motor can be moved in a vertical plane, for ascertaining synchronous interruption of the beams.

The detector is essentially the same as that described by KLUYVER (1952). However, the partition membrane which, originally, was made from aluminum foil of 30μ thickness, has been replaced by a nickel foil 8μ thick, kindly placed at our disposal by the "Nederlandsche Radio Unie". The nickel foil distorts less than the aluminum foil, so that the sensitivity of the apparatus remains unchanged for long periods.

The analyzer and reference tubes are of stainless steel, 30 cm long, and of 3 cm diameter. They are provided with mica windows. Diaphragms, mounted on the tubes, enable the beams to be balanced when the analyzer and reference tubes are filled with air of equal CO_2-concentrations.

All mechanical parts are mounted on top of a heavy I-shaped iron block, and the whole assembly is enclosed in an iron box.

A diagram of the electronic circuit for amplification and subsequent rectification of the signal induced in the microphone, as designed by Dr. STOLWIJK, is presented in fig. 2. The rectified signal is measured with a recording mA-meter (Metrawatt PC-120, internal resistance 6 kOhms, full scale deflection 120 mm for 1 mA).

II–3–2. The calibration of the CO_2-analyzer and the measurement of the CO_2-concentration in air samples. In the photosynthesis experiments, a sample

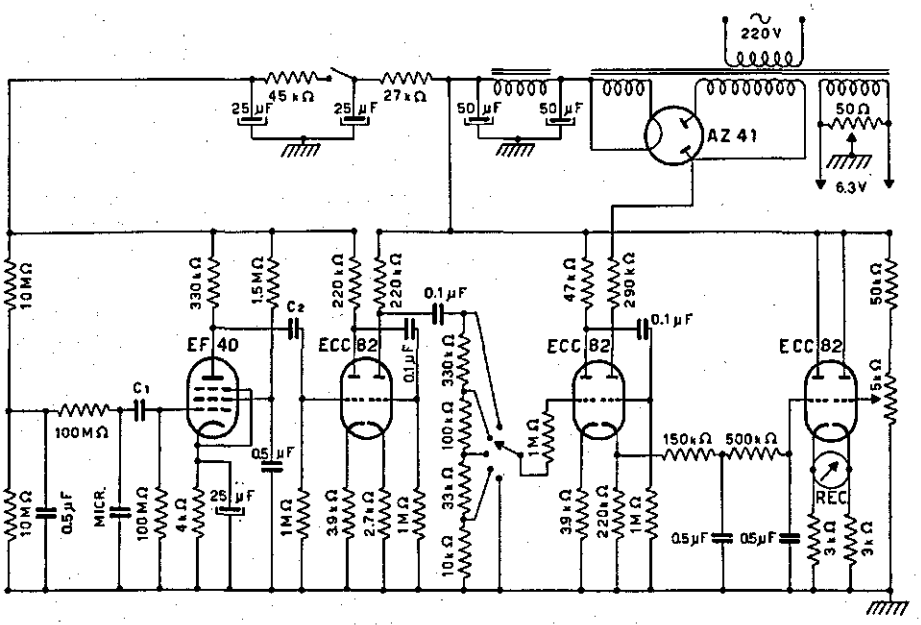


FIG. 2. Electronic circuit of the infrared CO_2-analyzer. MICR = condenser microphone (capacity 200 pF); REC = recorder (internal resistance 6 kOhms, full scale deflection 100 mA).

of the air which has passed the leaf, is supplied to the analyzer tube, while the reference tube is not supplied with nitrogen or CO_2-free air as usually, but with air with the same CO_2-concentration as that entering the assimilation chamber. This allows full scale deflection for small differences in CO_2-concentration of the air before and after its passage along the leaf. Furthermore, periodic control of the zero position of the recorder during an experiment is easy, *viz.*, by establishing a direct connection between the reference and analyzer tubes, so that reference air passes both tubes successively.

For the calibration of the set-up, two gas streams with slightly different CO_2-concentrations (c_{ref} and c_{anal}) are prepared. First, the zero point is determined, *i.e.* the position of the recorder when both tubes are flushed with the air sample with the highest CO_2-concentration (c_{ref}). Thereupon, the analyzer tube is provided with the other air sample, and the resulting galvanometer indication is compared with the zero point (ΔU). For a definite value of c_{ref}, an almost linear relation between ΔU and $c_{ref}-c_{anal}$ is found for small concentration differences, so that the sensitivity at the reference concentration is given by $(c_{ref}-c_{anal})/\Delta U$ ($\%CO_2$ per mm scale deflection). A calibration curve (fig. 3) is obtained by applying the same procedure at different values of c_{ref}. At c_{ref} = 0.03 % CO_2, the sensitivity of the apparatus was 6.3×10^{-5} $\%CO_2$ per mm scale deflection.

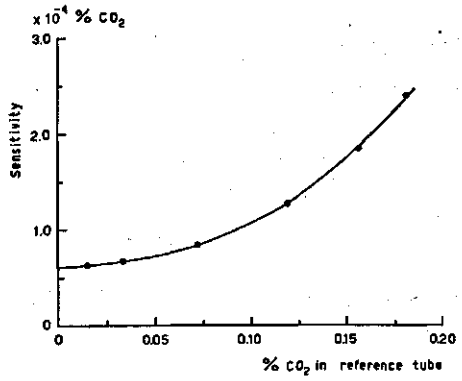


FIG. 3. Calibration curve of the infrared CO_2-analyzer. The sensitivity is expressed as the difference in CO_2-concentration of the air samples in the reference and analyzer tubes, causing a galvanometer deflection of 1 mm.

In photosynthesis experiments, the difference between the CO_2-concentrations in the air streams before and after having passed the leaf, $c_{ref}-c_{anal}$, has to be determined. In most experiments this value is approximated with sufficient accuracy by multiplying the deflection ΔU with the sensitivity value as defined above at the CO_2-concentration of the air entering the assimilation chamber. At high CO_2-concentrations, however, the relation between ΔU and $(c_{ref}-c_{anal})$ deviates from linearity, so that for large scale deflections the difference between the concentrations is then better approximated by multiplying ΔU with the sensitivity at the concentration $(c_{ref}-0.5 \times S_{ref} \times \Delta U)$ which is intermediate between c_{ref} and c_{anal}; S_{ref} is the sensitivity at the reference concentration.

II–4. APPARATUS FOR THE CONTINUOUS RECORDING OF THE WATER VAPOUR CONTENT OF THE AIR

For the continuous measurement of the transpiration rate, the moisture content of the air stream leaving the assimilation chamber has to be determined continuously. For this purpose an apparatus of small dimensions with a quick response and operating in streaming air was constructed. The author

is indebted to Dr. C. J. P. SPRUIT, of the staff of this laboratory, for this apparatus which is a modification of instruments described by DUNMORE (1938), STEIGER (1951), BRASTAD and BORCHARDT (1953), LIENEWEG (1955), and TANNER and SUOMI (1956). It is based on the principle that the electrical resistance of a film of lithium chloride is a function of the moisture content of an air stream led over it; this resistance is measured.

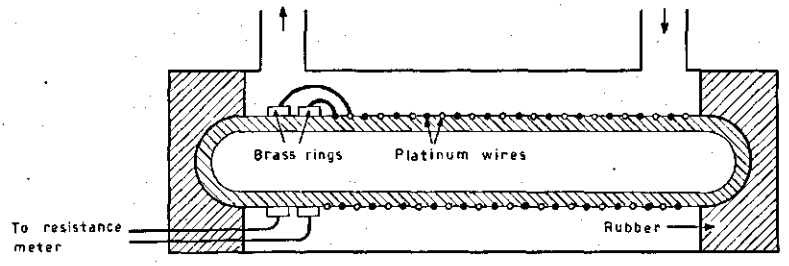


FIG. 4. Diagram, illustrating the lithium chloride hygrometer.

The detecting element (fig. 4) consists of two platinum wires (diameter 0.1 mm, length 120 mm), spirally wound side by side on a glass tube (diameter 10 mm). Tube and wires are covered with an aqueous solution of lithium chloride. The element is enclosed in a second glass tube, provided with an air inlet and an outlet.

After the wires are wound on the glass tube, the assembly is heated until the glass becomes soft, so that by blowing at one side of the tube, which is left open for the purpose, the wires become partially sealed in the glass surface. Each of the platinum wires is soldered to one of two brass rings, which are clamped around the tube and connected with the resistance meter. A small quantity of a 0.1 N LiCl-solution is then brushed over the tube and the wires, and allowed to dry.

The apparatus is placed in a thermostated waterbath, since the resistance depends on temperature. Before entering the apparatus, the air passes a copper tube, present in the same waterbath. At high relative humidities the results were first found to be irreproducible, which may have been due to small displacements of the diluted solution. In order to avoid this difficulty, the temperature of the waterbath was made a few degrees higher than the highest air temperature in the assimilation chamber, and, moreover, the surface of the glass tube was etched.

For the measurement of the resistance, the lithium chloride element is taken up in a voltage divider, supplied with a 900 $c.sec^{-1}$ tension in order to avoid polarization effects. The recorder is of the same type as that used for the CO_2-analyzer. A diagram of the electronic circuit, as designed by Dr. SPRUIT, is presented in fig. 5.

The moisture content is determined with an error of about 1 %, but the determination of the transpiration rate, which is based on the difference in moisture content of the air before and after passage of the leaf is less accurate. At high transpiration rates, the error is 2 %, and at low rates up to 7 %.

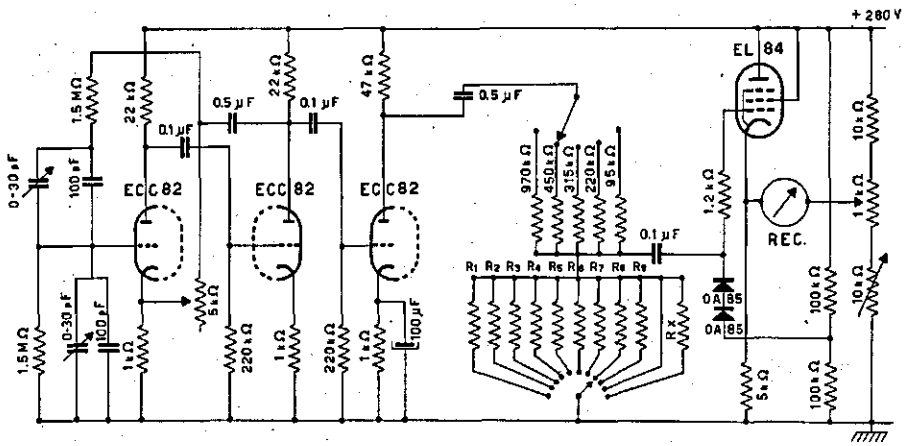


FIG. 5. Electronic circuit of the lithium chloride hygrometer. The resistances $R_{1\text{-}9}$ are used for the calibration of the set-up. R_X = the resistance of the detecting element; REC= recorder (internal resistance 6 kOhms, full scale deflection 100 mA).

II–5. THE GAS CIRCUIT

For reliable measurements of the photosynthetic rate, the air supply to the assimilation chamber should be in the order of 500 $l.h^{-1}$ (*cf.* section III–2), while the reference tube is supplied with air at a rate of 60 $l.h^{-1}$. Taking into account air losses in the overflow valves, air of the required CO_2-concentration should be available at a rate of 700 $l.h^{-1}$. At first, it was difficult to obtain reliable air pumps, inert to CO_2, and delivering a continuous output of 700 $l.h^{-1}$ at a head of approximately 100 cm H_2O. The electro-magnetic piston pumps finally adopted were found to operate quite satisfactorily (Reciprotor pumps, type 406G, Gentøfte, Denmark). For small air supplies and low pressures, electro-magnetic membrane pumps were used (Magnetos pump, B.A.S.F., Ludwigshafen, Germany).

The rate of the gas flow is measured with flow meters, consisting of a capillary tube, and a U-shaped manometer for measuring the pressure difference between both ends of the capillary tube. The manometer fluid of the air flow meters is water, coloured with a dye, while for the CO_2 flow meters liquid paraffin was used. The flow meters are calibrated by measuring the time required for the replacement of a certain volume of water by air or of a certain volume of paraffin by CO_2. The liquid is contained in a calibrated cylindrical glass tube, placed with its open end slightly below the level of the water or paraffin surface of a large vessel. The gas inlet is slightly below the open end of the cylinder. Appropriate corrections were made for temperature, hydrostatic pressure, and water vapour content.

A diagram of the gas circuit is presented in fig. 6. Air with the required CO_2-content is obtained by mixing pure CO_2 and CO_2-free air. A cylinder delivers CO_2 with a constant velocity to the vessel *M*1. The overflow valve *O*2 keeps pressure constant, and the flow is measured by the flow meter *F*2.

Air is freed from CO_2 by pumping it (*PR*1) first through 400 cm^3 of a 40 % KOH-solution, contained in a glass cylinder with a sintered glass plate near the air inlet, and then through a tube containing soda lime. After passing *O*1

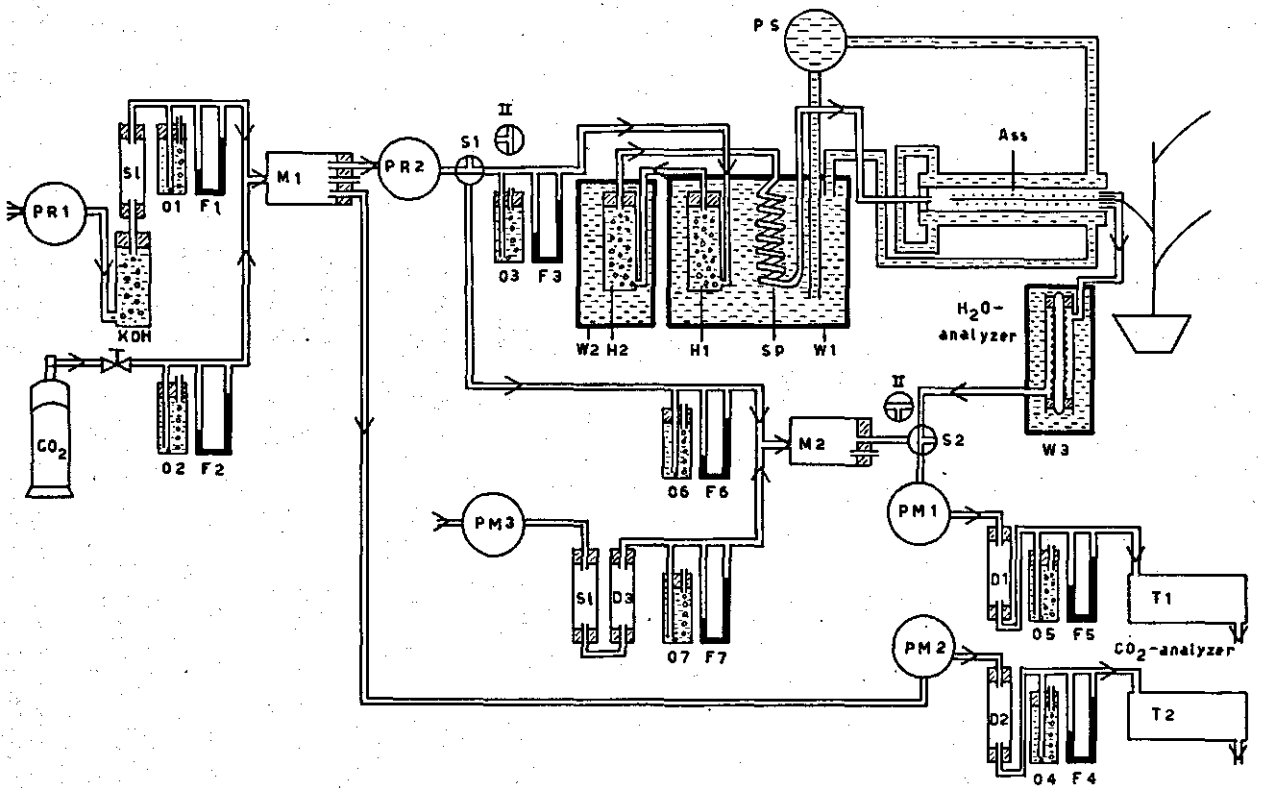


FIG. 6. Diagram of the gas circuit. Ass = assimilation chamber; D = drying tubes; F = flow meters; H = humidifiers; M = mixing vessels; O = overflow valves; PM = membrane pumps ("Magnetos"); PR = piston pumps ("Reciprotor"); PS = centrifugal pump ("Stuart"); S = stopcocks; Sl = soda lime; Sp = copper spiral; T1 and T2 = analyzer and reference tubes of the CO_2-analyzer.

and *F*1, the CO_2-free stream joins the CO_2 stream in a glass tube at a point 50 cm in front of the vessel *M*1, so that thorough mixing is obtained. *M*1 is provided with an open connection to the outside air, in order to prevent pressure changes in the system behind the pumps *PR*2 and *PM*2 affecting the pressure in *M*1 and the composition of the gas mixture by interference with the gas streams through the flow meters *F*1 and *F*2.

Part of the air in *M*1 is pumped (*PM*2) through a tube containing silica gel (*D*2) for drying, and then through *O*4 and *F*4 into the reference tube of the CO_2-analyzer. The overflow *O*4 is inserted in order to reduce pressure variation in the reference tube, since it was found that the accuracy of the CO_2-analysis was impaired by such variation: pressure changes, due to variations in flow rate, induce changes in the position of the mica windows and, consequently, in the reflection of the infrared beam.

Pump *PR*2 pumps air from *M*1 via *O*3 and *F*3 to the humidifier. The required water vapour pressure is obtained as follows: Air is saturated with water vapour by bubbling through a water column at a certain temperature, and this saturated air is then heated to the required temperature. The humidity of the air can be altered as required by varying the temperature of the water column. Gravimetric tests showed that complete saturation is not obtained when a single water column is used. Therefore, before being bubbled through the column at the required temperature, the air is led through a water column at a higher temperature, so that the air becomes oversaturated with respect to the temperature finally applied.

The humidifier consists of two glass tubes, *H*1 and *H*2, filled with distilled water, each provided with a sintered glass plate, and placed in the waterbaths *W*1 and *W*2 respectively. Both baths are provided with a thermo-relays, and with cooling, heating, and stirring equipment. The water in *W*1 is circulated by the centrifugal water pump *PS* through the double walls of the assimilation chamber. The temperature of *W*2 and *H*2 is lower than that of *W*1 and *H*1, and is adjusted in accordance with the desired vapour pressure. The temperature in *H*2 is indicated by a thermometer inserted through a hole in the rubber stopper, at the top of the tube. To prevent small droplets of water being carried by the air stream, a tube containing glass wool is in the circuit after *H*2. After humidification, the air passes through the copper spiral *Sp* (to obtain the correct temperature) and enters the assimilation chamber (*Ass*). After passing the leaf, the greater part of the air escapes to the external atmosphere, but a sample is drawn off by the pump *PM*1, and conducted first to the hygrometer, and then via *D*1, *O*5, and *F*5 to the analyzer tube (*T*1) of the CO_2-analyzer.

To calibrate the CO_2-analyzer, the stopcocks *S*1 and *S*2 are put in the position II. Again, air from *M*1 is led into the reference tube *T*2. By the action of pump *PR*2 another sample is carried from *M*1 via *O*6 and *F*6 to *M*2. Before reaching *M*2, it is mixed with a measured volume of CO_2-free air, delivered by pump *PM*3. The pump *PM*1 carries a sample of the mixture from *M*2 to the analyzer tube *T*1.

To check the zero point of the CO_2-analyzer, air from the reference tube can be supplied to the analyzer tube by a system of stopcocks, not represented in the diagram.

The hygrometer is calibrated with the same circuit as that used for transpiration measurements. Different vapour pressures are applied by varying the

temperature in *H*2. The humidified air is blown into the (empty) assimilation chamber, and a sample is flushed through the analyzer by pump *PM*1.

II–6. The assimilation chambers

The construction of the assimilation chambers should satisfy the following requirements (*cf.* also Chapter III): introduction of a leaf attached to the plant, reproducible position of the leaf in the centre-plane of the chamber, regular distribution of the air stream over the cross-section of the chamber, adaptation of the width of the chamber to the width of the leaf, double walls with circulating water for control of the leaf temperature.

Our "Plexiglass" chambers, inside dimensions 25 × 20 × 2 cm, have double, water-cooled walls. The upper wall can be taken off for the introduction of the leaf, and a small groove in one of the side walls admits the leaf petiole. After the introduction of the leaf into the chamber and application of the thermocouples, the front wall is screwed onto the lower part of the chamber. An air-tight fit is obtained by a rubber washer, and by sealing the groove which admits the leaf petiole.

The air inlet is at one side of the chamber, and consists of 18 small holes (⌀ 0.5 mm), regularly distributed over the width of the chamber. A regular air distribution is, furthermore, promoted by the fact that half of the inlet holes are directed upwards under an angle of 45°, whereas the other holes are facing downwards under the same angle.

After the air has passed along the leaf, a sample is drawn off for analysis at the side of the chamber opposite to the air inlet, through two tubes, each with nine small holes, and positioned close to the upper and lower walls respectively. The main air flow escapes through another set of 18 small holes, regularly distributed along the side of the chamber opposite to the air inlet. The groove admitting the leaf petiole is sealed during the experiments, so that the air can escape through the outlet holes only.

The leaf is kept in the centre-plane of the chamber by means of two networks of fine nylon wires. One of these is fixed to the lower part, the other to the upper wall of the chamber. The chamber closed, the distance between the leaf supports is 1 mm.

Plastic-covered rubber strips of different widths, and of the same length as the assimilation chamber, can be inserted into the chamber along the long sides, so that the effective width of the chamber can be adapted to the width of the leaf under investigation. During such experiments, the holes for the air inlet, sampling, and outlet which are outside the effective width of the chamber, are sealed.

II–7. The measurement of the leaf temperature

Leaf temperatures are measured with copper-constantan thermocouples (constantan ⌀ 0.08 mm, copper ⌀ 0.1 mm). The wires are joined side by side over a distance of 4 mm with acid-free solder and hammered as flat as possible. All measuring junctions are connected with the same reference junction, which is in a water-filled Dewar flask. The temperature of the reference junction is indicated by a thermometer, reading to 0.1 °C. By means of an eight-way switch the measuring junctions are successively connected with a portable

galvanometer (type A-70, KIPP, Delft, Holland), shunted in such a way that one cm scale deflection corresponds to 1 °C. An accuracy greater than 0.1 °C is obtained.

Introduction of the junctions into the leaves proved to be difficult, so that they were usually applied to the lower leaf surface.

II–8. THE IRRADIATION OF THE LEAVES

High light intensities were obtained with a set of five narrowly spaced high pressure mercury lamps (PHILIPS, *HO*-450 W) or with a set of four incandescent lamps with internal reflection (PHILIPS, Altrilux 500 W). A layer of eight cm running tap water was inserted between the lamps and the assimilation chamber. Behind this filter, 68 % of the radiation from the incandescent lamps was in the infrared ($\lambda > 700$ mμ), while this was only 6 % for the mercury lamps. The maximum intensities obtainable from both light sources were in the order of 30×10^4 erg.sec^{-1}.cm^{-2} in the spectral region between 400 and 700 mμ. Lower intensities were obtained by inserting various phosphorbronze screens of definite transmission values into the light path. The lamps were connected with the three phases of the AC-supply, to reduce cyclic flickering.

II–9. THE MEASUREMENT OF THE LIGHT INTENSITY

Light intensity was measured in absolute units (erg.sec^{-1}.cm^{-2}); for routine measurements barrier-layer photocells were used, because they have a quick response and are easy to handle. The non-uniform spectral sensitivity of the photocells necessitates calibration in absolute units for each type of light source used (see below).

The photocell (type 732, Electrocell G.m.b.H., Berlin-Dahlem) was mounted in a brass frame, and covered with a slightly convex opaline glass, in order to prevent damage. Furthermore, it was expected that closer agreement with the cosine law might be obtained in this way. Measurements in a beam of paralel light showed that a slight improvement only was obtained at angles of incidence greater than 65 °, whereas at angles between 30 ° and 65 ° the glass-covered cell showed a slightly greater deviation than an uncovered one (Table I). Since, however, in the photosynthesis experiments the angle of incidence rarely exceeded 40°, the opaline glass-covered cell was used throughout the experiments. If necessary, the deviation from the cosine law can be eliminated successfully, by using a design as described *e.g.* by PLEYEL and LONGMORE (1952) or HARTIG and HELWIG (1955).

TABLE I. Deviation from the cosine law (in %) for barrier-layer photocells with and without opaline glass cover.

	Angle of incidence								
	0°	10°	20°	30°	40°	50°	60°	70°	80°
Photocell + cover	0	0	1	1	3.5	5.5	8.5	18	35
Photocell — cover	0	0	0	1	1.5	2.0	7.0	20	48

The photocurrent was measured with a shock-proof galvanometer (type K, AEG, internal resistance 100 Ohms, full scale deflection 100 μA).

For calibration of the photocell-galvanometer system in absolute units, the relation between the galvanometer deflection and the light energy as measured with a calibrated thermopile, was determined. The calibration of the thermopile was checked periodically at the Physical Laboratory of the Utrecht University, and was 0.35 V/(W.cm^{-2}). The galvanometer used for measuring the thermopile output was calibrated with the device, described by STOLWIJK (1954).

With the thermopile, the intensity of the total radiation was measured and, separately, the intensity at wavelengths > 700 mμ by inserting a filter with a steep short wavelength cut-off near 700 mμ (Schott RG-8). The difference between the two measurements represents radiation at wavelengths shorter than 700 mμ. It includes radiation between 400 mμ and the short wavelength cut-off of the glass cover of the thermopile (320 mμ), whereas the spectral region between 400 and 700 mμ is mainly active in photosynthesis. For the high pressure mercury lamps, about 10% of the radiation between 320 and 700 mμ is present in the 365 mμ line, and the radiation between 400 and 700 mμ was obtained by multiplying the difference between the total energy and the energy transmitted by the RG-8-filter with 0.90. For the other light sources used, the radiation between 320 and 400 mμ is small as compared with that between 400 and 700 mμ, and corrections were omitted.

The sensitive surface of our thermopile is somewhat below the rim of the housing, so that large angles of incidence must be avoided during calibration. To this end, a hollow cylinder with internal non-reflecting diaphragms was inserted in the light beam, so that the maximum angle of incidence was 20°.

Originally, the galvanometer-photocell system was adapted to various ranges of light intensity by shunting the galvanometer. The effective resistances of the shunted galvanometer were 1.25, 6, 20, and 100 Ohms, yielding full scale deflection for photocurrents of 10, 2, 0.5, and 0.1 mA, respectively. Decrease in external resistance, applied with increasing photocurrents, results in an improved linearity of response (BERGER, 1956). Nevertheless, examination of the deviation from linearity of the photocell-shunt-galvanometer system showed that, even with these precautions, the deviation was considerable, so that the calibration had to be made at a range of light intensities.

Linearity tests were made in the light beam of a 500 W slide projector. Part of the infrared radiation was absorbed by a heat absorbing filter. At various distances from the projector, the light energy between 400 and 700 mμ was measured with the thermopile, while at the same points the current generated by the photocell was measured. For each intensity the relation between light energy and photocurrent was determined (Table II) for two photocells: cell *C1*,

TABLE II. Response of barrier-layer photocells to light intensity, when shunting the galvanometer or introducing neutral light filters, in relative units [incident energy/photocurrent, and incident energy/(galvanometer deflection/fractional transmission of the filter) respectively].

I	II	III	IV
	Response		
Light intensity ($\times 10^4$ erg.sec^{-1}.cm^{-2}, 400–700 mμ)	Photocell *C1* with shunt	Photocell *F5* with shunt	Photocell *C1* with filter
0.14	100	100	100
0.49	100	100	101
0.95	99	101	99
2.08	101	108	100
6.25	107	120	100
19.60	118	149	101

selected by the manufacturer for good linearity of response, and a non-selected cell *F5*. For *F5*, the deviation from linearity started at 10^4 erg.sec^{-1}.cm^{-2} (corresponding to a photocurrent of 210 μA), and was 49% at 19.6×10^4 erg.sec^{-1}.cm^{-2} (column III). For *C1* the deviation began at 2×10^4 erg.sec^{-1}.cm^{-2} (photocurrent 457 μA); the maximum deviation measured was 18% (column II).

In order to simplify the calibration procedure, shunts were not used in later experiments, but neutral filters with appropriate transmission factors were inserted between the photocell and the opaline glass cover. Since with this set-up the actual photocurrent never exceeded 100 μA, the response was linear over the entire range of intensities applied (column IV), and calibration could be restricted to one light intensity.

Neutral filters were made by grinding one side of each of two glass discs, and covering these sides with a thin layer of graphite. The discs then were cemented along the edges with the graphite sides facing each other.

CHAPTER III

EVALUATION OF THE EXPERIMENTAL CONDITIONS IN PHOTOSYNTHESIS EXPERIMENTS WITH LEAVES IN ASSIMILATION CHAMBERS

III–1. Introduction

For a quantitative interpretation of the experimental results, two requirements should be fulfilled: accurate measurement of the photosynthetic rate, and exact knowledge of the experimental conditions. In fact, the conditions directly determining the photosynthetic rate, *viz.* the light intensity, the CO_2-concentration, and the temperature in the chloroplasts, cannot be measured. The data actually collected, however, should allow an approximation of these conditions. With higher plants, the situation is particularly difficult for several reasons: The gaseous medium is a poor heat conductor, so that appreciable differences between air and chloroplast temperatures may occur; CO_2 has to diffuse from the air towards the chloroplasts through the complicated system of stomata, intercellular spaces, and mesophyll cells; under conditions of CO_2-limitation, the effective CO_2-concentration in the air has to be estimated. In addition, a comparison between the experiments of different investigators is hampered because different light sources have been used, and light intensities have been expressed in absolute units as well as in photometric units.

The present chapter, therefore, is devoted to a discussion of certain features of the measurement of the CO_2-concentration, the light intensity, and the temperature.

III–2. The effect of the rate of air supply upon the rate of photosynthesis under conditions of CO_2-limitation

The considerations will be restricted to methods involving gas analysis, with an intact leaf enclosed in an assimilation chamber, while the gas exchange is estimated from the difference in CO_2-concentration between the intake and outlet air. It is also presumed that the air is not recirculated in the chamber. Finally, the considerations are restricted to conditions of CO_2-limitation.

The actual rate of photosynthesis, P_{act}, is calculated from:

$$P_{act} = \frac{Q \cdot (c_0 - c_e) \cdot 10^{-2}}{A} \qquad (1)$$

in which P_{act} = the rate of photosynthesis ($cm^3\ CO_2 . cm^{-2} . sec^{-1}$)
c_0 = the CO_2-content of the inlet air (% CO_2)
c_e = the CO_2-content of the outlet air (% CO_2)
Q = the rate of air supply ($cm^3 . sec^{-1}$)
A = the leaf area (cm^2)

In the assimilation chamber, CO_2-gradients exist in the direction of the air flow, as well as in the direction perpendicular to the leaf surface. The former gradients, in particular, may assume large values when special precautions are not taken, so that corrections have to be applied. When the relation between the CO_2-concentration in the external air and the rate of photosynthesis is

investigated, the effective CO_2-concentration, c_{eff}, must be determined or closely approximated, *i.e.* the concentration which, when kept constant in the direction of the air flow, causes the same rate of photosynthesis as that actually observed. When the photosynthetic rate in normal air (0.03 % CO_2) has to be established, c_{eff} will generally deviate from 0.03 %, so that, under conditions of CO_2-limitation, P_{act} has to be reduced to $P_{0.03}$, *i.e.* the rate in air with $c_{eff} = 0.03$ % CO_2.

From the experimental data (c_0, c_e), the mean CO_2-concentration is usually calculated according to

$$\bar{c} = (c_0 + c_e)/2 \tag{2}$$

and it is then supposed that

$$\bar{c} = c_{eff}. \tag{2a}$$

When reducing P_{act} to $P_{0.03}$, a linear relation between photosynthesis and CO_2-concentration is usually assumed for concentrations between zero and 0.03 %, and this is approximately correct, see Chapters IV and V. The corrected rate is then calculated according to

$$P_{corr} = P_{act} \cdot 0.03/\bar{c} \tag{3}$$

and it is supposed that then

$$P_{corr} = P_{0.03} \tag{3a}$$

The reliability of the experiments depends on the validity of the suppositions 2a and 3a, and hence on the validity of the supposition that the CO_2-concentration decreases linearly in the direction of the air flow, and that the change in CO_2-concentration along both sides of the leaf is the same and amounts to $c_0 - c_e$. It is easily seen that the latter is not always true, *e.g.* in the case of a hypostomatous leaf, or when different quantities of air are flowing over the two leaf surfaces. In general, high flow rates will reduce the gradients and hence the influence of systematic errors, see HEINICKE and HOFFMAN (1933), and DECKER (1947).

As far as we know, no quantitative data are available concerning the effect of the rate of air supply upon the rate of photosynthesis of a single leaf, enclosed in an assimilation chamber, in which the effect of different CO_2-gradients over both leaf surfaces is considered. An empirical approach would require elaborate experiments, so that a theoretical treatment has been attempted for a somewhat idealized case, in which the following features are assumed (see also fig. 7A).

a. A rectangular leaf strip is considered (width 1 cm, length L cm), enclosed in a closely fitting chamber (also 1 cm wide).

b. The air flows in the direction of the long axis of the leaf.

c. The air stream is strongly turbulent, so that the CO_2-gradient perpendicular to the leaf surface can be neglected (this gradient is discussed in Chapter V).

d. The leaf may be situated at a variable height in the chamber, dividing the total air stream with rate Q into two separate streams with rates $(1-p)Q$ and pQ respectively, in which p and $1-p$ represent the fractions into which the height of the chamber is divided by the leaf.

e. The rate of photosynthesis is limited by the external CO_2-concentration, and more specifically by the rate of diffusion of CO_2 from the external air towards the reaction centre in the chloroplasts (see Chapter V).

For different experimental conditions (different values of the flow rate, Q, and of the position of the leaf in the chamber, p) and for different photosynthetic capacities of the leaf (different values of each of the diffusion resistances through upper and lower leaf surface, R_u and R_b, respectively) we should know the following quantities:

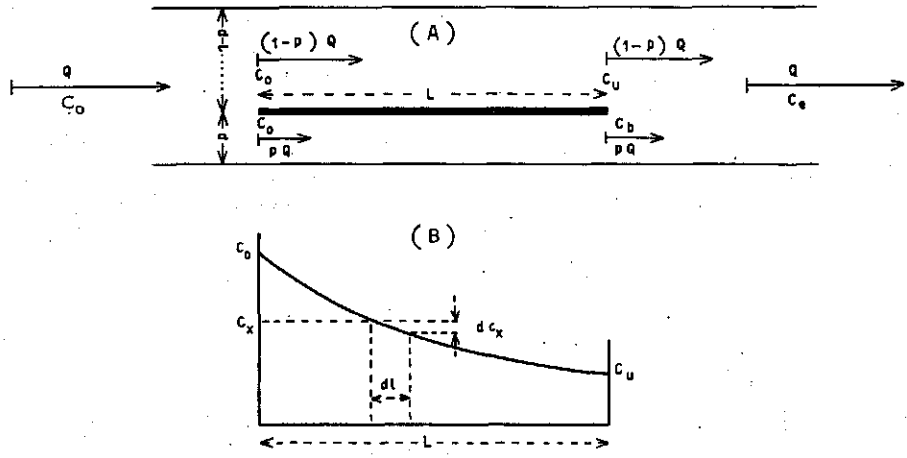


FIG. 7. A. Position of the leaf, distribution of the air, and CO_2-concentrations in the assimilation chamber. B. Distribution of the CO_2-concentration in the air stream along the upper leaf surface. For explanation see text.

1. The relation between the actual rate of photosynthesis, P_{act}, (equation 1) and the partial CO_2-uptakes through the upper and lower leaf surface respectively:

$$P_{act,u} = \frac{(1-p) \cdot Q \cdot (c_0 - c_u) \cdot 10^{-2}}{L} \tag{4}$$

and

$$P_{act,b} = \frac{p \cdot Q \cdot (c_0 - c_b) \cdot 10^{-2}}{L} \tag{5}$$

in which c_u and c_b are the CO_2-concentrations in the air streams along the upper and lower leaf surfaces respectively, immediately after having passed the leaf. When these separate streams are united, the concentration becomes c_e, *cf.* fig. 7A.

2. The relation between the mean CO_2-concentration $\bar{c} = (c_0 + c_e)/2$ and the physiologically important effective CO_2-concentrations in the separate air streams along the upper and lower leaf surfaces, $c_{eff,u}$ and $c_{eff,b}$, respectively.

3. The relation between the corrected rate of photosynthesis, P_{corr} (equation 3), and $P_{0.03} = P_{0.03,u} + P_{0.03,b}$, being the sum of the CO_2-uptakes through upper and lower leaf surface when $c_{eff,u} = c_{eff,b} = 0.03\,\%$ CO_2.

Generally, the rate of photosynthesis may be identified with the rate of diffusion of CO_2 from the external air towards the reaction centre in the chloroplasts. In Chapter V, we shall outline the principles according to which the rate of CO_2-diffusion, and hence the rate of photosynthesis, may be expressed as:

$$P = \frac{(c_{air} - c_{chl}) \cdot 10^{-2}}{R} \tag{6}$$

in which P = the rate of photosynthesis ($cm^3 CO_2 . cm^{-2} . sec^{-1}$)
c_{air} = the CO_2-concentration in the external air (% CO_2)
c_{chl} = the CO_2-concentration in the chloroplasts (% CO_2)
R = the diffusion resistance per cm^2 leaf surface ($sec . cm^{-1}$). R depends on the dimensions of the path of diffusion and on the diffusion constants of CO_2 in air and in the protoplasm of the mesophyll cells (see Chapter V).

When, as assumed, the rate of the diffusion process limits the rate of photosynthesis, c_{chl} is approximately zero (see Chapter V), so that, when the paths of diffusion via the upper and lower leaf surfaces do not interfere, the relation between the effective concentration and the actually occurring rate of photosynthesis for each of the leaf sides is given by

$$P_{act,u} = \frac{c_{eff,u} \cdot 10^{-2}}{R_u} \tag{7}$$

and

$$P_{act,b} = \frac{c_{eff,b} \cdot 10^{-2}}{R_b} \tag{8}$$

Combination of (7) and (4), and (8) and (5) respectively, gives

$$c_{eff,u} = \frac{(1-p) \cdot Q \cdot R_u \cdot (c_0 - c_u)}{L} \tag{9}$$

and

$$c_{eff,b} = \frac{p \cdot Q \cdot R_b \cdot (c_0 - c_b)}{L} \tag{10}$$

For expressing c_u (respectively c_b) in Q, p, and R_u (respectively R_b), consider a leaf section dL (fig. 7B). According to the equations 4 and 7, the rate of diffusion through dL cm^2 of the upper leaf surface is given by

$$-(1-p) \cdot Q \cdot dc_x \cdot 10^{-2} = \frac{c_x \cdot 10^{-2} \cdot dL}{R_u} \tag{11}$$

Hence

$$-R_u \cdot (1-p) \cdot Q \cdot \int_{c_0}^{c_u} \frac{dc_x}{c_x} = \int_0^L dL \tag{12}$$

Integrating, we get

$$\ln \frac{c_u}{c_0} = \frac{-L}{R_u \cdot (1-p) \cdot Q} \tag{13}$$

Hence

$$c_u = c_0 \cdot e^{\frac{-L}{R_u \cdot (1-p) \cdot Q}} \tag{14}$$

and

$$c_b = c_0 \cdot e^{\frac{-L}{R_b \cdot p \cdot Q}} \tag{15}$$

The quantity of CO_2 in the total air stream when the upper and lower streams are reunited, $Q \cdot 10^{-2} \cdot c_e$, equals the sum of the quantities of CO_2 in the separate streams having passed the leaf, $(1-p) \cdot Q \cdot 10^{-2} \cdot c_u + p \cdot Q \cdot 10^{-2} \cdot c_b$, so that

$$c_e = (1-p) \cdot c_u + p \cdot c_b \tag{16}$$

Substitution of the expressions for c_u and c_b (equations 14 and 15) in the equations 9, 10, and 16, gives the effective concentrations and c_e as a function of the experimental conditions (expressed by p, Q, L, c_0) and of the internal rate determining factors (R_u and R_b). Therefore, the validity of the assumptions 2a and 3a, and the reliability of photosynthesis measurements can now be examined by substituting commonly occurring values for Q, p, L, R_u, and R_b in the equations.

It may be remarked in passing that combination of the equations 13 and 9 gives

$$c_{eff,u} = (c_0 - c_u) / \ln \frac{c_0}{c_u}$$

so that the effective CO_2-concentration is better defined by the logarithmic mean than by the arithmetic mean of the CO_2-concentrations in the incoming and the outgoing air. However, as long as the ratio of the concentrations is <2, the error introduced by taking the arithmetic mean is less than 4%.

In all cases, we have taken $L = 10$ cm, and $c_0 = 0.03\%$ CO_2. For different values op p (different positions of the leaf in the assimilation chamber), two rates of air supply are considered, *viz.* $Q = 10$ and 1 cm^3 air.sec^{-1} respectively, corresponding to a high flow rate (3.6 $l.h^{-1}.cm^{-2}$) as applied in our experiments, and to a low rate (0.36 $l.h^{-1}.cm^{-2}$), as applied by many investigators.

Two extreme leaf types are considered: a hypostomatous leaf with $R_u = \infty$, and an amphistomatous leaf with $R_u = R_b$. For the hypostomatous leaf R_b is taken to be 10 $sec.cm^{-1}$; at 0.03% CO_2 this corresponds to a photosynthetic rate $P_{0.03} = 0.03 \times 10^{-2} / 10 = 3 \times 10^{-5}$ cm^3 $CO_2.cm^{-2}.sec^{-1} = 108$ mm^3 $CO_2.cm^{-2}.h^{-1}$. According to our experience, this rate is of the right order of magnitude. For the amphistomatous leaf we have taken $R_u = R_b = 10$ $sec.cm^{-1}$, with a rate of photosynthesis at 0.03 % CO_2 of $P_{0.03} = 6 \times 10^{-5}$ cm^3 $CO_2.cm^{-2}.sec^{-1}$. (The choice of the resistance values for the amphistomatous leaf is somewhat arbitrary, because it involves the assumption that, in principle, amphistomatous leaves have a higher actual photosynthetic capacity than hypostomatous leaves, and that the diffusion resistances R_u and R_b are, for the greater part, located in separate diffusion paths. We have not considered any other resistance values, since only an approximation to the situation is attempted).

The results are presented in Table III. We have calculated c_u, c_b, and c_e

TABLE III. Influence of the rate of air supply and the position of the leaf in the assimilation chamber on the CO_2-concentration and the rate of photosynthesis in hypostomatous and amphistomatous leaves. For explanation see text.

		I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV
		p	c_u	c_b	c_e	$P_{act,u}$	$P_{act,b}$	P_{act}	P_{act}	$c_{eff,u}$	$c_{eff,b}$	$\bar{c} = (c_o + c_e)/2$			P_{corr}	P_{corr}
		(% CO_2)				($\times 10^{-5}$ $cm^3 CO_2$ · $cm^{-2}.sec^{-1}$)			(% of $P_{0.03}$)	(% CO_2)		(% CO_2)	(% of $c_{eff,u}$)	(% of $c_{eff,b}$)	($\times 10^{-5}$ cm^3 CO_2 $.cm^{-2}$ $.sec^{-1}$)	(% of $P_{0.03}$)
Hypostomatous leaf ($R_u = \infty$; $R_b = 10$)	Q = 10	1	—	0.0271	0.0271	0	2.86	2.86	95	—	0.0286	0.0286	—	100	3.00	100
		0.75	0.0300	0.0262	0.0272	0	2.81	2.81	94	—	0.0281	0.0286	—	102	2.95	98
		0.50	0.0300	0.0246	0.0273	0	2.72	2.72	91	—	0.0272	0.0286	—	105	2.85	95
		0.25	0.0300	0.0201	0.0275	0	2.47	2.47	82	—	0.0247	0.0288	—	116	2.57	86
		0	0.0300	—	0.0300	0	0	0	0	—	—	0.0300	—	—	—	—
	Q = 1	1	—	0.0110	0.0110	0	1.90	1.90	63	—	0.0190	0.0205	—	108	2.78	93
		0.75	0.0300	0.0079	0.0134	0	1.66	1.66	55	—	0.0166	0.0217	—	131	2.30	77
		0.50	0.0300	0.0041	0.0170	0	1.30	1.30	43	—	0.0130	0.0235	—	182	1.66	55
		0.25	0.0300	0.0005	0.0226	0	0.74	0.74	24	—	0.0074	0.0263	—	357	0.84	28
		0	0.0300	—	0.0300	0	0	0	0	—	—	0.0300	—	—	—	—
Amphistomatous leaf ($R_u = R_b = 10$)	Q = 10	1	—	0.0271	0.0271	0	2.86	2.86	48	—	0.0286	0.0286	—	100	3.00	50
		0.75	0.0201	0.0262	0.0247	2.47	2.81	5.28	88	0.0247	0.0281	0.0274	110	97	5.78	96
		0.50	0.0246	0.0246	0.0246	2.72	2.72	5.44	91	0.0272	0.0272	0.0272	100	100	6.00	100
		0.25	0.0262	0.0201	0.0247	2.81	2.47	5.28	88	0.0281	0.0247	0.0274	97	110	5.78	96
		0	0.0271	—	0.0271	2.86	0	2.86	48	0.0286	—	0.0286	100	—	3.00	50
	Q = 1	1	—	0.0110	0.0110	0	1.90	1.90	32	—	0.0190	0.0205	—	108	2.77	46
		0.75	0.0005	0.0079	0.0061	0.74	1.66	2.39	40	0.0074	0.0166	0.0180	245	109	3.98	66
		0.50	0.0041	0.0041	0.0041	1.30	1.30	2.59	43	0.0130	0.0130	0.0170	132	132	4.55	76
		0.25	0.0079	0.0005	0.0061	1.66	0.74	2.39	40	0.0166	0.0074	0.0180	109	245	3.98	66
		0	0.0110	—	0.0110	1.90	0	1.90	32	0.0190	—	0.0205	108	—	2.77	46

(columns II, III, IV) for different positions of the leaf (column I). The actual photosynthetic rates and the relative values of P_{act} are given in columns V, VI, VII, VIII. The physiologically important effective concentrations, $c_{eff,u}$ and $c_{eff,b}$ (columns IX and X) are compared with the arithmetic mean of c_0 and c_e (colum XI) in columns XII and XIII, while the absolute values and relative values of the corrected photosynthetic rate (taking $P_{0.03} = 100$) are presented in columns XIV and XV.

Both the rate of air supply and the situation of the leaf in the assimilation chamber markedly affect the experimental results. With the leaf situated in the centre plane of the chamber ($p = 0.5$) and at the highest flow rate, $\bar{c}$ represents fairly well the effective CO_2-concentrations (deviations 5 % and zero for both leaf types), while P_{corr} gives a good estimate of $P_{0.03}$. At the lower flow rate, however, $\bar{c}$ gives an overestimation of 82 % and 32 % of the effective concentrations respectively, and the corresponding values for P_{corr} are only 55 % and 76 % of $P_{0.03}$.

Poorly reproducible results are obtained when no precautions are taken to ensure a reproducible position of the leaf in the assimilation chamber. For the relative situations $p = 0.75$ and 0.25, and for the low flow rate, in hypostomatous leaves P_{corr} is 77 % and 28 % of $P_{0.03}$ respectively.

Still greater deviations occur when the assimilation chamber does not fit the leaf closely. This effect was calculated for the amphistomatous leaf, with $Q = 10$, for a width of the chamber twice that of the leaf, and supposing that no exchange of CO_2 occurs in the direction of the width of the chamber. For $p = 0.75$ and 0.5, P_{corr} then becomes 85 % and 90 % of $P_{0.03}$ respectively, instead of 96 % and 100 % for the closely fitting chamber.

In the case considered, c_0 was equal to the concentration to which the photosynthetic rates were reduced. Smaller errors will be obtained when the latter is between c_0 and c_e.

It is clear that under conditions of CO_2-limitation, reproducible and comparable results can only be obtained when several precautions are taken, *e.g.* high rates of air supply, a reproducible position of the leaf in the assimilation chamber, and closely fitting chambers. In our opinion several conflicting data in literature can be explained by the fact that these requirements have been neglected. Our assimilation chambers (see Chapter II) assure a close fit around the leaf and a reproducible situation of the leaf in the chamber. Furthermore, care was taken to ensure an even distribution of the air stream throughout the chamber. With respect to the high rates of flow applied, an extremely sensitive CO_2-analyzer has been developed, so that small concentration differences could be detected accurately. An even better device would be to recirculate the air in the chamber (VAN DEN HONERT, 1930); with a rate of recirculation nQ, and an overall rate Q, the gradient over the leaf is only $(c_0-c_e) / (n+1)$, whereas the difference between incoming and outgoing air is c_0-c_e. DECKER (1947) applied rapid recirculation, but did not realize that the mean CO_2-concentration is much better approximated by $c_e + (c_0-c_e) / 2 (n+1)$ than by the arithmetic mean $c_e + (c_0-c_e) / 2$. The CO_2-concentrations in his fig. 6 are, therefore, considerably overestimated.

III–3. The relation between absolute and photometric units for different light sources in the 400 to 700 mμ region

The light reaction in photosynthesis is a photochemical process, so that the light conditions should preferably be characterized by the number of quanta absorbed. In most experimental set-ups, however, no facilities are available for the measurement of the spectral absorption curve of leaves, and the light conditions are usually defined as the energy incident upon a leaf (*e.g.* erg.sec^{-1}.cm^{-2}) or as the illuminance of the leaf (lux or ft-c). In fact, photometric units are unsuitable for this purpose, because the action spectra of photobiological processes in plants differ from the luminosity curve of the human eye, on which the photometric units are based, see section III–4, and Brackett (1935), Rabinowitch (1951), *Comm. Plant Irradiation (Netherlands)* (1953, 1955). Nevertheless, photometric units are still widely used. In order to facilitate the comparison between different experiments, the relation between both units in the spectral region between 400 and 700 mμ was calculated for various light sources. The calculations are based upon the luminosity curve of the human eye, the emission spectrum of the light source, and the mechanical equivalent of light. The luminosity curve (V_λ, fig. 8) was taken from Kohlrausch (1947); at 555 mμ, $V = 1$.

The emission spectra of the various lamps (E_λ), were supplied by the manufacturers or have been taken from Funke and Oranje (1951). The solar spectrum, as given by Moon (1940), holds for 30° solar angle, 20 mm precipitable water, 300 particles dust/mm^3, and 2.8 mm ozone. The following lamps are considered: incandescent lamps of 100 and 500 W, different types of fluorescent tubes, a high pressure mercury vapour lamp (*HO*–450 W, Philips), a super high pressure mercury vapour lamp with fluorescent bulb (*HPL*–400 W, Philips), and a super high pressure mercury vapour lamp blended with light from a filament lamp (*ML*–250 W, Philips). (In Great Britain the *HO*, *HPL*, and *ML* lamps are designated as *MA*, *MBF*, and *MBT* lamps, respectively).

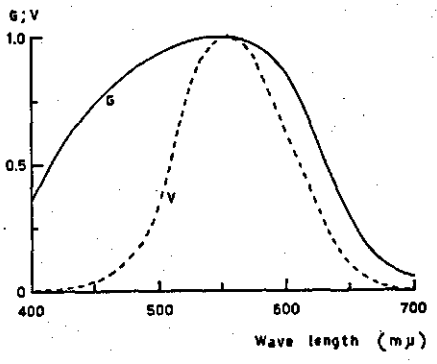


Fig. 8. Luminosity curve of the human eye (V), and spectral sensitivity of a "normal" photocell (G), taken from Berger (1956).

The mechanical equivalent of light, M_λ, is taken to be 650 lumen per watt for $\lambda = 555$ mμ, being the average of the values given by several authors.

From the definition of the lumen and the watt it follows that $M_{555} = 650$ lumen.W^{-1} $= 1.54 \cdot 10^{-3}$ W.lumen^{-1} $= 1.54 \cdot 10^{-3}$ W.m^{-2}. (lumen.m^{-2})$^{-1}$ $=$ 1.54 erg.sec^{-1}.cm^{-2}.lux^{-1}, because 1 lumen.m^{-2} $=$ 1 lux, and 1 W $= 10^7$ erg. sec^{-1}. Hence, $M_\lambda = 1.54/V_\lambda$ erg.sec^{-1}.cm^{-2}.lux^{-1}, and E_λ erg.sec^{-1}.cm^{-2} $=$ $E_\lambda \cdot V_\lambda / 1.54$ lux.

The average values of E_λ and $E_\lambda \cdot V_\lambda$ were calculated for wave length intervals of 20 mμ. For the 400 to 700 mμ region the radiometric/photometric ratio on a lux-basis (k_l) is then given by

$$k_l = \frac{1.54 \cdot \Sigma E_\lambda}{\Sigma E_\lambda \cdot V_\lambda} \text{ erg.sec}^{-1}.\text{cm}^{-2}.\text{lux}^{-1} \qquad (17)$$

while on a ft-c-basis, the ratio $k_f = 10.8 \times k_l$, since 1 ft-c equals 10.8 lux.

The values of k_l and k_f are listed in columns II and III of Table IV; between brackets the relative values are given, taking the value for a 100 W incandescent lamp equal to 100. The ratio is low for sources with a high radiation in the green region, which is due to large values of V. Hence, for *HO*-450W the relative value is only 58. As for the "white" light sources, the incandescent lamps have the highest ratio value, which is due to the high

TABLE IV. The relationship between energy units and photometric units for different light sources in the spectral region 400–700 mμ.
Column II: erg.sec^{-1}.cm^{-2} per lux; column III: erg.sec^{-1}.cm^{-2} per ft-c; column IV: erg.sec^{-1}.cm^{-2} per "lux", indicated by a luxmeter, containing a photocell with a spectral sensitivity, not corresponding to the luminosity curve of the human eye, and calibrated in lux only for an incandescent lamp of 100 W; between brackets: relative values, taking the values for a 100 W incandescent lamp equal to 100.

I	II	III	IV
Light source	erg.sec^{-1}.cm^{-2} per lux	erg.sec^{-1}.cm^{-2} per ft-c	erg.sec^{-1}.cm^{-2} per "lux" for uncorrected photocell
Sun	4.00 *(94)*	43.2 *(94)*	3.47 *(82)*
Incand. 500 W	4.16 *(98)*	44.9 *(98)*	4.10 *(97)*
Incand. 100 W	4.23 *(100)*	45.7 *(100)*	4.23 *(100)*
HPL 400 W	3.48 *(82)*	37.6 *(82)*	3.64 *(86)*
ML 250 W	3.47 *(82)*	37.6 *(82)*	3.66 *(86)*
HO 450 W	2.47 *(58)*	26.7 *(58)*	2.83 *(67)*
Fluorescent tubes (Philips)			
Warm tint (*TL*-29)	2.80 *(66)*	30.2 *(66)*	3.09 *(73)*
De luxe warm tint (*TL*-32)	3.64 *(86)*	39.3 *(86)*	3.70 *(87)*
White (*TL*-33)	3.11 *(73)*	33.6 *(73)*	3.09 *(73)*
De luxe white (*TL*-34)	3.61 *(85)*	39.0 *(85)*	3.32 *(78)*
Daylight (*TL*-55)	3.64 *(86)*	39.3 *(86)*	3.17 *(75)*
Blue (*TL*-18)	7.55 *(178)*	81.7 *(178)*	3.32 *(78)*
Green (*TL*-17)	2.26 *(53)*	24.4 *(53)*	2.58 *(61)*
Yellow (*TL*-16)	2.52 *(59)*	27.2 *(59)*	3.18 *(75)*
Red (*TL*-15)	14.68 *(347)*	158.6 *(347)*	9.16 *(216)*
Fluorescent tubes (Gen. Electric)			
Warm white	2.81 *(66)*	30.3 *(66)*	3.08 *(73)*
De luxe warm white	3.17 *(75)*	34.2 *(75)*	3.35 *(79)*
Cool white	3.13 *(74)*	33.8 *(74)*	3.02 *(71)*
De luxe cool white	3.41 *(80)*	36.8 *(80)*	3.05 *(72)*
Daylight	3.44 *(81)*	37.1 *(81)*	3.02 *(71)*
Blue	5.80 *(137)*	62.7 *(137)*	3.15 *(74)*
Green	2.07 *(49)*	22.4 *(49)*	2.55 *(60)*
Gold	2.28 *(54)*	24.6 *(54)*	2.96 *(70)*
Pink	3.96 *(93)*	42.8 *(93)*	3.96 *(93)*
Red	8.65 *(204)*	93.4 *(204)*	6.70 *(158)*

proportion of radiation in the red. In the coloured fluorescent lamps, very high values are reached by the red (347) and blue (178) ones, and very low values by the green (49) and yellow ones (59).

In order to obtain an impression of the ratio value in a crop, k_l was calculated for sunlight transmitted by one and three layers of leaves respectively. The fraction of light transmitted (t_λ) was calculated from the mean spectral absorption (a_λ) and reflection (r_λ) curves of four leaves of a "normal" type (bean, spinach, Swiss chard, tobacco), as given by MOSS and LOOMIS (1952). Average values of t_λ were calculated for 20 mμ intervals, and the value of k_l for sunlight after passing one leaf layer was obtained from

$$k_l' = \frac{1.54 \cdot \Sigma E_\lambda \cdot t_\lambda}{\Sigma E_\lambda \cdot t_\lambda \cdot V_\lambda} \qquad (18)$$

and in the case of three leaf layers from

$$k_l'' = \frac{1.54 \cdot \Sigma E_\lambda \cdot t^3{}_\lambda}{\Sigma E_\lambda \cdot t^3{}_\lambda \cdot V_\lambda} \qquad (19)$$

The relative values are only 55 and 47 respectively, as compared with 94 for full sunlight. Therefore, even for a comparison of the light energy conditions above and in a crop, photometric units will give erroneous results.

Most of the commercially available light meters are calibrated in lux or ft-c for incandescent light only. For this light source, the light energy corresponding to 1 lux and 1 ft-c is found in Table IV, columns II and III respectively. These columns also contain conversion factors for other light sources which, however, can only be used when the photocell is provided with a filter so as to make its spectral sensitivity equal to that of the human eye. When the photocell is not provided with such a filter, the meter does not indicate correct lux values when used with light sources for which the set-up has not been calibrated. In this case correct values of the incident energy cannot be computed by simply using the conversion factors of columns II and III.

Obviously, a certain meter indication (*e.g.* 1 "lux") under all conditions will correspond to the same photocurrent (expressed in arbitrary units). The problem, therefore, is to derive a general expression for the flux of energy generating the photocurrent corresponding to the meter indication of 1 "lux", assuming that the spectral energy distribution, $E\lambda$, of the light source, and the spectral sensitivity, $G\lambda$, of the photocell are known, and that the set-up has been calibrated for a 100 W incandescent lamp only. According to these assumptions, an incident energy of 1 erg.sec^{-1}.cm^{-2} generates a photocurrent of $G\lambda$ units for any wave length; for the spectral region from 400 to 700 mμ, the photocurrent generated by 1 erg.sec^{-1}.cm^{-2} amounts to $(\Sigma E\lambda \cdot G\lambda) / \Sigma E\lambda$ units, in which $E\lambda \cdot G\lambda$ and $E\lambda$ have been taken as mean values for 20 mμ intervals. Accordingly, 1 unit of photocurrent corresponds to $\Sigma E\lambda / (\Sigma E\lambda \cdot G\lambda)$ erg.sec^{-1}.cm^{-2}. (The photocurrent generated by light energy between 400 mμ and the short wavelength limit of G, and between 700 mμ and the long wavelength limit of G has been neglected, because the light energy distribution of most light sources was not known in these spectral regions. The maximum error introduced is estimated to be 3%).

Since for a 100 W incandescent lamp 1 lux equals 4.23 erg.sec^{-1}.cm^{-2} (see Table IV), a meter indication of 1 lux corresponds to a photocurrent of 4.23 $(\Sigma E\lambda' \cdot G\lambda) / \Sigma E\lambda'$ units, in which $E\lambda'$ represents the spectral energy distribution of the incandescent lamp.

For another light source, with spectral energy distribution $E\lambda'$, 1 unit of photocurrent is correspondingly generated by an energy of $\Sigma E\lambda' / (\Sigma E\lambda' \cdot G\lambda)$ erg.sec^{-1}.cm^{-2}, so that the meter indication of 1 lux represents an incident energy of $4.23\,[(\Sigma E\lambda' \cdot G\lambda) / \Sigma E\lambda'] \times [\Sigma E\lambda' / (\Sigma E\lambda' \cdot G\lambda)]$ erg.sec^{-1}.cm^{-2}.

For the calculation of the relevant data, the spectral sensitivity of the "normal" selenium barrier layer photocell (fig. 8) according to BERGER (1956) was used, which agrees with data of ZWORYKIN and RAMBERG (1949) as quoted by WITHROW and WITHROW (1956). It was assumed that the relation between light intensity and photocurrent is linear. As compared with the energy values on a lux-basis (Table IV, column II), those on the basis of an "indicated lux"

(column IV) are high for *HO*-450 W, and for warm white, green and yellow fluorescent tubes, whereas an important decrease obtains for the sun and for de luxe cool white, daylight, blue, and red fluorescent tubes.

When the incident energy is expressed in photometric units, usually it is not specified whether a corrected or an uncorrected photocell was used. In that case, it is not sure whether the ratios on a lux-basis, or those on an "indicated lux"-basis should be used for the calculation of the incident energy. The uncertainty is greatest for the light sources mentioned above, for which considerable differences between the ratios were found.

III–4. The relation between incident light, expressed in absolute units or photometric units, and the number of Einsteins absorbed for different light sources and different leaf types

In the previous section, it has already been pointed out that the physiologically interesting figure is the number of quanta absorbed by the leaf. This, however, requires knowledge of the incident energy, of the emission spectrum of the light source, and of the absorption spectrum of the leaf. Since no facilities were available for the measurement of the latter in non-monochromatic light, the number of Einsteins absorbed per unit incident energy was calculated, using published data on the spectral absorption of leaves. For a comparison with experiments in which photometric units are used for the measurement of the incident light, the number of Einsteins absorbed per ft-c was also calculated.

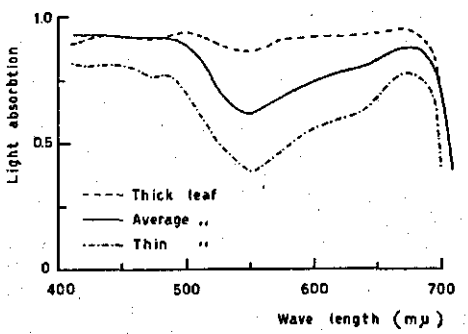


Fig. 9. Fractional light absorption by a thin leaf (*Lactuca*, taken from Rabideau *et al.*, 1946), by an average leaf (average of bean, spinach, Swiss chard, and tobacco, taken from Moss and Loomis, 1952), and by a thick leaf (*Ficus*, taken from Rabideau *et al.*, 1946).

The light sources considered are the same as in the previous section. Seybold and Weissweiler (1942, 1943), Rabideau, French, and Holt (1946), and Moss and Loomis (1952), have established comparable spectral absorption curves for a great number of leaves. The absorption spectra (fig. 9) of a thin, light green leaf (*Lactuca*, from Rabideau *et al.*), of a thick, dark green leaf (*Ficus*, from Rabideau *et al.*), and of a leaf with average absorption characteristics (the mean of leaves of bean, spinach, Swiss chard, and tobacco, from Moss *et al.*) were used in our calculations.

For intervals of 20 mμ between 400 and 700 mμ mean values of E_λ, a_λ, and Nhc/λ were determined, and the ratio Einsteins absorbed/incident erg, (k_φ), was calculated according to

$$k_\varphi = \frac{\Sigma E_\lambda \cdot a_\lambda \cdot \lambda \,/\, Nhc}{\Sigma E_\lambda} \qquad (20)$$

in which E_λ = the incident energy (erg)
a_λ = the fraction of the incident energy absorbed

Nhc/λ = the energy content of 1 Einstein (erg)
N = AVOGADRO's number (6.03×10^{23})
h = PLANCK's constant (6.60×10^{-27} erg.sec)
c = the velocity of light (3×10^{10} cm.sec^{-1})
λ = wave length (cm)

Combination of equations 20 and 17 gives the ratio Einsteins absorbed. cm^{-2}.sec^{-1} . lux^{-1}:

$$k_{\varphi}' = \frac{1.54 \, \Sigma E_{\lambda} \cdot a_{\lambda} \cdot \lambda \, / \, Nhc}{\Sigma E_{\lambda} \cdot V_{\lambda}} \qquad (21)$$

TABLE V. Absorption of light from different light sources (400–700 mμ) by three leaf types, *viz.*, a thin leaf (*Lactuca*, absorption spectrum taken from RABIDEAU *et al.*, 1946), an average leaf (mean of bean, spinach, Swiss chard, and tobacco, taken from MOSS and LOOMIS, 1952), and a thick leaf (*Ficus*, taken from RABIDEAU *et al.*, 1946).
Columns II, III, IV: Einsteins absorbed per incident erg; columns V, VI, VII: Einsteins absorbed.sec^{-1}.cm^{-2} per ft-c; between brackets: relative values, taking the values for a 500 W incandescent lamp equal to 100.

I	II	III	IV	V	VI	VII
Light source	× 10^{-13} Einsteins absorbed per incident erg			× 10^{-11} Einsteins absorbed · sec^{-1}.cm^{-2} per ft-c		
	Thin leaf	Aver. leaf	Thick leaf	Thin leaf	Aver. leaf	Thick leaf
Sun	2.97 *(93)*	3.78 *(94)*	4.27 *(93)*	1.28 *(90)*	1.63 *(90)*	1.84 *(88)*
Incand. 500 W	3.18 *(100)*	4.02 *(100)*	4.61 *(100)*	1.43 *(100)*	1.81 *(100)*	2.07 *(100)*
HPL 400 W	2.80 *(88)*	3.62 *(90)*	4.18 *(91)*	1.05 *(74)*	1.36 *(75)*	1.57 *(75)*
ML 250 W	2.93 *(92)*	3.78 *(94)*	4.38 *(95)*	1.10 *(77)*	1.42 *(60)*	1.65 *(79)*
HO 450 W	2.29 *(72)*	3.20 *(80)*	3.90 *(85)*	0.61 *(43)*	0.85 *(47)*	1.04 *(50)*
Fluorescent tubes (Philips)						
Warm tint (*TL*-29)	2.70 *(85)*	3.63 *(90)*	4.33 *(94)*	0.82 *(57)*	1.10 *(60)*	1.31 *(63)*
De luxe warm tint (*TL*-32)	2.98 *(93)*	3.90 *(97)*	4.52 *(98)*	1.17 *(82)*	1.53 *(84)*	1.78 *(85)*
White (*TL*-33)	2.72 *(85)*	3.60 *(89)*	4.20 *(91)*	0.91 *(64)*	1.21 *(66)*	1.41 *(68)*
De luxe white (*TL*-34)	2.90 *(91)*	4.03 *(100)*	4.31 *(94)*	1.13 *(79)*	1.57 *(86)*	1.68 *(81)*
Daylight (*TL*-55)	2.80 *(88)*	3.61 *(89)*	4.11 *(89)*	1.10 *(77)*	1.42 *(78)*	1.62 *(77)*
Blue (*TL*-18)	2.81 *(88)*	3.39 *(84)*	3.54 *(77)*	2.30 *(161)*	2.77 *(152)*	2.89 *(139)*
Green (*TL*-17)	2.34 *(73)*	3.20 *(80)*	3.90 *(85)*	0.57 *(40)*	0.78 *(43)*	0.95 *(46)*
Yellow (*TL*-16)	2.72 *(85)*	3.72 *(92)*	4.75 *(103)*	0.74 *(52)*	1.01 *(56)*	1.29 *(62)*
Red (*TL*-15)	3.83 *(120)*	4.65 *(115)*	5.10 *(111)*	6.07 *(425)*	7.37 *(405)*	8.08 *(388)*
Fluorescent tubes (Gen. Electric)						
Warm white	2.68 *(84)*	3.58 *(89)*	4.26 *(92)*	0.81 *(57)*	1.08 *(60)*	1.29 *(62)*
De luxe warm white	2.83 *(89)*	3.69 *(92)*	4.34 *(94)*	0.97 *(68)*	1.26 *(69)*	1.48 *(71)*
Cool white	2.69 *(84)*	3.55 *(88)*	4.12 *(89)*	0.91 *(64)*	1.20 *(66)*	1.39 *(67)*
De luxe cool white	2.75 *(86)*	3.52 *(87)*	4.02 *(87)*	1.01 *(71)*	1.30 *(71)*	1.48 *(71)*
Daylight	2.81 *(88)*	3.67 *(91)*	4.23 *(92)*	1.04 *(73)*	1.36 *(75)*	1.57 *(75)*
Blue	2.79 *(87)*	3.41 *(85)*	3.65 *(79)*	1.75 *(122)*	2.14 *(118)*	2.29 *(110)*
Green	2.25 *(71)*	3.15 *(78)*	3.93 *(85)*	0.50 *(35)*	0.71 *(39)*	0.88 *(42)*
Gold	2.60 *(81)*	3.61 *(90)*	4.50 *(98)*	0.64 *(45)*	0.89 *(49)*	1.11 *(53)*
Pink	3.06 *(96)*	3.96 *(98)*	4.58 *(99)*	1.31 *(92)*	1.69 *(93)*	1.96 *(94)*
Red	3.64 *(114)*	4.49 *(112)*	5.04 *(109)*	3.40 *(238)*	4.19 *(231)*	4.71 *(226)*

The results are presented in Table V. For the average leaf, the relative value of the ratio, $k\phi$, on the basis of lux or ft-c varies between 100 and 47 for various sources of "white" light, and between 39 and 405 for the coloured fluorescent tubes. The ratio $k\phi$, based on absolute units, varies between 100 and 80 for the white sources, and between 78 and 115 for the coloured tubes. Consequently, comparison of the incident light from different light sources in photometric units, gives a poor picture of the photosynthetic effectiveness of these light sources. The incident energy represents the number of Einsteins absorbed much more adequately, although deviations between light sources of the order of 20 % may occur.

To demonstrate the errors which may arise when photosynthetic efficiencies under different light sources are compared on a ft-c basis, data given by WENT (1957) were recalculated. Young tomato plants were grown for six days under various coloured fluorescent tubes. WENT expressed the efficiency of light utilization as dry weight increase per ft-c. These data and the calculated efficiencies, based on the incident energy and on the Einsteins absorbed, are presented in Table VI in relative units, taking the efficiencies in blue light equal to 100. In the calculations, the erg.sec^{-1}.cm^{-2}.ft-c^{-1} ratio value from Table IV was used, while the number of Einsteins absorbed was derived from the values for the average leaf in Table V. It can be seen that the photosynthetic effectiveness of green and gold lamps is underestimated by 2.5 to 3 times when the effectiveness is based on photometric units, while considerable deviations also occur for the pink and red lamps.

TABLE VI. Relative efficiencies of light utilization by young tomato plants, grown under fluorescent lamps of different colours. Column II: efficiencies based on foot-candles, according to WENT's data (1957). Columns III and IV: efficiencies based on incident ergs and on Einsteins absorbed respectively, calculated from WENT's data and from the data for an average leaf in Tables IV and V.

I	II	III	IV
Light source	Efficiency of light utilization		
	mg/ft-c	mg/erg.sec^{-1}.cm^{-2}	mg/Einsteins. sec^{-1}.cm^{-2}
Blue	100	100	100
Green	20	55	59
Gold	40	102	96
Pink	71	104	90
Red	62	42	32

III–5. LIGHT CURVES OF PHOTOSYNTHESIS, OBTAINED IN LIGHT OF DIFFERENT QUALITIES

In our experiments, artificial light sources, providing light intensities in the order of full sunlight, were required. The use of the carbon arc seemed impracticable, so that a choice had to be made between high pressure mercury vapour lamps and incandescent lamps. (Xenon lamps were not available at the start of the experiments, but their high output and the fact that their spectral composition in the visible region ressembles that of the sun, seem to offer important possibilities).

As far as the number of Einsteins absorbed per unit incident energy between 400 and 700 mμ is concerned (see Table V), incandescent light approaches sunlight better than mercury light. However, the measurement and control of

leaf temperature proved to be more difficult in incandescent light (see section III–6), so that in most of our experiments mercury lamps were used.

In order to assess the comparability between experiments in light of different qualities, the rates of photosynthesis of leaves of sugar beet and tomato were measured in light from mercury lamps (*HO*-450 W, PHILIPS), from fluorescent lamps (*TL*-33, PHILIPS), and from incandescent lamps (Altrilux, 500 W, PHILIPS). In six experiments, photosynthesis of each leaf was successively measured in mercury light and in incandescent light, while three experiments were made in fluorescent light only. The mean light curves are plotted in fig. 10.

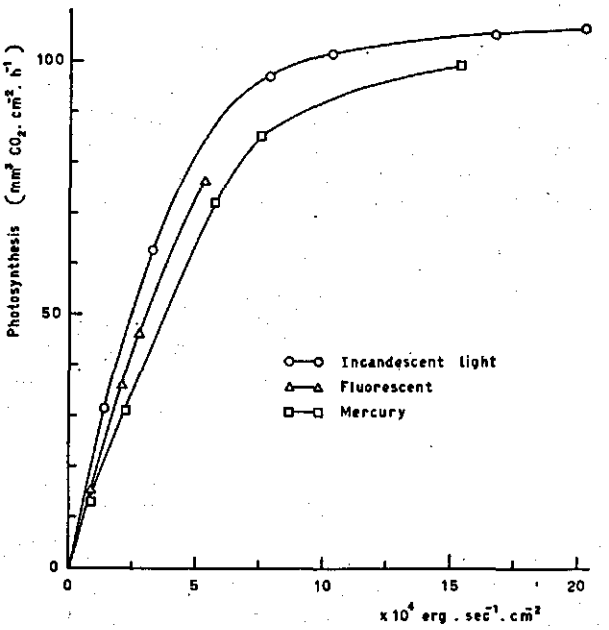


FIG. 10
Rate of photosynthesis in light from incandescent, high pressure mercury, and fluorescent lamps. Sugar beet leaf; 0.03 % CO_2; leaf temperature 21–25 °C.

As could be expected, within the range of limiting light intensities, photosynthesis was highest in incandescent light and lowest in mercury light. The maximum efficiency of light energy conversion was reached at low light intensities, and was in mercury light 70 % and in fluorescent light 80 % of the efficiency in incandescent light (Table VII, columns II and III). On account of the number of Einsteins absorbed per unit incident energy (Table V), the relative efficiencies of average leaves should be 80 % in mercury light and 89 % in fluorescent light, whereas in thin leaves the expected efficiencies should be 72 % and 85 % respectively (Table VII, columns IV and V). Probably, the spectral absorption of the beet and tomato leaves is best approached by that of the average leaves in Table V, so that the less efficient utilization of mercury light is not due solely to a less efficient absorption of light quanta, but also to an approximately 10 % lower quantum efficiency. This, probably, results from the relatively high proportion of blue radiation in mercury light, which may have been absorbed by pigments with a low efficiency of energy transfer, *cf.* GABRIELSEN (1940, 1948).

At high light intensities, photosynthesis in mercury light is still 5 % lower

TABLE VII. Maximum efficiency of light utilization in sugar beet and tomato leaves in mercury light and fluorescent light, compared with the maximum efficiency in incandescent light (columns II and III), together with the relative values of the ratio Einsteins absorbed/incident energy for average and thin leaves (columns IV and V), derived from Table V.

I	II	III	IV	V
	Maximum efficiency of light utilization		Einsteins absorbed/incident energy, 400–700 mμ	
	sugar beet	tomato	average leaf	thin leaf
TL-33/incandescent	0.80	—	0.89	0.85
HO-450 W/incandescent . .	0.70	0.69	0.80	0.72

than in incandescent light; this does not seem to be due to incomplete light saturation. Since, at the normal CO_2-concentrations used in these experiments, the capacity of the diffusion process determines the rate of photosynthesis (*cf.* Chapter IV), it may be that the diffusion resistance is slightly increased in mercury light.

The experiments reveal that light curves of photosynthesis, obtained in mercury light are not quantitatively valid for other light sources. The largest deviations occur at low light intensities. They are mainly due to differences in the number of Einsteins absorbed per unit of incident energy, so that the rates to be expected in other light sources can be approximated by taking into account the ratio values listed in Table V. In high light intensities, the differences amount to a few per cent only.

III–6. TEMPERATURE MEASUREMENTS IN AN IRRADIATED LEAF, ENCLOSED IN AN ASSIMILATION CHAMBER

In photosynthesis experiments, the temperature of the chloroplasts should be approximated. In the present experiments, thermocouples were attached to the under surface of the leaf, so that this surface temperature should be close to that of the chloroplasts in the neighbourhood of the thermojunction. Care must be taken to ensure that the thermocouple indicates the actual temperature of the lower leaf side; *e.g.* heating by direct absorption of radiant energy by the thermojunction should be avoided as much as possible.

The heat transfer from the leaf towards the air stream is a function of the temperature difference between the leaf surface and the air. At high light intensities, the low specific heat of the air, and the relatively small mass of air supplied to the leaf, will cause an appreciable increase in air temperature and, consequently, in leaf temperature in the direction of air flow. Therefore, several thermocouples should be used in order to obtain a reliable estimate of the mean leaf temperature.

As compared with outside conditions, the velocity of the air stream in the assimilation chamber usually is low, and this results in a low coefficient*) of heat transfer from the leaf towards the ambient air and, consequently, in relatively large differences between leaf temperature and air temperature. For

*) k, according to $Q = k\Delta t$ in which Q is the heat transfer and Δt the temperature difference between the leaf and the ambient air.

the same reason, the leaf temperature will increase strongly with increasing light intensity when no precautions are taken, as, *e.g.*, cooling of the walls of the assimilation chamber.

Some of the features, mentioned above, were examined in respect of the conditions prevailing in our experiments.

The direct heating of the thermojunctions by the absorption of radiant energy was evaluated by placing thermocouples in the empty assimilation chamber while filtering the incident light through a leaf, placed on the front wall of the chamber, so that the irradiation conditions occurring in actual experiments were approximated. At high light intensities (30×10^4 erg.sec^{-1}.cm^{-2}) from mercury lamps, the temperature readings were 0.1 to 0.15°C higher than in the dark. With incandescent lamps, the difference amounted to as much as 2.8°C, and this undoubtedly is due to the high proportion of near infrared radiation which is weakly absorbed by the leaf, so that the energy reaching the thermocouples is much higher than in the case of mercury lamps.

An approximate assessment of the relation between the temperature of the chloroplasts and that of the lower leaf surface (t_b) was attempted for enclosed leaves, exposed to mercury light. One cm^2 of a leaf model, 0.1 mm thick, was considered, introducing the following simplifications:

a. The upper 0.05 mm consist of pallissade cells without intercellular spaces, so that the heat conductivity is close to that of water ($\lambda_w = 0.0014$ cal.sec^{-1}.cm^{-1}.°C^{-1}). The coefficient of heat transfer of the "water" layer, k_w, then amounts to 0.28 cal.sec^{-1}.cm^{-2}.°C^{-1}.

b. The lower 0.05 mm consist almost entirely of air, and for this internal air layer $\lambda_{i.a.} = 0.000057$ cal.sec^{-1}.cm^{-1}.°C^{-1}, and $k_{i.a.} = 0.0114$ cal.sec^{-1}.cm^{-2}.°C^{-1}.

c. An irradiation of 31.5×10^4 erg.sec^{-1}.cm^{-2}, and an absorption (A) of 80% (0.0060 cal.sec^{-1}.cm^{-2}), which is fully localised in an infinitely thin upper leaf layer, are considered.

d. The energy used in photosynthesis is neglected, since this amounts to only a few per cent of A (GAASTRA, 1958).

e. Energy exchange by heat radiation between the leaf and the walls of the assimilation chamber is absent.

f. The transpiration rate, T, is taken as 1.5 gram H_2O.dm^{-2}.h^{-1}, which is a reasonable value for the conditions considered (*cf.* Chapter V). Since the heat of vaporization of water at 20°C is 585 cal.g^{-1}, about 0.0025 cal.sec.$^{-1}$.cm^{-2} are used for transpiration.

g. Following the assumption about the cross-section of the leaf (see *a* and *b*), the evaporation takes place in the middle of the leaf.

h. The temperature of the air streams (t_a) above and below the leaf is the same.

The coefficient of heat transfer from the leaf surfaces towards the external air is estimated from RASCHKE (1956) and taken as $k_{e.a.} = 0.00021$ cal.sec^{-1}.cm^{-2}.°C^{-1} (leaf width 10 cm, air velocity 0.1 m.sec^{-1}).

The rate of heat flow, q, through 1 cm^2 of a layer with a coefficient of heat transfer k, and a temperature difference Δt between both sides is given by

$$q = k\,\Delta t = \frac{\Delta t}{1/k} = \frac{\Delta t}{r} \qquad (22)$$

in which r is the heat resistance. When q flows through successive layers with different k-values, this equation is valid for each layer, but it can easily be derived that q is also given by the ratio total temperature difference/sum of successive resistances (ECKERT, 1949), *viz.*,

$$q = \frac{\Sigma\,\Delta t}{\Sigma r} \qquad (22a)$$

Since the heat resistances in our leaf model are: $r_{e.a.} = 1/0.00021 = 4750$, $r_w = 1/0.28 = 3.5$, and $r_{i.a.} = 1/0.0114 = 87.5$, the mode of heat dissipation can now be considered quantitatively. Denoting the quantities of heat transferred to the external air through the upper and lower leaf surfaces by q_u and q_b respectively, we obtain (see fig. 11):

$$A = q_u + q_b + T, \text{ hence } q_u + q_b = A - T = 0.0035 \qquad (23)$$

Furthermore, the external air temperatures on both sides of the leaf are equal, so that

$$4750\, q_u = 3.5\, (q_b + 0.0025) + (87.5 + 4750)\, q_b \qquad (24)$$

Solution of equations 23 and 24 gives $q_b = 0.00173$. The temperature difference between "chloroplasts" and lower leaf surface, $t_u - t_b$, equals $(t_u - t_m) - (t_m - t_b) = 3.5\, (q_b + 0.0025) + 87.5\, q_b = 0.166$°C.

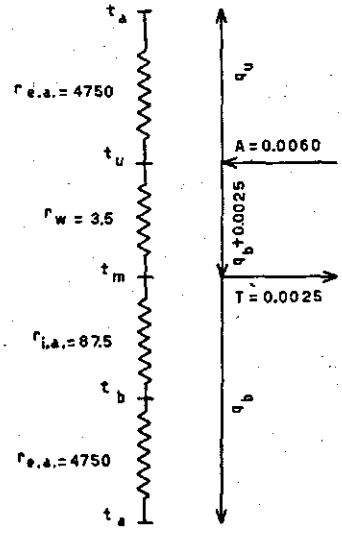


FIG. 11. Mode of energy dissipation in an irradiated leaf model. For explanation see text.

Analogous calculations showed that, with partial heat dissipation by heat radiation from the leaf surfaces towards the, 5 °C cooler, walls of the assimilation chamber, $t_u - t_b$ was of the same order of magnitude, whereas, in the extreme case of a non-transpiring leaf, the temperature difference was calculated to be 0.27 °C.

In actual leaves the temperature difference between chloroplasts and lower leaf surface is probably even less, because the assumption of complete light absorption in the upper leaf layer and very low heat conductivity of the lower portion of the leaf, tends to overestimate the differences. Moreover, the slight temperature increase of the thermojunctions by the absorption of radiant energy will partly compensate the actual differences, so that in mercury light the temperature of the chloroplasts will be fairly closely approximated. With incandescent light, at high light intensities, the temperatures are probably overestimated owing to the absorption of radiant energy by the thermojunctions (*cf.* p. 31). However, the error will be smaller than that observed in the experiments with thermocouples in the empty assimilation chamber, since the coefficient of heat transfer from the thermojunction towards the leaf surface is larger than that from the thermojunction towards the surrounding air.

In the photosynthesis experiments, the leaf temperature was measured by applying three or four thermocouples to the lower leaf surface at a distance of one sixth of the total leaf width from the mid vein and regularly distributed in the longitudinal direction of the leaf. At an irradiance of 30×10^4 erg.sec^{-1}.cm^{-2} from mercury lamps, the first couple was approximately 0.5 °C lower in temperature than the last one, but in incandescent light the difference was between 2 and 3 °C, so that the average chloroplast temperature can be more accurately approximated in mercury light than in incandescent light.

The relation between light intensity and leaf temperature in mercury light and in incandescent light is presented in Table VIII. The infrared radiation ($\lambda > 700$ mμ) was reduced to 6 and 65 % of the total intensity respectively, by an 8 cm water layer between the lamps and the assimilation chamber. The normal chamber with water-cooled walls was used, as well as the same chamber with air-filled lower wall, but with a single "Plexiglass" front wall without artificial cooling. Air with an initial relative humidity of 48 % was supplied at a rate of 300 l.h^{-1}. The temperature of the air entering the chamber, and the water temperature were 20.1 °C. As expected, the leaf temperature increased with increasing light intensity, but the differences between the temperatures in the dark and at 30×10^4 erg.sec^{-1}.cm^{-2} were consistently smaller in mercury light than in incandescent light. Cooling of the walls resulted in lower leaf temperatures with both light sources.

It is clear that the relatively small temperature increase in mercury light can more easily be reduced further by increasing the transpiration rate (*e.g.* by

TABLE VIII. Relation between light intensity and average temperature of a turnip leaf enclosed in an assimilation chamber with and without cooled walls, in mercury light and in incandescent light, filtered through an 8 cm water layer. Water temperature in cooled walls and inlet air temperature 20.1 °C.

I	II	III	IV	V
Light intensity ($erg.sec^{-1}.cm^{-2}$, 400-700 mμ)	Mercury lamps		Incandescent lamps	
	with cooled walls	without cooled walls	with cooled walls	without cooled walls
0	19.5 °C	20.1 °C	19.5 °C	20.6 °C
5×10^4	20.6 „	22.1 „	21.5 „	24.6 „
10×10^4	21.5 „	23.8 „	22.9 „	27.1 „
20×10^4	23.8 „	27.1 „	26.5 „	31.8 „
30×10^4	26.6 „	30.7 „	31.0 „	36.5 „

decreasing the water vapour tension of the air) or by increasing the transfer of energy to the surroundings (by lowering air and wall temperatures). Such effects are shown in Table IX for a leaf exposed to 30×10^4 $erg.sec^{-1}.cm^{-2}$, with dry air entering the chamber. With walls and incoming air at 12 °C, the leaf temperature in mercury light was 21.6 °C, in incandescent light 28.6 °C. Table IX shows further that with increasing temperature, the difference between leaf temperature and wall and air temperatures becomes smaller, indicating an increasing contribution of transpiration to the energy dissipation.

TABLE IX. Effect of inlet air temperature and wall temperature upon average temperature of turnip leaves enclosed in an assimilation chamber, in mercury light and in incandescent light, filtered through an 8 cm water layer. Light intensity 30×10^4 $erg.sec^{-1}.cm^{-2}$, air supply 300 $l.h^{-1}$.

I	II	III
Air and wall temperature	Leaf temperature	
	Mercury lamps	Incandescent lamps
12 °C	21.6 °C	28.6 °C
20 „	26.0 „	32.4 „
29 „	31.7 „	37.4 „

The experiments show that in mercury light, the mean leaf temperature can be measured to within 0.2 °C, provided three or four thermocouples, regularly distributed over the leaf surface, are used. In incandescent light, filtered through an 8 cm water layer, the uncertainty of the temperature measurements amounts to, perhaps, one or more degrees centigrade. Probably, the error can be reduced by using near-infrared absorbing filters (TRANQUILLINI, 1954), but these cannot easily be used where large areas have to be irradiated. Moreover, most filters show appreciable absorption in the red region of the spectrum, so that the photosynthetically utilizible energy is also considerably reduced.

III–7. Discussion

The considerations presented in this chapter demonstrate that the comparability and the reproducibility of experiments on photosynthesis in higher plants may be difficult because important differences can exist between the conditions actually measured, and those directly determining photosynthesis, *viz.* the chloroplast temperature, the number of absorbed light quanta, and the CO_2-concentration in the external air close to the leaf surface through which CO_2 is taken up. Moreover, the relation between the measured values and the physiologically important values of the various factors depends upon the environmental conditions.

With low rates of air supply *e.g.*, the mean CO_2-concentration as computed from the concentrations in the total air mass before and after passing the leaf, may be several times higher than the effective CO_2-concentrations at each leaf surface, especially in the case of hypostomatous leaves. Failure to reproduce the position of the leaf in the chamber will also yield varying results under conditions of CO_2-limitation, when the air is supplied at a low rate.

The temperature of the leaf surface seems to give a reasonable approximation to the chloroplast temperature; it depends strongly on the light intensity and on the infrared radiation of the light source. In sunlight, Tranquillini (1954) observed temperatures of 52.1 °C in leaves enclosed in an assimilation chamber so that the photosynthetic apparatus was damaged, and CO_2 was given off in the middle of the day. On the other hand, in less extreme temperatures, photosynthesis will be strongly enhanced when a temperature-sensitive process with a high Q_{10}-value limits photosynthesis.

The light intensity may also be inadequately defined when the linearity of response of the photocells is not checked (section II–9) or when photometric units are used irrespective of the light source employed. The latter is especially important with regard to coloured light, as could be demonstrated by a recalculation of data published by Went (1957) on the efficiency of light energy conversion based on photometric units: in green and yellow light the efficiencies were probably underestimated by a factor 2.5.

The great variation in photosynthetic rates published by different authors and measured under apparently identical conditions, (see, *e.g.* a survey by Rabinowitch, 1951, Table 28.VI) probably are due more to actual variation in the photosynthetically important conditions, than to differences in the properties of the photosynthetic apparatus. Under conditions of CO_2-limitation, quantitatively valid data will be obtained only, at high rates of air supply (see also Heinicke and Hoffman (1953) and Decker (1947)), while at moderate rates of air supply a reproducible position of the leaf in the centre plane of the assimilation chamber is required. Further, leaf temperatures should be measured, and the effect of light intensity upon leaf temperature should be eliminated as far as possible, while light intensities must be determined correctly in absolute units.

CHAPTER IV

THE EFFECT OF LIGHT INTENSITY AND CARBON DIOXIDE CONCENTRATION UPON PHOTOSYNTHESIS

IV–1. INTRODUCTION

In normal air (0.03 % CO_2) and at light saturation, photosynthesis is strongly affected by the rate of air supply (*cf.* Chapter III, and KOSTYTSCHEW, BAZYRINA and WASSILIEFF (1927), HEINICKE and HOFFMAN (1933), DECKER (1947)), a fact which demonstrates that external CO_2-concentrations up to at least 0.03 % CO_2 limit photosynthesis. Remarkably little and, moreover, conflicting information is available on the effect on photosynthesis in crop plants of CO_2-concentrations higher than normal, see Table 27.I in RABINOWITCH (1951).

Some observations of previous investigators, together with the relevant experimental data are given in Table X. In many experiments, CO_2-saturation was not reached (column II) which, taking into account the variation in photosynthetic rates (column IV), would appear to be largely due to insufficient control of the experimental conditions. Leaf temperatures were not measured, and in the experiments with a low rate of air supply, steep CO_2-gradients in the external air probably occurred. Furthermore, high leaf temperatures may have created sub-optimal physiological conditions in the leaf.

CO_2-saturation was obtained in experiments of HOOVER *et al.* (0.11 %), SINGH and LAL (0.172 %), and MÜLLER (10 %). The high saturating concentration obtained by MÜLLER undoubtedly is due to the low rate of air supply, which is estimated to be in the order of 6 $l.h^{-1}$.

In MÜLLER's experiments, the O_2-concentration was recorded by the paramagnetic method. It follows from the sensitivity indicated (1 % O_2 = 50 units of scale deflection) and the greatest deflection obtained (15 units) that $X \times 10^6 \times 10^{-2} \times 15/50$ mm^3 $O_2.h^{-1}$ are produced (X = flow rate in $l.h^{-1}$). The maximum O_2-exchange observed was 25 mg $O_2.h^{-1}$ which, taking into account the molecular weight (32) and the molvolume (22.41), equals approximately $22.4 \times 10^3 \times 25/32 = 17500$ $mm^3.h^{-1}$. Hence $X = 10^{-4} \times 17500 \times 50/15 = 6$ $l.h^{-1}$.

The maximum light intensity in the experiments of SINGH and LAL is not clearly defined ("1875 candle power") but was probably low, judging by the low rate of photosynthesis.

Extensive and reliable data are given by HOOVER, JOHNSTON and BRACKETT. For each of six light intensities, photosynthesis in young wheat plants was measured at several CO_2-concentrations, in rapidly circulated air. Although the experiments lasted about a week, so that considerable corrections for growth had to be applied, very consistent data were obtained. The highest light intensity at the leaf surface was 950 ft-c which, according to Table IV of this paper, corresponds to approximately 4.3×10^4 $erg.sec^{-1}.cm^{-2}$. The plants were irradiated from all directions, and the maximum incident energy amounted to about 8.6×10^4 $erg.sec^{-1}.cm^{-2}$.

Probably, the actual value is lower, because part of the red radiation will have been absorbed by the copper sulphate filter, resulting in a lower $erg.sec^{-1}.cm^{-2}.ft\text{-}c^{-1}$ ratio. In a discussion of these data RABINOWITCH (1951, fig. 27.3) mentions a maximum intensity of 68×10^4 $erg.sec^{-1}.cm^{-2}$. However, in this value the infrared radiation is included, whereas, moreover, the intensity on the surface of the leaves is $^1/_4$ of this value, *cf.* HOOVER *et al.*, p. 5.

TABLE X. Summary of previous experiments on the relation between carbon dioxide concentration and photosynthesis in crop plants.

I	II	III	IV	V	VI	VII	VIII
Author and Plant material	Saturating CO_2-concentration as found	Rate of air supply ($l.h^{-1}.cm^{-2}$)	Maximum photosynthesis (mm^3 $CO_2.cm^{-2}.h^{-1}$)	Light source	Saturating light intensity (if not reached, maximum intensity applied [M])	Temperature	
						Wall of assimilation chamber	Leaf
LUNDEGÅRDH (1922)							
Phaseolus vulgaris(excised leaf)	> 0.09%	Still air	119	sun	Full sun [M]	20°C	?
Potato („ „)	> 0.18%	„	225	„	„	21°C	?
LUNDEGÅRDH (1924)							
Avena sativa („ „)	> 0.236%	„	394	„	„	?	?
HOOVER *et al.* (1933)							
Wheat (young plants)	0.11%	Recirculated	17 $cm^3.min^{-1}.plant^{-1}$	Mazda lamps +$CuSO_4$-filter	2 × 950 ft-c = ≈8.6 × 10^4 $erg.sec^{-1}.cm^{-2}$	22°C	?
SINGH and LAL (1935)							
Sugar cane (excised leaf)	0.172%	?	39	Incandescent lamp	1875 c.p.	30°C	?
Wheat („ „)	0.172%	?	37	„	„	30°C	?
Linseed („ „)	0.172%	?	46	„	„	30°C	?
WASSINK (1946)							
Strawberry (leaf discs)	> 1%	Still air	52	Sodium lamps	7 × 10^4 $erg.sec^{-1}.cm^{-2}$[M]	25°C	?
Tomato („ „)	> 2%	„	48	„	„	25°C	?
CHAPMAN and LOOMIS (1953)							
Potato (attached leaf)	> 0.15%	1 $l.h^{-1}.cm^{-2}$	250	Sun	High [M]	—	?
THOMAS and HILL (1949)							
Tomato	> 0.24%	high	30 $mm^3.cm^{-2}$ soil.h^{-1}	„	Full sun [M]	—	?
Sugar beet (6 × 6 feet field or sand culture plots)	> 0.37%	„	72 „	„	„	—	?
Alfalfa	> 0.2%	„	55 „	„	„	—	?
MÜLLER (1958)							
Asparagus sprengerii (detached shoot)	10%	6 $l.h^{-1}$ (estimated)		Nitraphot lamp	20000 Lux	—	?

In view of the uncertain experimental conditions and their bearing upon the results in most of the experiments discussed, an attempt was made to collect some more information about the relation between CO_2-concentration and photosynthesis at different light intensities. In contrast to HOOVER *et al.*, corrections for growth were avoided by completing the experiments in one or, occasionally, two days. This meant, however, that at the most three or four CO_2-concentrations could be combined with three or four light intensities, and less extensive series of curves were obtained. For technical reasons, the maximum CO_2-concentration applied was about 0.15 %, so that in some experiments CO_2-saturation was not obtained.

IV–2. TECHNIQUE AND EXPERIMENTAL RESULTS

Young, fully developed leaves from sugar beet, turnip, tomato, and cucumber plants, grown in gravel culture under controlled conditions, were used in the majority of the experiments. By using mercury lamps as a light source and by controlling the water temperature in the walls of the assimilation chamber, the leaf temperature was kept between 21 and 24 °C during the experiments. The rate of air flow was between 2 and 3 $l.h^{-1}.cm^{-2}$ leaf.

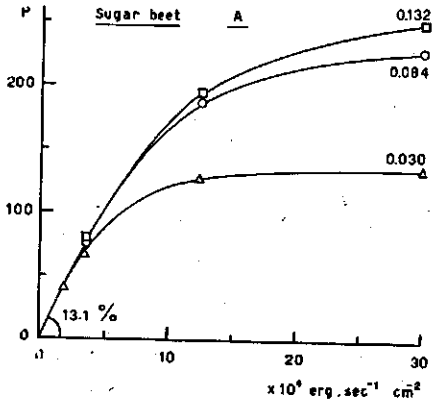
In a specially constructed assimilation chamber with a water-cooled upper wall, experiments were made with young intact spinach plants, grown in soil. The light intensity was measured at the top of the plants.

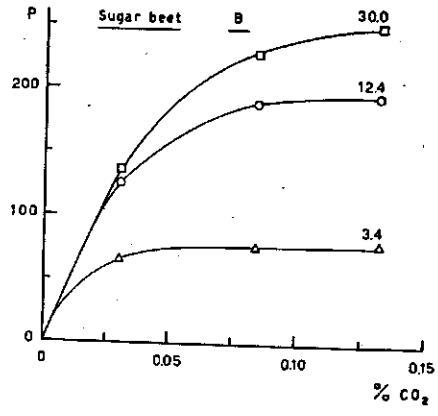
At a given CO_2-concentration, the rate of photosynthesis was measured at different light intensities, after which the same procedure was repeated in air with other CO_2-concentrations. The respiration rate was determined at the beginning and, occasionally, also at the end of the experiments. Usually it had increased by 10 to 20 %, but the maximum rate was 10 $mm^3.cm^{-2}.h^{-1}$, so that the measurement of photosynthesis was not seriously affected by the increased respiration rate.

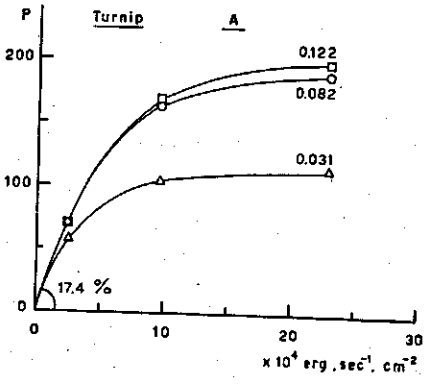
Representative curves are shown in figs. 12-A and B. At the highest light intensity used, in all leaves, except cucumber, CO_2-saturation was reached, or nearly so, at concentrations in the order of 0.1 % CO_2 (figs. 12-B). The figs. 12-A show that light saturation was also reached, so that the maximum rates of photosynthesis for the given leaf temperature (21 to 24 °C) have been obtained.

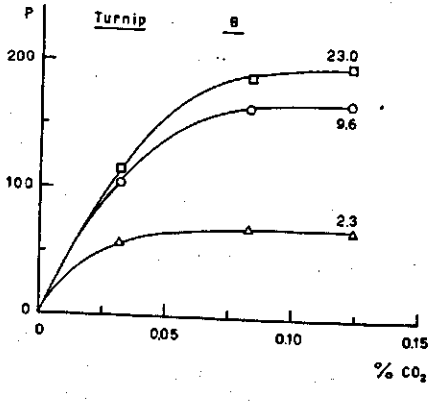
IV–3. DISCUSSION

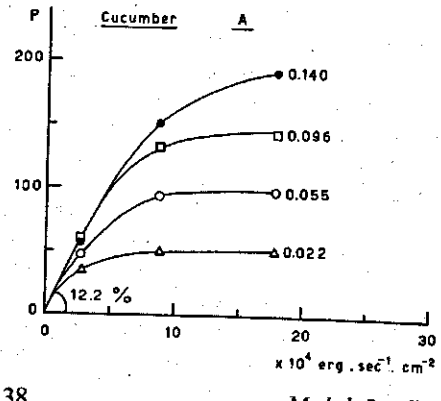
Compared with the data given by SINGH and LAL, WASSINK, and MÜLLER (see Table X), CO_2-saturation was obtained at low concentrations (about 0.1 % CO_2) while the maximum rates (between 180 and 250 mm^3 $CO_2.cm^{-2}.h^{-1}$), in spite of the low CO_2-concentration, are higher at comparable light intensities. This suggests that, in the previous investigations mentioned, either the effective CO_2-concentrations were overestimated, or the photosynthetic apparatus was below optimum efficiency, a possibility recognized by WASSINK for his plant material (which had been forwarded by mail). The rates obtained by LUNDEGÅRDH, and by CHAPMAN and LOOMIS were of the same order of magnitude as those in the present investigation, but photosynthesis was almost linearly increasing with the CO_2-concentration up to the highest concentrations applied (0.236 % and 0.15 % CO_2 respectively). Although the difference may

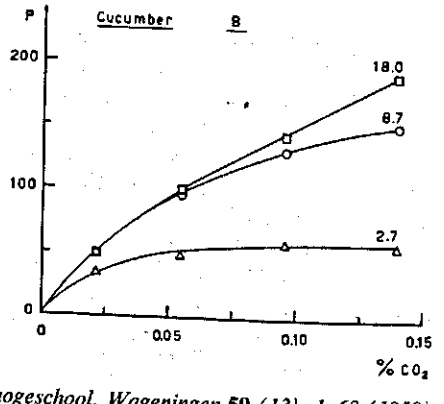

P
Sugar beet
A
0.132
0.084
0.030
200
100
0
13.1 %
10
20
30
x 10⁴ erg . sec⁻¹ cm⁻²


P
Sugar beet
B
30.0
12.4
3.4
200
100
0
0.05
0.10
0.15
% CO₂


P
Turnip
A
0.122
0.082
0.031
200
100
0
17.4 %
10
20
30
x 10⁴ erg . sec⁻¹ . cm⁻²


P
Turnip
B
23.0
9.6
2.3
200
100
0
0.05
0.10
0.15
% CO₂


P
Cucumber
A
0.140
0.096
0.055
0.022
200
100
0
12.2 %
10
20
30
x 10⁴ erg . sec⁻¹ . cm⁻²


P
Cucumber
B
18.0
8.7
2.7
200
100
0
0.05
0.10
0.15
% CO₂

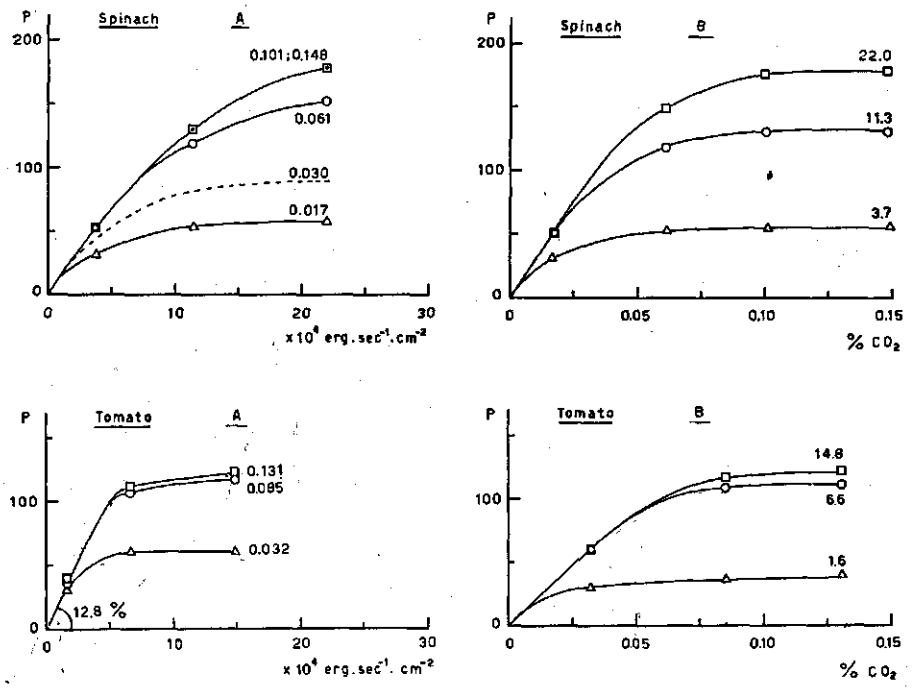


FIG. 12. Photosynthesis (P, in mm^3 $CO_2.cm^{-2}.h^{-1}$) in relation to light intensity at different CO_2-concentrations (figs. A), and in relation to CO_2-concentration at different light intensities (figs. B). Leaves of sugar beet, turnip, tomato, cucumber; young spinach plants; leaf temperature 21 °–24 °C. Parameters in A: % CO_2; in B: light intensity in 10^4 $erg.sec^{-1}.cm^{-2}$. In A the maximum efficiency of light energy conversion is indicated in per cent.

be due to the different growth conditions of the plants (outdoors as opposed to the controlled environment of the present study), a more plausible explanation seems to be that in full sunlight the leaf temperature was higher than 21 °C, resulting in higher saturating concentrations of CO_2, as indicated by fig. 13.

Light intensity curves of photosynthesis were determined for tomato and turnip leaves at different leaf temperatures and at normal and high CO_2-concentrations, and for cucumber leaves at a high CO_2-concentration only (fig. 13). At 0.03 % CO_2, photosynthesis is almost independent of leaf temperature, while at higher concentrations the rate is strongly affected by temperature, so that light saturation was not reached at the highest temperatures (31 to 35 °C).

At CO_2-concentrations around 0.03 % and at light saturation, photosynthesis is almost independent of temperature, but it is affected by the CO_2-concentration, indicating that the rate of diffusion of CO_2 from the external air towards the chloroplasts determines the rate of photosynthesis (VAN DEN HONERT, 1930). The temperature-sensitivity of photosynthesis at higher CO_2-concentrations indicates that, under these conditions, a biochemical process limits the rate. The Q_{10}-values, then observed, are lower than those normally occurring in such processes, due, probably, to the fact that light saturation was not yet complete, so that the temperature-independent photochemical process was determining the rate in part of the system.

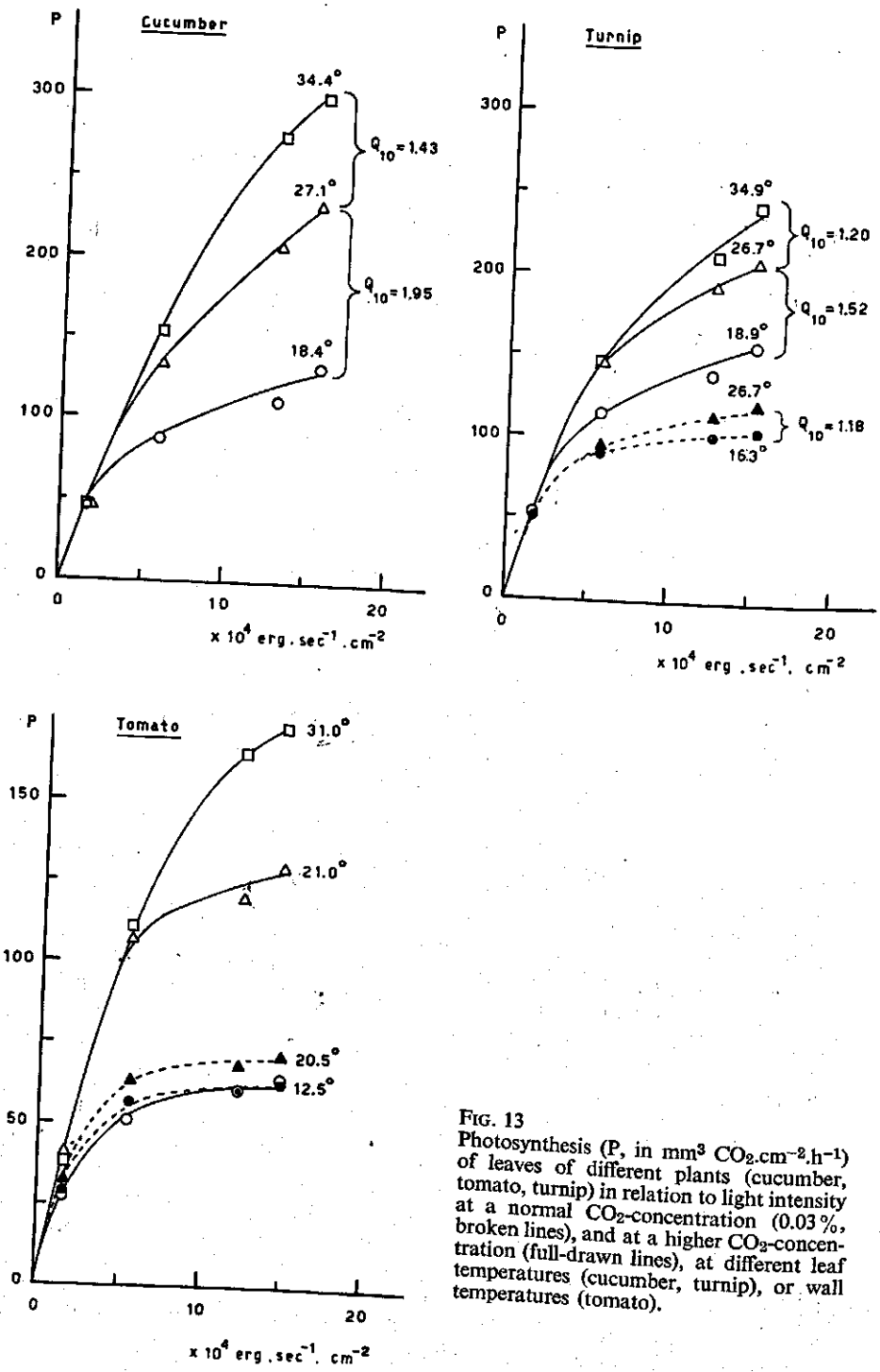


FIG. 13
Photosynthesis (P, in mm^3 $CO_2.cm^{-2}.h^{-1}$) of leaves of different plants (cucumber, tomato, turnip) in relation to light intensity at a normal CO_2-concentration (0.03 %, broken lines), and at a higher CO_2-concentration (full-drawn lines), at different leaf temperatures (cucumber, turnip), or wall temperatures (tomato).

The slope of the light curves at low light intensities indicates the maximum efficiency of light energy conversion. The percentage values are shown in figs. 12-A. The quantum efficiency, Φ, is approximated by taking into account the number of Einsteins absorbed per unit incident energy for mercury light and "average" leaves as given in Table V. Discarding the data for spinach (where the determination of the light intensity incident upon the leaves necessarily was less accurate, because whole plants were used), the quantum efficiences for cucumber, turnip, beet, and tomato were 0.081, 0.116, 0.087, and 0.085 moles CO_2 per Einstein absorbed respectively. Since mercury light is probably used 10 % less efficiently than incandescent light and sunlight (*cf.* section III–5), the maximum quantum efficiences ($\Phi = 0.1$), obtained in higher plants by WASSINK (1946) and GABRIELSEN (1947) are fairly well approximated in these experiments.

In the present experiments we were primarily interested in the maximum capacity of photosynthesis, so that CO_2-concentrations lower than normal were used only incidentally. Experiments to be described in the next section (see figs. 19 and 23) show that at high light intensities, the relation between photosynthesis and CO_2-concentration is linear in the concentration range from zero to 0.03 % CO_2.

The results presented here are in agreement with those of HOOVER *et al.*, which seem to be the only experiments listed in Table X using both low CO_2-gradients in the external air (which was rapidly recirculated) and correctly estimated leaf temperatures (incandescent light was filtered through $CuSO_4$-filters). Thus, in many higher plants CO_2-saturation seems to be reached in concentrations of approximately 0.1 % CO_2 at temperatures between 21 and 24 °C.

CHAPTER V

THE RELATION BETWEEN STOMATAL DIFFUSION RESISTANCE AND PHOTOSYNTHESIS, AS INFLUENCED BY LIGHT INTENSITY AND CARBON DIOXIDE CONCENTRATION

V–1. INTRODUCTION

In the previous chapter it was concluded that, under conditions of CO_2-limitation, the rate of photosynthesis is governed by the rate of diffusion of CO_2 from the external air towards the reaction centre in the chloroplasts. Since the stomata form part of the diffusion path, and their opening depends on external conditions, an investigation into the influence of the stomata upon the rate of photosynthesis seemed of value.

In leaves well provided with water, an increase in light intensity causes stomatal opening, while most investigators (*cf.* HEATH and RUSSELL, 1954) have found that an increase in the external CO_2-concentration induces closing. Thus, the influence of light and CO_2 upon photosynthesis may be direct (by way of the photosynthetic process proper) or indirect (by way of the degree of stomatal opening and, hence, by influencing the capacity of the diffusion process). In the experiments to be described in this chapter, an attempt was made to distinguish these direct and indirect effects. In order to do so, the relation

between the stomatal opening and the capacity of the total diffusion path, and that between the capacity of the diffusion process and the capacity of other possible rate-determining processes must be established. An appreciable influence of the rate of CO_2-supply upon photosynthesis can be expected only when no other processes, *e.g.* the photochemical process, are limiting the photosynthetic rate.

V–2. The measurement of stomatal opening

V–2–1. Requirements. Since the transport of CO_2 through the stomata is a diffusion process, the degree of stomatal opening should be characterized as a diffusion resistance. In investigations into the stomatal control of photosynthesis, the diffusion resistance and the rate of photosynthesis should be measured in the same leaf, in order to avoid individual differences between leaves, while both should be examined under the same experimental conditions, in particular with regard to CO_2-concentration and light intensity. Moreover, the average resistance of a large number of stomata must be determined, in order to compensate differences between individual stomata.

The reaction of the stomata upon a change in light intensity or CO_2-concentration is slow (see section V–3) as compared with that of the photosynthetic process proper. Therefore, valuable information about the stomatal control of photosynthesis can be obtained when the methods used allow simultaneous and continuous measurements of stomatal resistance and photosynthesis.

Various methods commonly applied for the investigation of stomatal opening are not suitable for our purpose. For obvious reasons, Lloyd's method, infiltration methods, microscopic observation, and impression methods are inadequate.

With the porometer technique, quantitative data are obtained only when certain precautions are taken (Spanner and Heath, 1951). Primarily, the resistance to viscous flow through a leaf segment enclosed by a cup, sealed on to the leaf, is measured. To convert this resistance into a stomatal diffusion resistance, corrections must be made for the resistance of the intercellular space system and for the influence of stomata outside the cup. Finally, the relation between resistance to viscous flow and diffusion resistance must be established (see Penman, 1942). It is necessary to ensure that the CO_2-concentration of the air in the cup and of that outside it are the same, because the stomatal opening depends on the CO_2-concentration of the air. Many investigators, however, have used cups which were permanently attached to the leaf and contained stagnant air, so that the CO_2-concentration of the air within the cup must have been severely reduced by photosynthetic CO_2-uptake. As a consequence, their results are questionable, as Heath (1950) has pointed out. To overcome these difficulties, Spanner and Heath (1951) recommended the use of cups, which are detached or swept with air between measurements. This means, however, that continuous measurements are impossible, while the CO_2-concentration may still be reduced considerably during the measurement. (Assuming *e.g.* 1 cm^3 of air with 0.03 % CO_2 originally available per cm^2 leaf, and a photosynthetic rate of 100 mm^3 $CO_2.cm^{-2}.h^{-1}$, the entire CO_2-content of the sample would be consumed in 11 seconds). Therefore, after preliminary experiments with the porometer technique, this method was discarded for the present experiments.

In the method finally adopted, the data needed for the calculation of the stomatal diffusion resistance are derived from the continuously measured transpiration rates and leaf temperatures, simultaneously with the continuously measured photosynthetic rates. The underlying principles are treated in the next section.

V–2–2. The quantitative estimation of the stomatal diffusion resistance. In transpiration, water evaporates from the wet cell walls, lining the intercellular space. The water vapour diffuses through the intercellular space and the stomata to the leaf surface, and further to the surrounding air. In photosynthesis, CO_2 in the external air is transported towards the leaf surface; it then diffuses through the stomata and the intercellular space and, after dissolving in the aqueous medium of the walls of the mesophyll cells, to the reaction centre in the chloroplasts.

BROWN and ESCOMBE (1900) calculated the stomatal diffusion resistance from the dimensions of the stomata, while RENNER (1910) and MASKELL (1928) computed the diffusion resistance in the external air. Along similar lines BANGE (1953) calculated the diffusion resistance in *Zebrina* leaves. He found a close agreement between the theoretically expected transpiration rate and that actually observed. The method used in the present investigation for the calculation of the stomatal diffusion resistance is based upon BANGE's results. Before discussing it in detail, the nature of the diffusion process will be briefly outlined. An extensive treatment is given by the above mentioned authors and by PENMAN and SCHOFIELD (1951).

BROWN and ESCOMBE (1900) used FICK's diffusion law for the calculation of the rate of diffusion in a stomatal tube:

$$Q = \frac{D \cdot (c_0 - c_1) \cdot a}{l} \tag{25}$$

in which Q = the diffusion rate (cm^3 diffusing matter.sec^{-1})
D = the diffusion constant (cm^2. sec^{-1})
c_0 and c_1 = cm^3 diffusing substance per cm^3 air, at both ends of the tube
a = the cross-section of the tube (cm^2)
l = the length of the tube (cm)

By analogy with OHM's law for the flow of electricity, c_0–c_1 can be regarded as corresponding to a "potential difference", while the diffusion resistance can be defined as $r = l/Da$ (sec.cm^{-3}).

Several diffusion resistances are successively encountered in photosynthesis and transpiration, *e.g.* in the external air during diffusion towards the leaf as a whole, or away from it (r_a), and through the stomata (r_s). The latter again can be divided into resistances encountered during diffusion towards the stomatal opening (r_{s1}), inside the stomatal tube (r_{s2}) and away from the latter (r_{s3}).

In a system of successive resistances, the total resistance equals the sum of the separate resistances. This was demonstrated experimentally by BANGE (1953) for diffusion of water vapour from the intercellular spaces in a leaf towards the external air. The total resistance in the gaseous part of the diffusion path thus amounts to

$$r = r_a + r_s \tag{26}$$

while the stomatal resistance amounts to

$$r_s = r_{s1} + r_{s2} + r_{s3} \tag{27}$$

The resistances can easily be calculated for a circular leaf (radius S cm), containing N equally spaced pores with circular cross-section (radius s cm, length l cm), *cf.* BROWN and ESCOMBE (1900), PENMAN and SCHOFIELD (1951), BANGE (1953). Per cm^2 leaf area, in still air, they amount to

$$r_{s2} = \frac{l}{\pi s^2 \cdot D} \cdot \frac{A}{N} \quad \text{sec.cm}^{-1} \tag{28}$$

$$r_{s1} = r_{s3} = \frac{1}{4s \cdot D} \cdot \frac{A}{N} \quad \text{sec. cm}^{-1} \tag{29}$$

$$r_a = \frac{1}{4S \cdot D} \cdot A \quad \text{sec.cm}^{-1} \tag{30}$$

In the steady state, the diffusion rate through successive resistances equals the ratio of the total concentration difference to the total diffusion resistance, as well as the ratio of the concentration difference to the resistance along each separate part of the diffusion path. The rate of photosynthesis, P (cm^{3}CO$_2$. cm^{-2}.sec^{-1}), which under all conditions is equal to the rate of CO$_2$-diffusion, may, therefore, be expressed as follows

$$P = \frac{[CO_2]_a - [CO_2]_{surf}}{r_{a,CO_2}} = \frac{[CO_2]_{surf} - [CO_2]_{int}}{r_{s,CO_2}} = \frac{[CO_2]_a - [CO_2]_{int}}{r_{a,CO_2} + r_{s,CO_2}} \tag{31}$$

in which $[CO_2]_a$, $[CO_2]_{surf}$, and $[CO_2]_{int}$ refer to concentrations (cm^{3}CO$_2$. cm^{-3} air) in the unchanged external air, in the air very close to the leaf surface, and in the intercellular spaces, respectively.

Accordingly, the rate of transpiration, T (cm^3 water vapour.cm^{-2}.sec^{-1}), is expressed by

$$T = \frac{[H_2O]_{int} - [H_2O]_{surf}}{r_{s,H_2O}} = \frac{[H_2O]_{surf} - [H_2O]_a}{r_{a,H_2O}} = \frac{[H_2O]_{int} - [H_2O]_a}{r_{s,H_2O} + r_{a,H_2O}} \tag{32}$$

in which the water vapour concentrations are expressed in cm^3 vapour.cm^{-3} air.

The diffusion resistance of stomata with an idealized shape can easily be calculated according to equations 28, 29, and 27, but the calculations cannot be made frequently and simultaneously with the photosynthetic rate. Moreover, for the naturally occurring complex shape of the stomata, the calculation is difficult (*cf.* BANGE, 1953).

According to equations 31 and 32, however, the diffusion resistance of any part of the diffusion path can also be obtained from the rates of photosynthesis or transpiration and the concentration difference of the CO$_2$ or water vapour at the two ends of the diffusion path considered. In this way $(r_s + r_a)_{H_2O}$ was calculated according to

$$(r_s + r_a)_{H_2O} = \frac{[H_2O]_{int} - [H_2O]_a}{T} \tag{33}$$

The transpiration rate, T, and $[H_2O]_a$ are measured, while $[H_2O]_{int}$ can be derived from the leaf temperature. When the leaf is well supplied with water, the air in the intercellular space is assumed to be saturated with water vapour, so that $[H_2O]_{int}$ corresponds to the saturated water vapour pressure at the measured leaf temperature in the interior of the leaf.

It is impossible to prove by direct measurements that the air in the intercellular space is saturated with water vapour. GRADMANN (1928) and VAN DEN HONERT (1948) have pointed out that the relative humidity of air, in equilibrium with solutions with a diffusion pressure deficit (*DPD*) corresponding to the *DPD* of the cell sap in mesophyll cells, is very high (see also SHULL, 1939), so that the air in the intercellular space is nearly saturated with water vapour. However, this is only true, when small resistances occur during the transport of water through the membranes of the cells lining the intercellular space (BANGE, 1953, SLAVÍK, 1958). SLAVÍK analysed the rate of water loss by detached leaves according to HYGEN's method (1951, 1953), and concluded that the air in the intercellular space is not saturated with water vapour. The same conclusion was reached by KLEMM (1956) from his measurements of the rate of water loss by detached leaves, the epidermis of which had been stripped off. However, KLEMM's quantitative comparison between membrane resistance and stomatal resistance seems incorrect. In his Table 4 the transpiration rates of leaves with and without epidermis ($+E$ and $-E$ respectively) are compared with the evaporation rate of a 0.25 molar sugar solution (Z). The relative values of the membrane resistance (W_m), of the stomatal resistance (W_s) and of the total resistance (W_t) are calculated according to: $W_m = Z/-E$; $W_s = (-E)/(+E)$; $W_t = Z/(+E)$. In this calculation, however, the external air resistance, W_a, is neglected. Applying the principle of addition of successive resistances, the following expressions for these relative resistances are obtained: $W_a = 1/Z$; $W_a + W_m = 1/(-E)$; $W_t = W_a + W_s + W_m = 1/(+E)$. Treating the average data in KLEMM's Table 4 in this way, W_s/W_m is found to be 9.7 instead of 2.6, so KLEMM appears to have overestimated the membrane resistance considerably.

Nevertheless, the experiments of SLAVÍK and KLEMM indicate that, with large water losses and a relatively small water supply, the air in the intercellular space may not be saturated with water vapour. On the other hand, STÅLFELT (1956) and MILTHORPE and SPENCER (1957) could not detect appreciable membrane resistances in leaves well provided with water. In the present experiments with attached leaves, no decrease in the transpiration rate was observed, even when the leaves were exposed to high light intensities for several hours, so that the resistance in the mesophyll membranes must have been low. Therefore, the assumption that the air in the intercellular space was saturated with water vapour, was approximately correct in this case.

The external air resistance, $r_{a,\,H_2O}$, cannot be derived from the transpiration measurements, because $[H_2O]_{surf}$ is not known. This resistance, however, can be approximated by measuring the rate of evaporation from a piece of moist blotting paper of the same shape as the leaf, and exposed to the same conditions. For this leaf model, $[H_2O]_{surf}$ can be derived from the measured surface temperature. Hence,

$$r_{a,\,H_2O} = \frac{[H_2O]_{surf} - [H_2O]_a}{\text{Evaporation}} \tag{34}$$

in which the evaporation rate is expressed in cm^3 water vapour.cm^{-2}.sec^{-1}, and the concentrations in cm^3 water vapour.cm^{-3}air.

The stomatal diffusion resistance for CO_2 can now be calculated according to

$$r_{s,\,CO_2} = \frac{D_{H_2O}}{D_{CO_2}} \cdot \left\{ (r_a + r_s)_{H_2O} - r_{a,\,H_2O} \right\} \tag{35}$$

in which D_{H_2O} and D_{CO_2} are the diffusion constants of water vapour and CO_2 in air (cm^2.sec^{-1}).

In the present investigation, the stomatal diffusion resistance was calculated according to the equations presented in this section from the simulta-

neously measured leaf temperatures and rates of photosynthesis and transpiration, and from the evaporation rates of leaf models. For D_{CO_2} and D_{H_2O} the values 0.14 and 0.24 $cm^2.sec^{-1}$ respectively were accepted.

The resistance in the intercellular spaces is not considered separately. This resistance is usually low as compared to the others. In the following considerations it is combined with the stomatal resistance. Also, cuticular transpiration and cuticular CO_2-diffusion are not considered, because, so far, the capacities of these processes proved to be much smaller than the capacity of the stomatal diffusion, as soon as the stomata are only slightly opened.

V–3. Qualitative observations on the effect of CO_2 and light upon the stomatal diffusion resistance

Before facilities for measuring the transpiration rate were available, only leaf temperature and photosynthesis were measured simultaneously. These experiments provide a qualitative measure of the stomatal opening, as can be concluded from a consideration of the energy balance of the leaf:

$$A = P + T + E - R \tag{36}$$

in which A = the absorbed light energy
P = the energy fixed in photosynthesis
T = the energy dissipated by transpiration
E = the energy transferred to the environment of the leaf by conduction, convection, and radiation
R = the respiratorye nergy

Depending on the light intensity, P is between 2 and 20 % of the absorbed energy, whereas R is negligible for most light intensities. Therefore, the greater part of the absorbed energy is dissipated via T and E. E is approximately proportional to the temperature difference between the leaf and its surroundings, so that any effect of the stomatal resistance upon transpiration is reflected in a change of leaf temperature; opening of the stomata results in a decrease in leaf temperature.

Some representative results, obtained with sugar beet leaves are presented in fig. 14. The effect of the CO_2-concentration upon leaf temperature and photosynthesis was investigated at two light intensities (11.5×10^4 and 38.2×10^4 $erg.sec^{-1}.cm^{-2}$). At a light intensity of 38.2×10^4 $erg.sec^{-1}.cm^{-2}$, the leaf temperature is higher than at 11.5×10^4 $erg.sec^{-1}.cm^{-2}$, because the larger quantity of energy absorbed is not completely dissipated by increased transpiration. At each light intensity, however, (*i.e.* for the same amount of energy absorbed), the leaf temperature increases considerably when the CO_2-concentration increases from 0.03 % to 0.10 %, thus demonstrating an increase in stomatal resistance within this range of CO_2-concentrations.

Simultaneously measured transpiration rates and temperatures of turnip leaves at two different light intensities (20.7×10^4 and 1.65×10^4 $erg.sec^{-1}.cm^{-2}$) as a function of the CO_2-concentration are presented in fig. 15. As expected, transpiration and leaf temperature are closely related. At 20.7×10^4 $erg.sec^{-1}.cm^{-2}$, the transpiration rate is highest, while between 0.04 % and 0.10 % CO_2 partial closure of the stomata is obvious. At the lower light in-

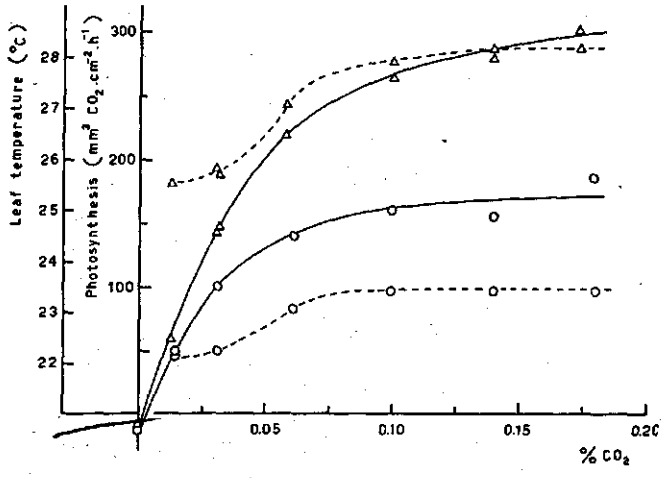


FIG. 14. Influence of CO_2-concentration upon leaf temperature and photosynthesis at two light intensities. Sugar beet; △ = 38.2×10^4 erg.sec^{-1}.cm^{-2}; ○ = 11.5×10^4 erg.sec^{-1}.cm^{-2}; - - - = leaf temperature; —— = photosynthesis.

tensity, closure already occurs at lower CO_2-concentrations, *viz.*, between 0.01 % and 0.04 %.

The curve, representing the influence of light intensity upon transpiration and leaf temperature (fig. 16) offers difficulties for interpretation in terms of stomatal opening, because transpiration and leaf temperature are directly affected by light intensity. The decrease in slope above 6×10^4 erg.sec^{-1}.cm^{-2}

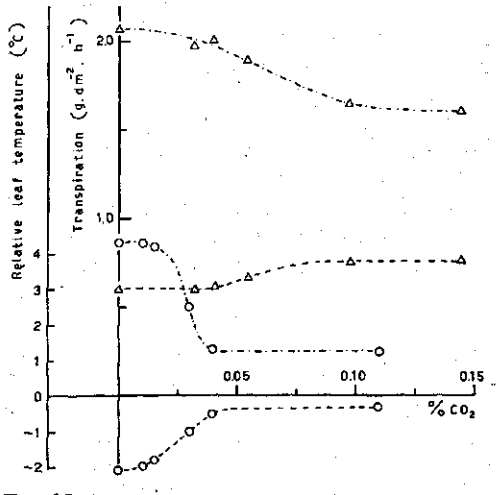


FIG. 15
Influence of CO_2-concentration upon relative leaf temperature and transpiration at two light intensities. Turnip; inlet air temperature 19 °C; △ = 20.7×10^4 erg.sec^{-1}.cm^{-2}; ○ = 1.65×10^4 erg.sec^{-1}.cm^{-2}; ····· = transpiration; - - - = difference between leaf temperature and inlet air temperature.

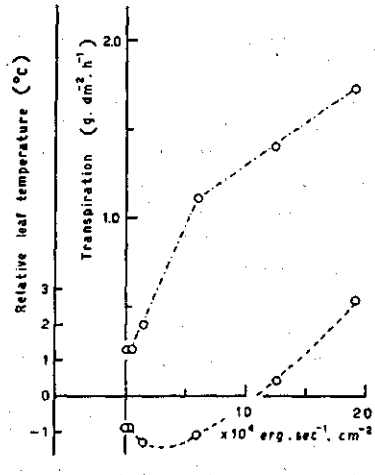


FIG. 16
Influence of light intensity upon relative leaf temperature and transpiration. Turnip; 0.03 % CO_2; inlet air temperature 19.6 °C; ····· = transpiration; - - - = difference between leaf temperature and inlet air temperature.

suggests that the influence of higher light intensities upon the stomatal opening is relatively small.

The time course of the stomatal reaction to a change in CO_2-concentration, as reflected in the time course of transpiration, is presented in fig. 17 for two light intensities, *viz.* 20.7×10^4 and 1.65×10^4 erg.sec^{-1}.cm^{-2}. The time required to reach half of the change in steady state value after a change in CO_2-concentration, varies between 5.5 and 15 minutes for the high light intensity, and between 17 and 22 minutes for the low one. No clear-cut relation was found between reaction time and CO_2-concentration. This may be due to the unequal exposure times at the different concentrations. At both light intensities, the series ended in an atmosphere without CO_2. After imposing darkness, the transpiration rate decreased sharply, due to the decrease in leaf temperature, and, hence, in $[H_2O]_{int}-[H_2O]_a$. Within twenty minutes, no distinct closure of the stomata was observed, but the closing reaction started immediately after the introduction of 0.13 % CO_2.

Simultaneously measured leaf temperatures and rates of photosynthesis and transpiration in a leaf of turnip are presented in fig. 18. All curves show a sharp initial rise upon illumination at an intensity of 19.9×10^4 erg.sec^{-1}.cm^{-2}, after a dark period. This rise, which is particularly pronounced in the case of leaf temperature, can be interpreted as indicating that, in darkness and immediately after illumination, the stomata are almost closed, and that the height of the initial rise represents the maximum rates of photosynthesis and transpiration which are possible at the given light intensity and high diffusion resistance. Due to the low transpiration rate, most energy must be dissipated by conduction, convection, and radiation, so that the leaf temperature increases sharply. After some minutes, the opening reaction of the stomata sets in, and more energy is dissipated via transpiration, resulting in a decrease in leaf temperature. Transpiration and photosynthesis almost simultaneously reach the steady state, which suggests that the equilibration time of photosynthesis on a change from darkness to high light intensity in this case is governed by the rate of opening of the stomata.

A decrease in light intensity from 19.9×10^4 to 13.1×10^4 erg.sec^{-1}.cm^{-2} results in a rapid equilibration of all three curves, apparently because the stomatal opening does not change. A further decrease in light intensity to 0.65×10^4 erg.sec^{-1}.cm^{-2} shows a different picture. Photosynthesis quickly reaches the steady rate, but transpiration and leaf temperature at first show an appreciable decrease, due to the smaller quantity of energy absorbed; thereafter, transpiration decreases more gradually, whereas the leaf temperature increases, reflecting the closure of the stomata.

The reaction of transpiration and leaf temperature upon a subsequent stepwise increase in light intensity shows that the stomata partly open at 1.77×10^4 erg.sec^{-1}.cm^{-2}, while further opening occurs at 6.3×10^4 erg.sec^{-1}.cm^{-2}. No effect is observed on further increasing the light intensity. Photosynthesis quickly reaches equilibrium at all three intensities. Apparently, with a stepwise increase in light intensity, the potential diffusion capacity at the lowest intensity is greater than that required by the rate of the photochemical process at the next higher intensity. Increase in light intensity from 6.3×10^4 to 19.9×10^4 erg.sec^{-1}.cm^{-2} does not result in any further opening of the stomata (as indicated by the quick equilibration of transpiration and leaf temperature), but photosynthesis increases, because at 6.3×10^4 erg.sec^{-1}.cm^{-2}

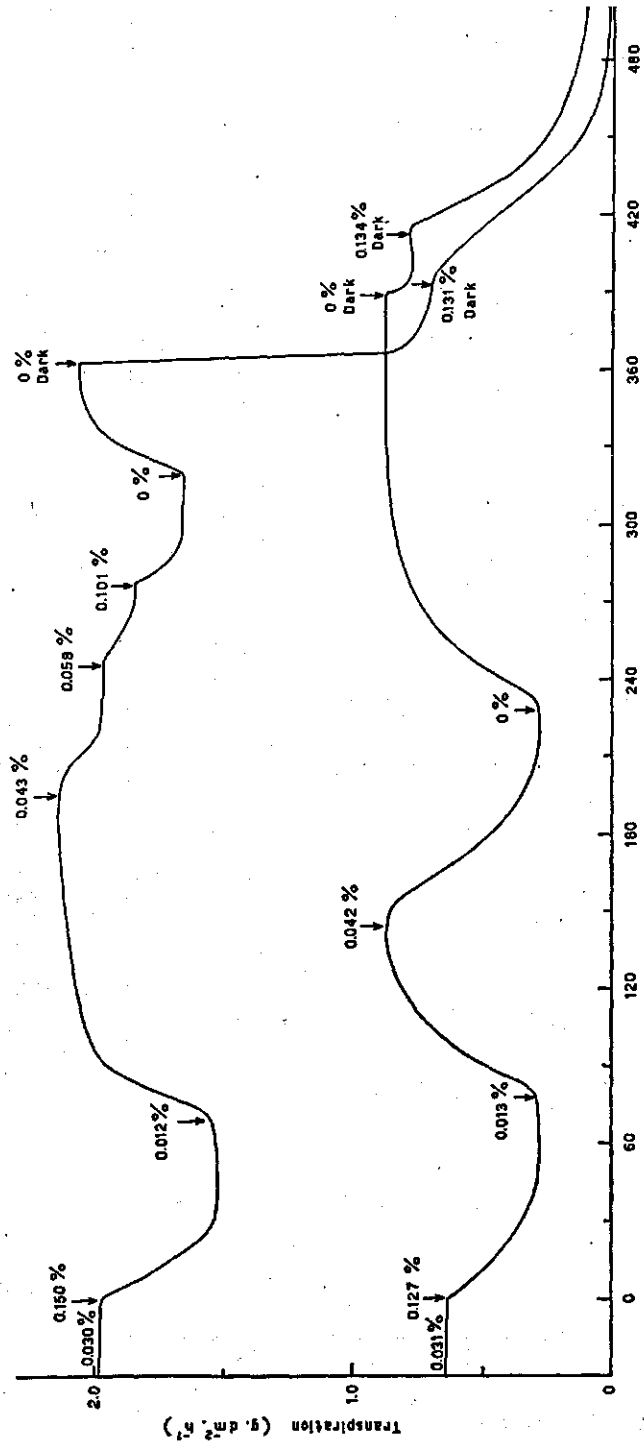


FIG. 17. Time course of transpiration in response to changes in CO_2-concentration, at two light intensities: 20.7×10^4 erg.sec^{-1}.cm^{-2} (upper curve), and 1.65×10^4 erg.sec^{-1}.cm^{-2} (lower curve). Turnip; inlet air temperature 19.6 °C.

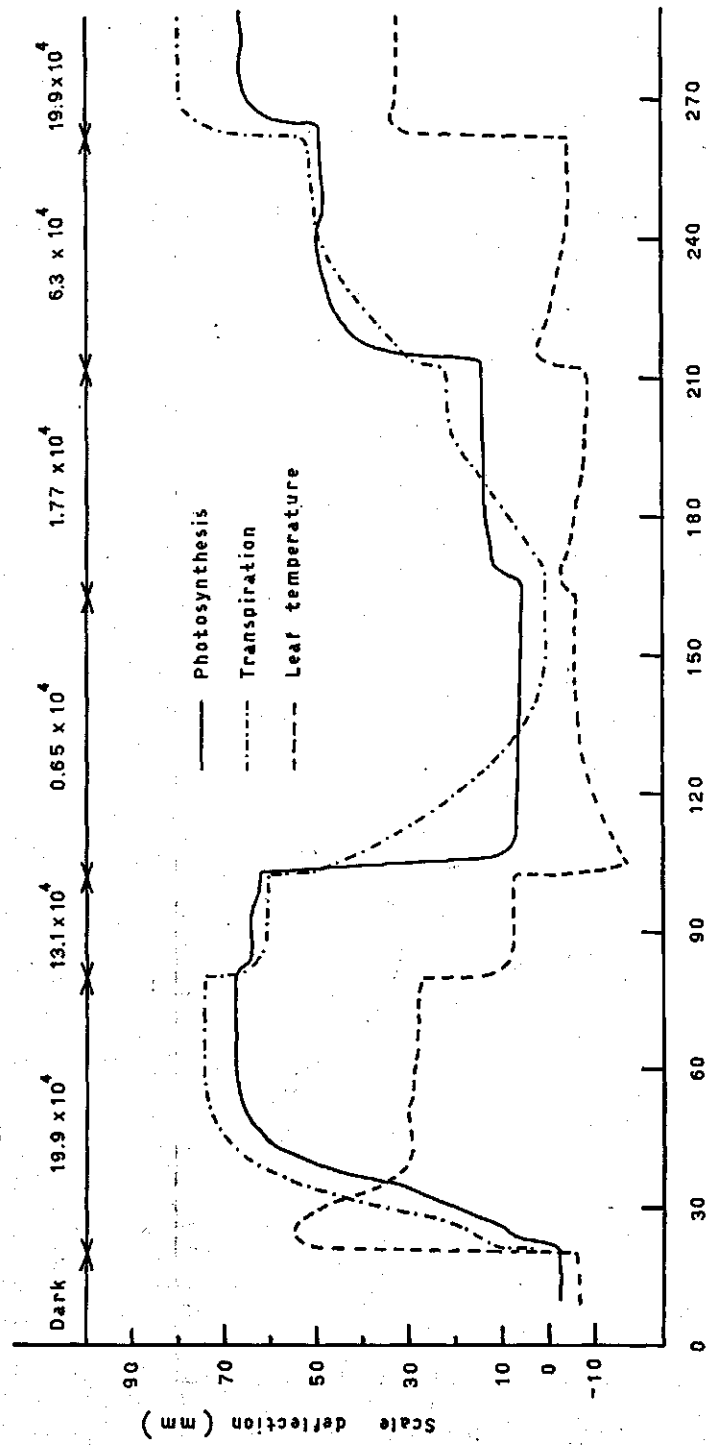


FIG. 18. Time course of photosynthesis, transpiration, and leaf temperature as compared with inlet air temperature, in response to changes in light intensity, in mm scale deflection. Turnip; 0.03 % CO_2; inlet air temperature 21.1 °C. 1 mm scale deflection = 0.1 °C.

the photochemical process limits the rate. The level finally reached is determined by the capacity of the diffusion process, which is indicated by the observation, mentioned already, that at a light intensity of 19.9×10^4 erg.sec^{-1}.cm^{-2}, after a dark period, photosynthesis and transpiration simultaneously reach the steady rate.

V–4. Quantitative estimation of the stomatal diffusion resistance and of the stomatal control of photosynthesis

The stomatal diffusion resistance for CO_2 (r_{s,CO_2}), was calculated according to equations 33, 34, and 35, using the simultaneously measured rates of photosynthesis and transpiration, and the leaf temperature. The stomatal conductance is defined by $1/r_{s,CO_2}$.

The stomatal diffusion resistance, the conductance, and the rate of photosynthesis are plotted in figs. 19-A and B in relation to the CO_2-concentration for a high light intensity and a low one (20.7×10^4 and 1.65×10^4 erg.sec^{-1}.cm^{-2}). An interaction between stomatal opening, CO_2-concentration and light intensity is clearly demonstrated: at the high light intensity, partial closure begins at 0.04 % CO_2 and is almost complete at 0.1 % CO_2. At the low light intensity, the stomatal resistance is much higher, and closure occurs already between 0.01 % and 0.04 % CO_2.

In the high light intensity experiment, photosynthesis is independent of stomatal opening: between 0.04 % and 0.1 % CO_2 the conductance decreases by 37 %, while the rate of photosynthesis remains at the same level.

At the low light intensity, photosynthesis increases between 0.01 % and

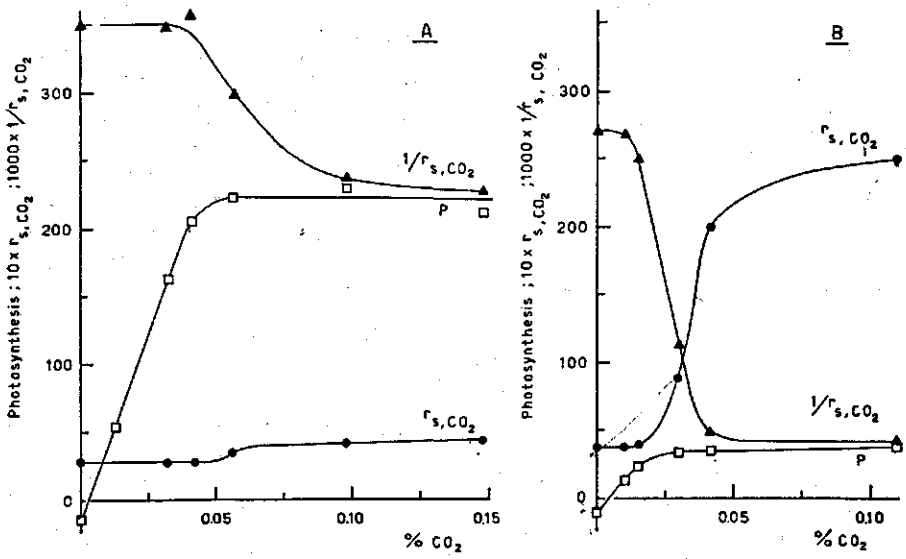


FIG. 19. Photosynthesis (P, in mm^{3}CO$_2$.cm^{-2}.h^{-1}), stomatal diffusion resistance ($r_{s,\,CO_2}$, in sec.cm^{-1}), and stomatal conductance ($1/r_{s,\,CO_2}$, in cm.sec^{-1}) in relation to CO_2-concentration at two light intensities: 20.7×10^4 (fig. A) and 1.65×10^4 erg.sec^{-1}.cm^{-2} (fig. B). Turnip; inlet air temperature 20.1° C.

0.02 % CO_2, in spite of a decrease in conductance, apparently because $[CO_2]_a - [CO_2]_{chl}$ increases relatively more than the conductance of the entire diffusion path decreases, so that the product of conductance and concentration difference (*i.e.* the diffusion rate) increases. The maximum rate of photosynthesis is determined by the capacity of the photochemical process: the efficiency of light energy conversion then is high, *viz.* 15.5 %.

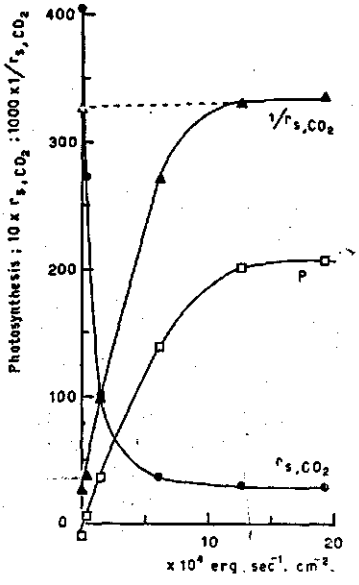


FIG. 20
Photosynthesis (P, in $mm^3 CO_2.cm^{-2}.h^{-1}$), stomatal diffusion resistance (r_{s,CO_2}, in $sec.cm^{-1}$), and stomatal conductance ($1/r_{s,CO_2}$, in $cm.sec^{-1}$) in relation to light intensity. Turnip; 0.03 % CO_2; inlet air temperature 20.1 °C.

The relation between light intensity and resistance, conductance, and photosynthesis was examined in greater detail for the ecologically important normal CO_2-concentration, about 0.03 %. The average data obtained with three leaves with high rates of photosynthesis at light saturation are plotted in fig. 20. Stomatal conductance and photosynthesis both increase with increasing light intensities, but at high intensities, conductance is less influenced than photosynthesis by increasing light intensity: between 6.1×10^4 and 19.2×10^4 $erg.sec^{-1}.cm^{-2}$, conductance increases by 20 %, while photosynthesis does so by 50 %. Therefore, the increased rate of photosynthesis can only partially be due to increased conductance.

The dotted triangle at zero light refers to the stomatal conductance in darkness, immediately after illumination with 19.2×10^4 $erg.sec^{-1}.cm^{-2}$. On imposing darkness, the recordings showed a sharp initial drop in transpiration and temperature, in a way analogous to that observed for the change from 13.1×10^4 to 0.65×10^4 $erg.sec^{-1}.cm^{-2}$ in fig. 18. Thereafter, temperature and transpiration gradually changed owing to stomatal closure. From the temperature and transpiration rate at the intersection of the steeply and gradually changing parts of the time curves, r_{s,CO_2} and the conductance were calculated. As expected, the conductance corresponds to the conductance at the high light intensity, because in the short dark period the stomata could not have closed appreciably. This result demonstrates that the method applied allows correct estimation of the stomatal diffusion resistance.

The data in fig. 20 do not allow a further analysis of the stomatal control of photosynthesis, because the diffusion capacity of the leaf is not determined by the stomatal diffusion resistance alone, but also by the resistances along other parts of the diffusion path. A comparison between these resistances will now be attempted.

In the diffusion of CO_2 from the external air towards the reaction centre in the chloroplasts, resistances are successively encountered in the external air, in the stomata, in the intercellular space, and, in the dissolved state, in the mesophyll cells. Therefore, equation 31 can be extended to

$$P = \frac{[CO_2]_a - [CO_2]_{chl}}{(r_a + r_s + r_{me})_{CO_2}} \qquad (37)$$

in which $[CO_2]_{chl}$ represents the CO_2-concentration in the chloroplasts, and r_a, r_s, and r_{me} the resistances of the external air, the stomata including the intercellular space, and the mesophyll cells, respectively. This equation demonstrates that an appreciable stomatal control of photosynthesis can be expected only when r_s constitutes a considerable part of the total resistance.

P and $[CO_2]_a$ can be determined experimentally, so that the sum of the resistances can be calculated, applying equation 37, when $[CO_2]_{chl}$ is known.

Under conditions of CO_2-limitation (*i.e.* at high light intensities in an atmosphere with relatively low CO_2-concentrations) an almost linear relation between the CO_2-concentration and the rate of photosynthesis was found (see figs. 19 and 23) for CO_2-concentrations between zero and at least 0.03 %. Moreover, fig. 19-A shows that at high light intensities the stomatal resistance remains constant between zero and 0.04 % CO_2. Assuming that the mesophyll resistance is independent of the CO_2-concentration, the observed linear relation between the CO_2-concentration in the external air and the rate of photosynthesis strongly suggests that the CO_2-concentration in the chloroplasts is approximately zero under conditions of CO_2-limitation (*cf.* VAN DEN HONERT, 1930). Under these conditions, the sum of the diffusion resistances can, therefore, be calculated, using equation 37.

The value of r_{a,H_2O} is known from evaporation experiments with leaf models (equation 34) so that r_{a,CO_2} can be calculated according to

$$r_{a,CO_2} = \frac{D_{H_2O}}{D_{CO_2}} \cdot r_{a,H_2O} \qquad (38)$$

Since r_{s,CO_2} is also known (equation 35), r_{me,CO_2} is obtained by subtracting $(r_a + r_s)_{CO_2}$ from the sum of the resistances.

The cell wall may be considered as an aqueous medium in which CO_2 dissolves. According to VAN DEN HONERT (1930) the CO_2-concentration in the cell wall is in equilibrium with that in the air of the intercellular space. The ratio between the two concentrations is determined by the absorption coefficient of CO_2 in the cell wall. We have considered the change of concentration at the air-cell boundary to be due to part of the mesophyll resistance, so that corrections could be omitted.

For several reasons, the validity of equation 38 is not beyond doubt. The transport of water vapour and CO_2 in the external air stream can partly proceed by mass-exchange, due to turbulence of the air. In that case the ratio $r_{a,CO_2} / r_{a,H_2O}$ will be closer to unity than the ratio D_{H_2O} / D_{CO_2}. Attempts were made to determine this relation in experiments with leaf models, consisting of pieces of blotting paper saturated with water or potassium hydroxide solution. R_{a,H_2O} was calculated according to equation 34, and, similarly, r_{a,CO_2} was derived from $\{[CO_2]_a - [CO_2]_{KOH}\} / CO_2$-uptake, in which $[CO_2]_{KOH}$, the CO_2-concentration at the surface of the leaf model, is assumed to be zero. Typical results are presented in Table XI. As expected, r_{a,H_2O} and r_{a,CO_2} both decrease with increasing flow rates, but the ratio $r_{a,CO_2} / r_{a,H_2O}$ increases, whereas a decrease was expected. A similar effect was observed by GRADMANN (1923), using discs with water and KOH-solution in the open air. It may be due to incomplete absorption of CO_2 at the surface of the KOH-solution, so that the layer with zero CO_2-concentration is at some distance below the surface. That this may easily occur is indicated by the fact that owing to the low value of the diffusion coefficient of CO_2 in water (0.16×10^{-4} cm^2. sec^{-1}), a water layer of $1 \times 0.16 \times 10^{-4}$ cm = 0.16 μ, represents a resistance of 1 sec.cm^{-1}.

Notwithstanding the uncertainties discussed, equation 38 has, so far, been used for the calculation of r_{a,CO_2}, because the choice of any other proportionality factor would have been still more arbitrary. In future experiments, a rapid recirculation of the air in the assimilation chamber is envisaged, resulting in smaller values of r_{a,CO_2}, so that small errors in the calculation of this resistance will have less effect on the calculation of r_{me,CO_2}.

TABLE XI. External air resistances, r_{a,H_2O} and r_{a,CO_2}, determined from the evaporation rate and the CO_2-uptake of leaf models for two leaf areas (69 and 148 cm^2), and two rates of air supply (254 and 385 $l.h^{-1}$).

Leaf area (cm^2)	Air supply ($l.h^{-1}$)	Air velocity ($cm.sec^{-1}$)	r_{a,CO_2} ($sec.cm^{-1}$)	r_{a,H_2O} ($sec.cm^{-1}$)	$\frac{r_{a,CO_2}}{r_{a,H_2O}}$
69	255	4.21	1.78	0.71	2.51
69	384	6.38	1.69	0.57	2.97
148	253	2.70	2.16	1.03	2.11
148	387	4.13	2.03	0.88	2.31

In fig. 21, the stomatal resistance of different leaves has been plotted against light intensity, while in separate columns the values of the resistance in the mesophyll and in the external air are indicated. All experiments were made in air with 0.03 % CO_2. At high light intensities, *i.e.* when the capacity of the diffusion process limits the rate of photosynthesis, r_{me} is from 1 to 4 times r_s, so that r_{me} is an important rate-determining factor under these conditions. At high light intensities the variation of r_{me} is much larger than that of r_s, indicating that differences in the photosynthetic rate of the various leaves are due mainly to differences in mesophyll resistance.

Under conditions of CO_2-limitation, the effect of partial closure of the stomata (as *e.g.* induced by water shortage) on photosynthesis is mainly determined by the ratio $(r_{me} + r_a) / r_s$. The lowest value observed of this ratio is about 1. In that case, doubling of the stomatal resistance causes a decrease of one third in the rate of photosynthesis. For $(r_{me} + r_a) / r_s = 4$, the decrease is only one sixth for doubling of the stomatal resistance, (see equation 37).

Under conditions of light limitation, variations in stomatal resistance affect the rate of photosynthesis only when the diffusion capacity is small as compared with the maximum rate of the photochemical process at the prevailing light intensity. For reasons of comparison, it has been tentatively assumed that the mesophyll resistance does not change with light intensity. The maximum diffusion capacity at various light intensities was then calculated according to equation 37 for $[CO_2]_a = 0.03$ % and $r_a = 1$ $sec.cm^{-1}$, while r_s was

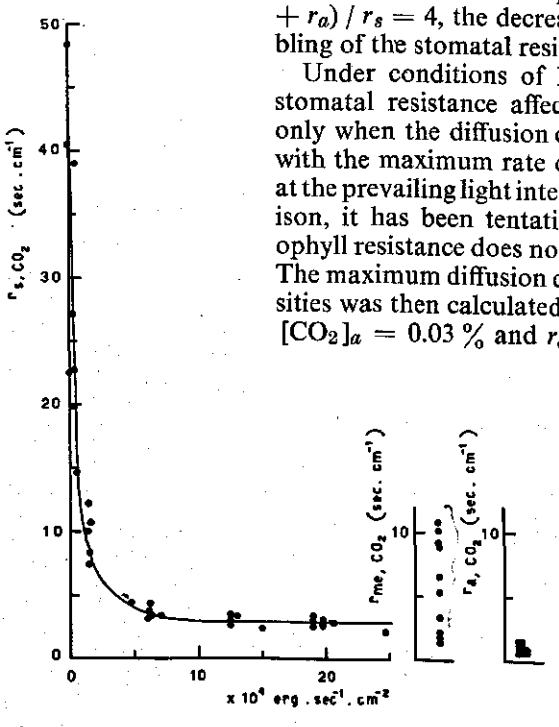


FIG. 21
Stomatal diffusion resistance (r_{s,CO_2}) in relation to light intensity. In separate columns external air resistance (r_{a,CO_2}), and mesophyll resistance (r_{me,CO_2}). Turnip; 0.03 % CO_2; inlet air temperature 20.3 °C.

taken from the average curve of fig. 21. Two values of r_{me} were used, *viz.* 2 and 10 sec.cm^{-1}, while in all cases $[CO_2]_{chl}$ was assumed to be zero. The maximum rate of the photochemical process was calculated assuming a quantum efficiency of 0.1. The number of Einsteins (A) absorbed per incident erg by a leaf with average absorption characteristics (see Table V) was used in the calculation. Thus, for an incident intensity I erg.sec^{-1}.cm^{-2}, $I \times A \times 0.1$ moles CO_2.sec^{-1}.cm^{-2} are absorbed, or $I \times A \times 0.1 \times 22.4 \times 10^6 \times 3600$ mm^3 CO_2.cm^{-2}.h^{-1}.

The maximum diffusion rate, and the maximum rate of the photochemical process are plotted against the light intensity in fig. 22 (see also Table XII). In the case of low r_{me}, when thediffusion capacity is largely determined by the stomatal resistance, the maximum rate of diffusion is much greater than that of the photochemical process at limiting light intensities. Consequently, a considerable degree of stomatal closure will not affect the rate of photosynthesis under these conditions. This is expressed for a number of light intensities by the ratio r'_s / r_s in Table XII, in which r'_s represents the stomatal resistance at which the diffusion capacity would just allow the maximum rate of the photochemical process.

The calculated differences between the maximum rates of the photochemical process and the maximum diffusion rate are in accordance with the rapid equilibration of photosynthesis on stepwise increase in light intensity (fig. 18), discussed at p. 48.

It may be concluded that appreciable stomatal control of photosynthesis can be expected under conditions of CO_2-limitation at relatively low values of the mesophyll resistance. The influence of the stomata is relatively small at limiting light intensities, and at high values of the mesophyll resistance under conditions of CO_2-limitation.

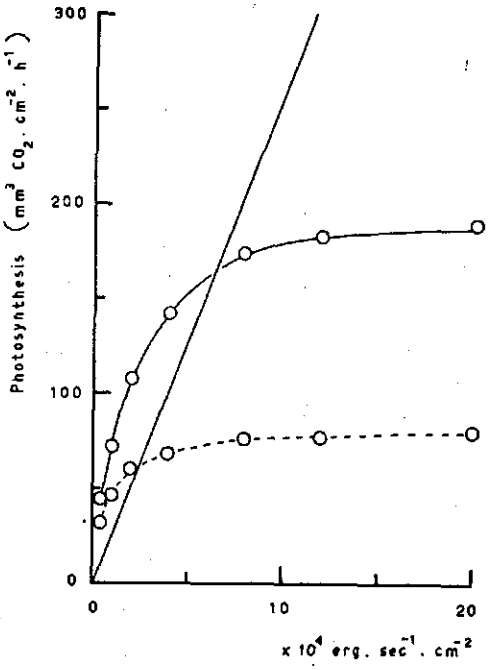


FIG. 22. Comparison between the maximum rate of the photochemical process (straight line) and that of the diffusion process for, respectively, a low mesophyll resistance (fulldrawn curve) and a high one (broken curve), as a function of light intensity. Turnip; 0.03 % CO_2; inlet air temperature 20.3 °C.

V–5. Discussion

The calculation of the total diffusion resistance from the rate of photosynthesis under CO_2-limiting conditions and the difference between the CO_2-concentrations in the external air and in the chloroplasts, was based on the assumptions that the CO_2-concentration in the chloroplasts approaches zero, and that, in the mesophyll cells, CO_2

TABLE XII. Comparison between the maximum rates of the photochemical process and the diffusion process, and between the mean observed stomatal resistance (r_{s,CO_2}, taken from fig. 21) and the resistance (r'_{s,CO_2}) inducing equal rates of the photochemical and the diffusion process at a number of light intensities. Maximum diffusion rate calculated according to equation 37 for $[CO_2]_a = 0.03\%$, $r_{a,CO_2} = 1$ sec.cm^{-1}, and $r_{me} = 2$ and 10 sec. cm^{-1} respectively. Maximum rate of the photochemical process calculated from the number of Einsteins absorbed per incident erg for an average leaf (Table V) and for a quantum efficiency $\Phi = 0.1$.

Light intensity ($\times 10^4$ erg.sec^{-1}. cm^{-2})		0.5	1	2	4	5	6	8	12	20
r_{s,CO_2} (sec.cm^{-1})		22.0	12.0	7.0	4.6	4.15	3.8	3.2	3.0	2.7
$r_{me} = 2$ sec.cm^{-1}	diffusion rate / photochem. rate	3.35	2.80	2.10	1.39	1.18	1.03	0.85	0.59	0.37
	r'_{s,CO_2} (sec.cm^{-1})	81	39	18	7.5	5.5	4.0	2.2	0.5	
	$r'_{s,CO_2}/r_{s,CO_2}$	3.7	3.3	2.6	1.6	1.3	1.05	0.69	0.17	
$r_{me} = 10$ sec.cm^{-1}	diffusion rate / photochem. rate	2.53	1.84	1.17	0.68	0.56	0.47	0.37	0.25	0.15
	r'_{s,CO_2} (sec.cm^{-1})	73	31	10						
	$r'_{s,CO_2}/r_{s,CO_2}$	3.3	2.6	1.4						

is transported by diffusion. The validity of the latter supposition has been questioned, *e.g.* by ROMELL (1927), STÅLFELT (1935), and VERDUIN (1954). Indeed, the process may not be as simple as the dissolution of CO_2 in the wet cell walls, followed by its diffusion towards the chloroplasts. Adsorption phenomena and chemical absorption by cell constituents may occur, but ultimately they will also depend upon the CO_2-concentration, and the complex formed must be transported to the chloroplasts. For the present purpose, the nature of the process is immaterial if it has a linear relation to the CO_2-concentration. The diffusion resistance of the mesophyll then simply includes an additional factor, *viz.*, the reciprocal of the proportionality factor of the CO_2-dependent process.

The linear relation between photosynthesis and external CO_2-concentration under conditions of CO_2-limitation (see section V–4), indicates that the rate of each partial process, active in the transport of CO_2, indeed is proportional to the CO_2-concentration. Moreover, it is not improbable that CO_2-transport in the mesophyll cells is simply due to diffusion; in experiments with *Hormidium*, VAN DEN HONERT (1930) found that the observed rate of photosynthesis under conditions of CO_2-limitation corresponds to the diffusion rate as calculated from the dimensions of the diffusion path, when the cell was considered as an aqueous medium, and $[CO_2]_{chl}$ was assumed to be zero.

The assumption that $[CO_2]_{chl}$ is approximately zero and the observation that a linear relation between photosynthesis and $[CO_2]_a$ starts at CO_2-concentrations very close to zero (see below), seems contrary to data of GABRIEL-

SEN (1948, 1949) and HEATH (1949). GABRIELSEN, in closed and open circuits, and HEATH, in open circuits with air flushed over or through a leaf, found that in the external air a minimum CO_2-concentration of about 0.009 % was maintained, irrespective of the initial CO_2-concentration. The authors considered this as a threshold concentration, below which no photosynthesis occurs. It seems, however, that this concentration in the external air does not represent $[CO_2]_{chl}$. In a closed circuit, *e.g.*, $[CO_2]_{chl}$ will be lower than the steady state value of $[CO_2]_a$, and the difference between these concentrations will increase with increasing respiration rate and with increasing diffusion resistance between the average site of respiration and the chloroplasts. As expected, the equilibrium value of $[CO_2]_a$ was found to increase with increasing respiration rate by EGLE and SCHENK (1953).

In our open system, the CO_2-exchange of leaves was measured in rapidly flowing air, avoiding large CO_2-gradients along the leaf. Typical results obtained in low CO_2-concentrations at an incident light intensity of 4.05×10^4 erg.sec^{-1}.cm^{-2} are represented in fig. 23. Even at very low CO_2-concentrations the rate of photosynthesis increases linearly with increasing $[CO_2]_a$, suggesting that $[CO_2]_{chl}$ was very low in these experiments.

The occurrence of very low CO_2-concentrations in the chloroplasts implies that the equilibrium in the first chemical reaction of CO_2 is largely at the side of the reaction products. In modern concepts of photosynthesis, this first chemical step is a reaction of CO_2 with ribulose diphosphate to form phosphoglyceric acid. This reaction was observed by JAKOBY, BRUMMOND and OCHOA (1956), and by WEISSBACH, HORECKER and HURWITZ (1956) in enzyme preparations from spinach leaves. No indications could be obtained for its reversibility, suggesting that the steady state concentration of CO_2 in the chloroplasts is very low during photosynthesis.

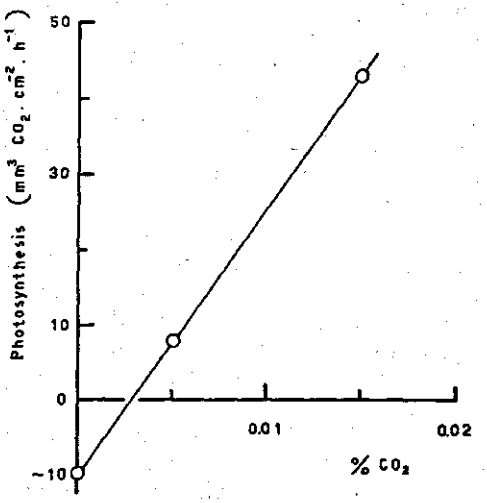


FIG. 23. Rate of photosynthesis at low CO_2-concentrations. Turnip; light intensity 4.05×10^4 erg.sec^{-1}.cm^{-2}; inlet air temperature 20.3 °C.

The assumptions, made above in calculating the sum of the diffusion resistances seem, therefore, acceptable. Certain advantages of our method are: The diffusion resistance of the stomata is obtained in absolute units during their normal, undisturbed operation; synchronous and simultaneous measurements of this resistance and of photosynthesis and transpiration is possible, eliminating the influence of individual differences between leaves; the mean stomatal resistance of the whole leaf is determined, and the stomatal resistance can be compared quantitatively with other resistances along the diffusion path.

Many conflicting data on stomatal control of photosynthesis are found in the literature, see the reviews by STÅLFELT (1935) and RABINOWITCH (1951). In most cases, however, the stomatal diffusion resistance was not determined quantitatively. Consequently, comparisons with the resistances in other parts of the diffusion path cannot be made, and moreover, comparison between the capacity of the diffusion process and the rate of the photochemical process at the prevailing light intensity is impossible. In that case, a correlation between stomatal opening and photosynthesis is observed, when both increase with increasing light intensities, but it is obvious that this correlation has no significance in limiting the rate of photosynthesis when the photochemical process determines the photosynthetic rate.

In experiments on the influence of water supply on stomatal opening and photosynthesis, a simultaneous change of the stomatal and mesophyll resistances may give rise to erroneous conclusions regarding the effect of stomatal opening on photosynthesis. PISEK and WINKLER (1956) *e.g.*, worked with leaves saturated with water at the beginning of the experiments, and measured photosynthesis and stomatal opening during water loss. A parallelism between photosynthesis and stomatal opening was observed. At first both increased until an optimum was reached; with increasing water deficit both decreased again until finally the stomata were completely closed and photosynthesis stopped. PISEK and WINKLER concluded from these observations that the influence of water deficit upon photosynthesis is due to an effect upon stomatal opening. This conclusion, however, is not justified. Comparison of the photosynthetic rates for the same stomatal width in the opening and closing phase respectively (*loc. cit.* fig. 6), shows that the photosynthetic rate in the opening phase is 2.4 to 3.5 times that in the closing phase. A plausible explanation is that water loss brought about a greater increase in mesophyll resistance than in stomatal resistance.

The most extensive experiments on stomatal control of photosynthesis at different light intensities are those of STÅLFELT (1935). At light saturation, photosynthesis was correlated with stomatal opening, which suggests an important stomatal control of photosynthesis. However, different degrees of stomatal opening were obtained by exposing the plants to different degrees of humidity and to different light intensities prior to the experiments, so that the water balance of the leaves under investigation was different. Consequently, not only the stomatal resistance but also the mesophyll resistance may have been different, and the causal relationship between stomatal opening and photosynthesis may have been less obvious than is suggested by STÅLFELT's results. The true relation can only be discovered when the various diffusion resistances are compared.

Since BROWN and ESCOMBE's (1900) fundamental investigations into the nature of the stomatal diffusion process, the diffusion capacity of widely opened stomata has usually been calculated from idealized dimensions, or from data obtained by the porometer technique, *e.g.* by MASKELL (1928), PENMAN and SCHOFIELD (1951), and VERDUIN (1954). Simultaneous measurements of photosynthesis have not been made, but comparison between the maximum diffusion capacity for a drop of 0.03 % CO_2 in the stomata and actually observed rates of photosynthesis showed that, during photosynthesis, the CO_2-concentration drop in the stomata was small as compared with the concentration in the external air, indicating a high mesophyll resistance. HILL and WHITTINGHAM (1955) compared rates of transpiration and photosynthesis, observed by BROWN and ESCOMBE in separate experiments and under different conditions. In *Helianthus* leaves,

they found the mesophyll resistance to be 14 times the stomatal resistance.

PENMAN and SCHOFIELD (1951) estimated the total water loss and dry matter production of an acre of turf under outdoor conditions over an entire season. They compared the "transpiration ratio" (weight of water lost/dry matter produced) with the ratio transpiration/photosynthesis $= D_{H_2O}([H_2O]_{int} - [H_2O]_a)/D_{CO_2}([CO_2]_a - [CO_2]_{int})$, (see equations 31 and 32), in which the water vapour concentrations were estimated from meteorological data. For $[CO_2]_a - [CO_2]_{int} =$ 0.03 % CO_2, the ratio transpiration/photosynthesis was much smaller than the transpiration ratio, which is based on the actually measured dry matter production, so that the CO_2-concentration in the intercellular space must have been close to the external CO_2-concentration. PENMAN and SCHOFIELD estimated that for favourable outdoor conditions $r_{me,CO_2}/r_{s,CO_2} = 30$ and for normal conditions this ratio might even be 100. It seems, however, that r_{me} was greatly overestimated, because the calculations were based on the assumption that the diffusion process was limiting the rate of photosynthesis. During a large part of the season, however, the light intensity will have been the limiting factor, resulting in a high CO_2-concentration in the intercellular space, because the concentration near the chloroplasts will then be above zero.

Very careful calculations of the stomatal resistance at different stomatal apertures were made for *Zebrina* leaves by BANGE (1953). We have calculated r_{s,CO_2} and the diffusion capacity for a concentration drop of 0.03 % CO_2 in the stomata from his data (Table XIII). The resistance of widely opened stomata is of the same order of magnitude as found in our experiments, while the maximum diffusion capacity is much higher than the rates actually observed in 0.03 % CO_2, again indicating a high mesophyll resistance.

TABLE XIII. Stomatal diffusion resistance (r_{s,CO_2}) and stomatal diffusion capacity in *Zebrina* leaves for $[CO_2]_a - [CO_2]_{int} = 0.03\%$. Derived from BANGE (1953).

Stomatal aperture (μ)	2.5	5	7.5	10	12.5	15	17.5	20
r_{s,CO_2} (sec.cm^{-1})	6.40	4.10	3.08	2.50	2.14	1.89	1.70	1.53
Stomatal diffusion capacity (mm^3 CO_2.cm^{-2}.h^{-1})	148	230	306	380	458	500	558	618

The effect of CO_2 upon stomatal opening has been investigated by LINSBAUER (1917), CHAPMAN, COOK and THOMPSON (1924), SCARTH (1932), FREUDENBERGER (1940), HEATH (1950), HEATH and MILTHORPE (1950), SCARTH and SHAW (1951), HEATH and RUSSELL (1954). Most of these investigators compared the opening in normal air (0.03 % CO_2) with that in very low (usually zero) or very high CO_2-concentrations. In general, increase in CO_2-concentration induced stomatal closure. HEATH and RUSSELL (1954*b*) applied different light intensities and a series of photosynthetically interesting CO_2-concentrations (zero, 0.010, 0.017, 0.029, 0.049, and 0.084 % CO_2). In wheat, they found similar interactions between CO_2-concentration, light intensity, and stomatal resistance (measured with the porometer technique) as observed in the present experiments. At the highest light intensity (800 ft-c from a 500 W projector lamp which approximately equals 3.6×10^4 erg.sec^{-1}.cm^{-2}), the lowest resistance was observed, and it remained constant between zero and 0.049 % CO_2. At 90 ft-c, a gradual increase of the resistance began already at 0.01 % CO_2.

CHAPTER VI

GENERAL DISCUSSION

Photosynthesis depends on the temperature, the light intensity, and the CO_2-concentration in the chloroplasts; the experimental conditions actually measured should allow an approximation of the conditions *in situ*, *cf.* Chapter III.

Under conditions of CO_2-limitation, one should know the effective CO_2-concentrations in the air streams along the upper and lower leaf sides, $c_{eff,u}$ and $c_{eff,b}$ respectively, which concentrations determine the rate of photosynthesis. Normally, however, only the CO_2-concentrations in the total mass of incoming and outgoing air are known, and the CO_2-concentration around the leaf is then assumed to be characterized by the arithmetic mean, $\bar{c}$, of these concentrations. It has been shown that $\bar{c}$ approaches $c_{eff,u}$ and $c_{eff,b}$ satisfactorily only when high flow rates are applied and when care is taken to ensure a reproducible position of the leaf in the centre plane of the assimilation chamber.

In agreement with HOOVER, JOHNSTON and BRACKETT (1933), CO_2-limitation was found to occur up to 0.1 % CO_2 at high light intensities and at about 23 °C. Therefore, CO_2 will limit photosynthesis in experiments carried out in normal air (containing about 0.03 % CO_2) at high light intensities. The observed rates of photosynthesis then will depend on the rate of air supply and on the position of the leaf in the assimilation chamber, see HEINICKE and HOFFMAN (1933), and DECKER (1947).

According to VERDUIN and LOOMIS (1944), however, photosynthesis at high light intensities should not be appreciably limited by CO_2, even at concentrations considerably below normal. They measured photosynthesis of maize leaves (70–130 cm^2), enclosed in cellophane envelopes and provided with normal air at a rate of 0.74-1.37 $l.cm^{-2}.h^{-1}$. The rate of photosynthesis per unit leaf area was plotted against the residual CO_2-content of the air stream for all experiments at light intensities of 4000 ft-c or more (*loc. cit.* fig. 6, p. 289). Photosynthesis was inversely related to residual CO_2-content, while the slope of the curve was only slightly affected by the degree of depletion, from which the authors concluded that "photosynthesis was affected surprisingly little" even by 70 % depletion. This conclusion, however, is not justified, since the relation was derived from experiments with different leaves, while the experimental conditions, *e.g.* the leaf area, the initial CO_2-concentration, and the position of the leaves in the cellophane envelopes, were not kept constant. No information on the influence of the CO_2-concentration in a single leaf was obtained. Qualitatively, the relation observed can be explained only by an irreproducible position of the leaves in the envelopes (*cf.* Chapter III), and by differences in the photosynthetic activity of various leaves. According to the viewpoints developed in Chapter IV, these differences probably are due to different diffusion resistances, so that the rate of diffusion in the separate leaves then depends on the CO_2-concentration.

At high light intensities, the leaf temperature may deviate considerably from the temperature of the air or of the wall, so that, particularly under CO_2- and light saturating conditions, when the rate of photosynthesis is strongly affected by temperature (Chapter IV), measurement and control of leaf temperature are necessary. Under CO_2-limiting conditions and at moderate temperatures, the rate of photosynthesis is almost independent of temperature. Excessive temperatures, however, should be avoided also under these conditions, because other-

wise even negative rates of apparent photosynthesis may be measured or irreversible damage to the plants may occur (TRANQUILLINI, 1954). Furthermore, increased transpiration by the action of high temperatures, combined with insufficient water supply to the leaves, may result in a decreased rate of photosynthesis (ASHTON, 1956).

The need for accurate control and measurement of the experimental conditions is evidenced by comparing experiments on the influence of the CO_2-concentration upon photosynthesis at ambient temperatures of about 23 °C (Chapter IV). With higher plants, in the experiments of HOOVER, JOHNSTON and BRACKETT (1933) and in those presented in Chapter IV, did CO_2-saturation occur at low CO_2-concentrations (about 0.1 %), as observed by VAN DEN HONERT (1930) for *Hormidium*. In the other investigations reviewed, much higher values were reported, which may have been due either to inadequate air supply or to insufficient control of leaf temperature.

The combined measurements of photosynthesis, transpiration, and leaf temperature (Chapter V) are useful in that the diffusion resistances in different parts of the diffusion path can be computed quantitatively. For the limited number of plants so far used, it has been found that photosynthesis is affected relatively little by the stomata under conditions of CO_2-limitation, because the mesophyll resistance was usually high as compared with the stomatal resistance. Under these conditions, differences in photosynthetic activity between different plants were due mainly to variation in the mesophyll resistance, so that the latter seems to be an important rate-determining factor.

Diffusion takes place through a series of resistances, and the concept that the rate is determined by the sum of these resistances implies that transpiration (T) depends upon the stomatal opening more than photosynthesis (P). For a given set of experimental conditions, and under CO_2-limitation, the relation between P and T is determined by the ratio $(r_a + r_s) / (r_a + r_s + r_{me})$, *cf.* PENMAN and SCHOFIELD (1951), so that plants utilizing water economically are expected to show high ratio values. It seems, therefore, that the application to other species of the principles outlined in Chapter V, might give valuable information concerning the factors determining the production capacity and the water economy in crop plants.

SUMMARY

The present paper deals with experiments designed to analyse the photosynthetic activity of crop plants. The following features are successively discussed: technical requirements and details of the experimental set-up, the influence of light intensity, CO_2-concentration, and leaf temperature on photosynthesis of cucumber, tomato, spinach, turnip, and sugar beet, the effect of light intensity and CO_2-concentration on the stomatal diffusion resistance, and the stomatal control of photosynthesis.

The experimental set-up (Chapter II) allows the simultaneous and continuous measurement of the rate of photosynthesis (infrared gas analyzer), the transpiration rate (lithium chloride hygrometer), and the temperature (thermocouples) of a single leaf, attached to the plant. The set-up was designed to obtain accurate measurement and independent variation of light intensity, CO_2- and water vapour concentration of the air, and leaf temperature.

Theoretical considerations showed that high rates of air supply and a repro-

ducible position of the leaf in a narrow assimilation chamber are required in order to obtain accurate estimates of the average CO_2-concentration. The temperature-increasing effect of light was reduced by the use of assimilation chambers with double, water-cooled walls, and by reduction of the infrared radiation of the light source, resulting also in increased accuracy of the temperature measurement (Chapter III).

The light, incident upon the leaf, was measured in absolute units. Computation of the number of Einsteins absorbed per unit incident energy and per unit of illuminance (ft-c, lux) provided a quantitative demonstration of the well-known fact that the light conditions should not be expressed in photometric units. In order to facilitate the comparison of the present experiments with those in which photometric units are used, the relation between absolute and photometric units was computed for a large number of light sources (Chapter III, Tables IV and V).

For leaf temperatures between 21 and 24°C, CO_2-saturation of photosynthesis was reached in a gas phase containing approximately 0.1 % CO_2. The higher values observed by several investigators at about the same ambient temperature, are to be ascribed to low rates of air supply or insufficient control of leaf temperature (Chapter IV).

The diffusion resistances in the external air, in the stomata, and in the mesophyll cells were computed from the simultaneously and continuously measured temperature, transpiration, and photosynthesis of a single leaf, and from evaporation experiments with leaf models (Chapter V–2).

Time curves of transpiration, leaf temperature, and photosynthesis, following a change in light intensity, and of transpiration following a change in CO_2-concentration, reflect the time course of stomatal opening. These experiments showed that the time course of photosynthesis was not determined by the stomatal reaction when the light intensity was stepwise increased in the range of light limitation. At high light intensities, given immediately after a dark period in which the stomata had been closed, however, the time course of photosynthesis was determined by the opening reaction of the stomata (Chapter V–3).

In normal air, the stomatal conductance increased linearly with light intensity between zero and 6×10^4 erg.sec^{-1}.cm^{-2}. At a light intensity of 20×10^4 erg.sec^{-1}.cm^{-2}, the maximum conductance was observed in CO_2-concentrations between zero and 0.04 %, while a partial closure of the stomata occurred at CO_2-concentrations between 0.04 % and 0.1 %. At a light intensity of 1.65×10^4 erg.sec^{-1}.cm^{-2}, a lower value of the stomatal conductance was found, and the closing reaction occurred already between 0.01 % and 0.04 % CO_2 (Chapter V–4).

It follows from a comparison of the maximum capacity of the diffusion process and the maximum rate of the photochemical process at a given light intensity, that, under conditions of light limitation, a considerable closure of the stomata may occur without an effect on the rate of photosynthesis. Under light saturation and at normal CO_2-concentrations, the rate of diffusion determines the rate of photosynthesis, but the stomatal control depends upon the relation between the stomatal resistance (r_s) and the sum of the resistances in the external air, in the stomata, and in the mesophyll cells ($r_a + r_s + r_{me}$). In the leaves used so far, the ratio $(r_a + r_s + r_{me})/r_s$ ranged between 1 and 5. For the latter value, doubling of the stomatal diffusion resistance results in a decrease in the photosynthetic rate of only 16 %. In the leaves used, variation in the value of the ratio

was mainly due to variation in the mesophyll resistance. An examination of the relevant literature suggest that the relatively high value and the great variability of the mesophyll resistance is a widespread phenomenon. Consequently, the mesophyll resistance seems to be an important yield-determining factor in crop plants (Chapter V–4).

For leaves with an adequate water supply, transpiration depends upon the sum of the diffusion resistances in the external air and in the stomata, while, under conditions of CO_2-limitation, the rate of diffusion of CO_2 from the external air towards the chloroplasts is governed by the sum of these resistances and the mesophyll resistance. Consequently, transpiration is more dependent on the stomatal condition than is photosynthesis. In this connection, the method employed offers possibilities for a quantitative analysis of the water economy of crop plants.

SAMENVATTING

Het doel van het beschreven onderzoek is de meting van de photosynthese bij cultuurgewassen. Achtereenvolgens worden besproken: bijzonderheden van de apparatuur en van de methodiek; de invloed van de lichtintensiteit, de koolzuurconcentratie en de bladtemperatuur op de photosynthese van komkommer, tomaat, spinazie, stoppelknol en suikerbiet; de invloed van licht en koolzuur op de diffusieweerstand van de huidmondjes en de invloed van deze weerstand op de photosynthese.

De photosynthese, de transpiratie en de temperatuur van een aan de plant bevestigd blad werden gelijktijdig en continu gemeten, respectievelijk met de infrarood-absorptiemethodiek, met een lithiumchloride-hygrometer en met thermokoppels (Hoofdstuk II). De proeven werden ingericht op nauwkeurige meting en onafhankelijke variatie van de lichtintensiteit, het koolzuur- en waterdampgehalte van de lucht en van de bladtemperatuur.

Op grond van theoretische overwegingen werd aangetoond dat een grote stroomsnelheid van de lucht en een reproduceerbare positie van het blad in de assimilatiekamer noodzakelijk zijn voor een nauwkeurige bepaling van het gemiddelde CO_2-gehalte van de lucht in de assimilatiekamer. De temperatuurverhogende werking van licht werd beperkt door de toepassing van assimilatiekamers met dubbele, met water gekoelde wanden. Hierdoor werd eveneens een nauwkeuriger meting van de bladtemperatuur verkregen (Hoofdstuk III).

Het ingestraalde licht werd in absolute eenheden gemeten. Meting van de lichtsterkte in photometrische eenheden (lux, foot-candle) is niet aan te bevelen, hetgeen werd aangetoond door de berekening van het aantal geabsorbeerde Einsteins per eenheid van ingestraalde lichtenergie en per lux en ft-c. Ter vergelijking van de beschreven proeven met experimenten waarin photometrische eenheden worden gebruikt, werd de relatie tussen absolute en photometrische eenheden voor verschillende lichtbronnen berekend (Hoofdstuk III, Tabellen IV en V).

Bij bladtemperaturen van 21 tot 24 °C en hoge lichtintensiteiten trad koolzuurverzadiging op bij een concentratie van ongeveer 0.1 % CO_2 in de lucht buiten het blad. De hogere koolzuurconcentraties die hiervoor door verschillende onderzoekers werden gevonden, zijn waarschijnlijk het gevolg van een te lage stroomsnelheid van de lucht, of van onvoldoende beheersing van de bladtemperatuur (Hoofdstuk IV).

Kwalitatieve gegevens over de invloed van de lichtintensiteit en het CO_2-gehalte van de lucht op de openingstoestand van de huidmondjes werden verkregen door de transpiratiesnelheid en de bladtemperatuur in afhankelijkheid van deze factoren te meten. In overeenstemming met literatuurgegevens, bleek licht de opening, CO_2 de sluiting van de huidmondjes te bevorderen (Hoofdstuk V–3).

Vergeleken met het photosyntheseproces, reageren de huidmondjes langzaam op een verandering in lichtintensiteit of CO_2-gehalte. De stomataire regeling van de photosynthese kon daarom kwalitatief worden beoordeeld door het verloop van de transpiratie, de bladtemperatuur, en de photosynthese te vervolgen na een verandering van de lichtintensiteit. Bij een trapsgewijze verhoging van de lichtintensiteit binnen het gebied van lichtlimitering der photosynthese, bleek de verandering van de photosynthesesnelheid niet te worden bepaald door de reactie van de huidmondjes. Dit was wel het geval wanneer na een donkerperiode, waarin de huidmondjes gesloten waren, plotseling een hoge lichtintensiteit werd gegeven (Hoofdstuk V–3).

Voor de kwantitatieve beoordeling van de openingstoestand van de huidmondjes en van de stomataire regeling van de photosynthese, werden de diffusieweerstanden in de lucht buiten het blad, in de huidmondjes inclusief de intercellulaire ruimte, en in de mesophylcellen berekend. De berekeningen waren gebaseerd op de gelijktijdig en ononderbroken gemeten bladtemperaturen en photosynthese- en transpiratiesnelheden en op de in afzonderlijke proeven gemeten evaporatiesnelheid van bladmodellen (Hoofdstuk V–2).

In normale lucht nam het geleidingsvermogen van de huidmondjes rechtlijnig toe met verhoging van de lichtintensiteit tot 6×10^4 erg.sec^{-1}.cm^{-2}. Bij 20×10^4 erg.sec^{-1}.cm^{-2} werd het grootste geleidingsvermogen waargenomen in CO_2-concentraties tussen nul en 0.04 %, terwijl een gedeeltelijke sluiting van de huidmondjes optrad tussen 0.04 % en 0.1 % CO_2. Bij een lagere lichtintensiteit (1.65×10^4 erg.sec^{-1}.cm^{-2}) was het geleidingsvermogen kleiner, terwijl sluiting reeds optrad tussen 0.01 % en 0.04 % CO_2 (Hoofdstuk V–4).

Uit een vergelijking van de maximale capaciteit van het diffusieproces en de maximale snelheid van het photochemische proces bij een reeks van lichtintensiteiten bleek dat bij lichtlimitering een aanzienlijke mate van sluiting van de huidmondjes kan optreden voordat de photosynthese wordt beïnvloed. Bij lichtverzadiging en normale CO_2-concentraties, werd de photosynthesesnelheid bepaald door de capaciteit van het diffusieproces. De invloed van de huidmondjes hangt dan echter af van de verhouding tussen de diffusieweerstand in de huidmondjes (r_s) en de som van de weerstanden in de lucht buiten het blad, in de huidmondjes en in de mesophylcellen ($r_a + r_s + r_{me}$). Tot dusverre werden voor de verhouding $(r_a + r_s + r_{me})/r_s$ waarden tussen 1 en 5 gevonden. Bij de laatste waarde veroorzaakt een verdubbeling van r_s, slechts 16 % vermindering van de photosynthesesnelheid. Bij de onderzochte bladen bleek de variatie van de bovengenoemde verhouding voornamelijk te worden veroorzaakt door verschillen in r_{me}. Uit een overzicht van literatuurgegevens bleek dat deze schommelingen en de betrekkelijk hoge waarden van r_{me} algemeen voorkomen. Deze weerstand lijkt daarom een belangrijke opbrengstbepalende factor te zijn (Hoofdstuk V–4).

Bij een goede vochtvoorziening van het blad, wordt de transpiratiesnelheid geregeld door de som van de diffusieweerstanden in de buitenlucht en in de huidmondjes, terwijl bij CO_2-limitering de photosynthese wordt bepaald door de som van deze zelfde weerstanden en de mesophylweerstand. De transpiratie wordt daarom meer door de huidmondjes beïnvloed dan de photosynthese. In

dit verband biedt de toegepaste methodiek perspectieven voor de analyse van de watereconomie van cultuurgewassen.

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