RESPIRATION OF LEAVES DURING PHOTOSYNTHESIS

III.* RESPIRATION RATE AND MESOPHYLL RESISTANCE IN TURGID COTTON LEAVES, WITH STOMATAL CONTROL ELIMINATED

By J. V. LAKE† and R. O. SLATYER‡

[*Manuscript received December* 12, 1969]

Summary

Mixtures of carbon dioxide in air, nitrogen, or 1% oxygen in nitrogen, prepared beforehand in plastic balloons, were passed through illuminated cotton leaves in a laboratory leaf chamber. This procedure enabled much more accurate calculation of intercellular carbon dioxide concentration than conventional gas flow techniques. It also made it possible to calculate leaf temperature from a measurement of the vapour pressure of the air leaving the chamber. From the measured flow rates and changes in the carbon dioxide concentration of the gas mixtures, the relation between the net rate of carbon dioxide exchange and the intercellular carbon dioxide concentration was calculated.

It was inferred from the results that in bright light at 27°C the basal rate of respiratory carbon dioxide production during photosynthesis in air was at least $16 \cdot 3$ ng cm^{-2} s^{-1}, with additional respiratory production taking place at a rate equivalent to 38% of the rate of photosynthetic fixation.

The liquid phase resistance to carbon dioxide transport within the mesophyll cells was $3 \cdot 0$ s cm^{-1}; this compares with a value of $4 \cdot 8$ s cm^{-1} which would have been inferred if respiration had not been allowed for.

I. INTRODUCTION

In an earlier paper in this series, Lake (1967*b*) proposed to improve the measurement of mesophyll resistance to carbon dioxide transport by passing known gas mixtures through a leaf. This has now been done and, by varying the oxygen content of the air as well as the carbon dioxide content, estimates of respiration rate and its variation with photosynthesis have also been made.

Measurement of the carbon dioxide concentration $\Gamma(\mu g\, l^{-1})$ at which net photosynthesis is zero and measurement of the rate at which carbon dioxide is released into a stream of carbon dioxide-free air have been used separately or together to provide indices of the rate of respiratory carbon dioxide production by leaves during photosynthesis in bright light (see, for example, Krotkov 1963; Moss 1966; Poskuta, Nelson, and Krotkov 1967). However, neither measurement alone provides a quantitative estimate of this rate; an estimate based on Γ requires a knowledge of the magnitude of one or more resistances to carbon dioxide transport within the photosynthesizing cells, and the alternative method requires in addition a knowledge of the resistance between the intercellular spaces and the ambient air (Lake 1967*a*, eqn 6 and 12; Bravdo 1968). As these resistances may vary with environment,

* Part II, *Aust. J. biol. Sci.*, 1967, **20**, 495–9.

† Rothamsted Experimental Station, Harpenden, England.

‡ Research School of Biological Sciences, Australian National University, P.O. Box 475, Canberra City, A.C.T. 2601.

failure to measure them can lead to equivocal conclusions about the effects of environment on respiration. For example, Zelawski (1967) found that the rate at which carbon dioxide was released into carbon dioxide-free air depended on illumination over a certain range, whereas Γ remained constant. The interpretation was that the rate of respiration during photosynthesis varied with illumination, but it is also possible that the respiration rate was constant and illumination influenced the stomatal resistance to carbon dioxide transport.

The concentration, Γ, and the rate of release of carbon dioxide into carbon dioxide-free air in bright light represent two points on the curve relating the rate of carbon dioxide exchange per unit leaf area, F (ng cm^{-2} s^{-1}), to the ambient concentration, c_t. The relationship is usually linear at values of c_t less than that in ordinary air (for exceptions, see Holmgren and Jarvis 1967; Heath and Orchard 1968); the equation of the line depends on the sum of the resistances in the carbon dioxide pathway between the ambient air and the chloroplasts and on the rate of release of respiratory carbon dioxide into the intercellular spaces, which may possibly vary with the rate of photosynthesis (Decker 1957). Because of reassimilation at the chloroplasts, this rate of release will be less than the actual respiration rate, B, and will have a value βB where β depends on the magnitudes of the relevant intracellular resistances (Lake 1967*a*).

These problems are simplified considerably if air is passed through the leaf, from one side to the other, so that F can be plotted against c_w, the concentration at the walls of the mesophyll cells (Heath 1951). The equation of this straight line depends on the rate of release of respiratory carbon dioxide, βB (ng cm^{-2} s^{-1}), and on only one resistance, that in the liquid phase in the mesophyll cells, r_m (s cm^{-1}), provided that the carboxylation resistance is negligible (Lake 1967*b*). Estimation of βB and its dependence, if any, on photosynthesis is thus possible if r_m can first be estimated in conditions where the release of respiratory carbon dioxide is negligible. Such release can be prevented by withholding oxygen (Fock and Egle 1966; Forrester, Krotkov, and Nelson 1966; Tregunna, Krotkov, and Nelson 1966), e.g. by using nitrogen instead of air as a carrier gas for the carbon dioxide passing through the leaf; $\mathrm{d}c_w/\mathrm{d}F$ then provides a direct estimate of r_m.

II. Method

Cotton plants (*Gossypium hirsutum* cv. Deltapine Smooth Leaf) were grown from seed for 5–8 weeks in a controlled-environment cabinet. The air temperature was 25°C during the night and 30°C during the 12·5-hr day. Illumination was provided by fluorescent tubes and incandescent lamps giving 100–120 W m^{-2} (0·4–0·7 μm). The roots were in aerated modified Hoaglands nutrient solution, contained in opaque 3-litre beakers.

Plants were transferred to a room held at 27°C for the experiments and the third, fourth, or fifth leaf from the top was placed in the leaf chamber.

The leaf chamber and ancillary equipment were as described by Jarvis and Slatyer (1966) except for the following modifications: the leaf was held in position in the chamber with a "plasticine" O-ring replacing the lower rubber gasket used by Jarvis and Slatyer and a solid rubber O-ring replacing the upper one. The area of the disk of leaf held between the O-rings and through which gas mixtures were passed was 31 cm^2. β-Particle gauging verified that this part of the leaf remained turgid, with unchanged water content throughout the experiments, which continued for up to 3 days.

The required mixtures of gases—carbon dioxide in air, nitrogen, or nitrogen with 1% oxygen—were prepared in balloons (capacity $\simeq$ 4000 litres) made of polyvinyl chloride sheet

(thickness 0·4 mm). The carbon dioxide concentrations were measured using a conductimetric analyser (Begg and Lake 1968) calibrated by means of gas mixtures prepared in proportioning pumps (H. Wösthoff, Bochum, Germany). The resistance of the balloon material to carbon dioxide transport was nominally $\simeq 7\times10^6$ s cm^{-1} and when the concentration in the balloon (kept more than half full) was between zero and that in ordinary air ($\simeq$ 600 μg l^{-1}) it drifted by only about 2 μg l^{-1} in a week. The resistance to oxygen transport was greater ($\simeq 3\times10^7$ s cm^{-1}) and, although the concentration gradient was also greater, the weekly increase of oxygen concentration in a balloon initially containing pure nitrogen was calculated to be only 0·02%, i.e. negligibly small for the present purpose. The balloons made it possible to use the same known gas mixtures in successive experiments, thus greatly improving the reproducibility as compared with conventional gas mixing techniques.

The gas inlet and exit tubes on the leaf chamber were fitted with capillaries; solenoid valves, actuated successively by means of the switch on a multipoint 1 mV chart recorder, connected the ends of the capillaries to a differential micromanometer (Mercury Electronics Ltd.) which gave a direct current output proportional to the pressure difference and thus to the rate of gas flow.

The direction of gas flow through the leaf was usually from the lower (abaxial) surface to the upper one and the rate of flow was about 10 (range 6·2–13·6) ml s^{-1}, corresponding to a velocity of 0·9 cm s^{-1}. However, on three occasions the direction of flow was reversed and the rate of flow was varied from 9·7 to 18·2 ml s^{-1}.

A necessary preliminary to each set of measurements was to illuminate the leaf and pass carbon dioxide-free air over it to cause the stomata to open. The gas mixtures passed through the leaves during the experiments all had carbon dioxide concentrations in the range 0–400 μg l^{-1}, i.e. less than ordinary air, and the stomata remained open sufficiently for the desired flow rate to be sustained with a water gauge pressure difference not exceeding 31 cm and usually only $\simeq$ 5 cm. Tests in darkness, with closed stomata, showed that no leakage occurred with a pressure difference of 31 cm.

Although the gas was dried before entering the leaf chamber, it left the upper surface of the leaf nearly saturated with water and to avoid condensation in the outlet tube it was necessary to supply an additional stream of dry gas to the upper part of the leaf chamber, so that the total rate of outflow was about 16·7 (range 15·8–19·7) ml s^{-1}.

Calculation of the mean concentration of carbon dioxide at the mesophyll cell walls, c_w, was then based on the measured rates of gas flow through the leaf (V_1) and out of the upper part of the leaf chamber (V_2), so that

$$c_w = c_{\text{in}} - V_2(c_{\text{in}} - c_{\text{out}})/2V_1, \tag{1}$$

where c_{in} and c_{out} were the concentrations in the air streams entering and leaving the chamber. Although c probably changed exponentially as the air passed through the leaf, the use of this linear approximation introduced no serious error.

As estimates of stomatal resistance were not required, measurements of transpiration rate were of no direct interest; however, during 25 of the 99 sets of measurements reported here, the water vapour contents of the air streams entering and leaving the leaf chamber were measured by differential thermocouple psychrometry (Slatyer and Bierhuizen 1964) as this appeared in principle to provide a possible means for estimating leaf temperature, which invariably presents difficulties in transpiration experiments.

If the air leaving the upper leaf surface is taken to be saturated with water vapour at the leaf temperature, T_{leaf}, then its water vapour content (g m^{-3}) is given by

$$\chi_{\text{sat.}}(T_{\text{leaf}}) = \chi_{\text{in}} + V_2(\chi_{\text{out}} - \chi_{\text{in}})/V_1, \tag{2}$$

where the subscripts "in" and "out" have the same meanings as in equation (1). Thus T_{leaf} can be inferred from a table of the temperature dependence of the water vapour content of saturated air. In addition, leaf temperature was measured by a thermocouple (40 wire gauge) threaded from the abaxial side into a large vein.

For the majority of the sets of measurements the light flux density (0·4–0·7 μm) at the leaf surface was 270 W m^{-2}, but during 24 sets the lamp was raised to reduce it to 150 W m^{-2} and on two occasions it was reduced still further, to 54 W m^{-2}.

III. Results

When the results obtained from a single leaf were plotted (Fig. 1) there were linear relationships between the rate of carbon dioxide exchange, F (ng cm^{-2} s^{-1}),* and the concentration, c_w (μg l^{-1}), in air or nitrogen, and there was no obvious difference between nitrogen and 1% oxygen in nitrogen. All the measurements made

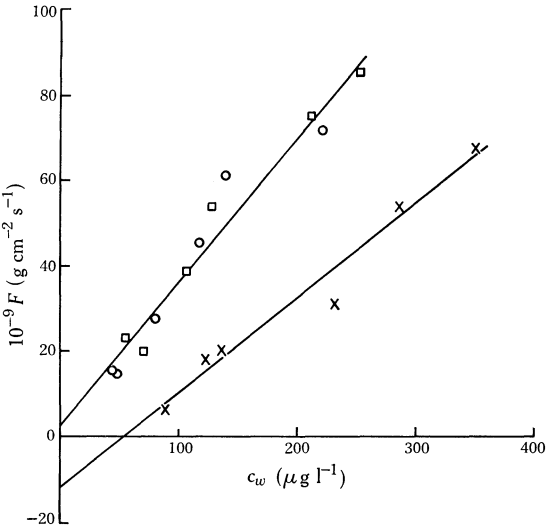

Fig. 1.—Effects of intercellular carbon dioxide concentration, c_w, on the net rate of carbon dioxide exchange, F, of a cotton leaf in air (×), nitrogen (○), or 1% oxygen (□). Lines represent regressions, with F the dependent variable.

on 10 leaves were used together for calculation of the coefficients of correlation and regression between F and c_w (Table 1), taking F as the dependent variable, i.e.

$$F = ac_w - b. \tag{3}$$

The difference between the values of a for measurements in air and in nitrogen was significant ($P < 0{\cdot}01$); that between values in nitrogen and in 1% oxygen was not.

Table 1

Correlation coefficients, gradients (a), and intercepts (b) for the relation between the net rate of carbon dioxide exchange of illuminated cotton leaves and the intercellular concentration of carbon dioxide in nitrogen, 1% oxygen, or air as carrier gas

Carrier Gas	No. of Observations	Correlation Coefficient	a (cm s^{-1})	Standard Error of a	b (ng cm^{-2} s^{-1})
Nitrogen	33	+0·89	0·34	0·03	4·6
1% oxygen	26	+0·91	0·32	0·03	4·9
Air	40	+0·91	0·21	0·02	16·4

In the ranges tested, there were no detectable effects of brightness of illumination, direction or rate of air flow through the leaf, or leaf temperature.

* Note that the units for F have been changed from mg dm^{-2} hr^{-1} (used in papers I and II) to ng cm^{-2} s^{-1} to facilitate linking fluxes with concentrations in μg l^{-1} and resistances in s cm^{-1}.

Although over the complete set of experiments the measured leaf temperature ranged from 23 to 31°C, the range in the experiments with upwards air flow and which included psychrometric measurements was only 27·6–30·2°C. Over this range, the calculated leaf temperatures, found from equation (2) and the appropriate psychrometric tables, were linearly related to the measured values (Fig. 2), but exceeded them by an amount which was small near 27°C and increased as the leaf became warmer.

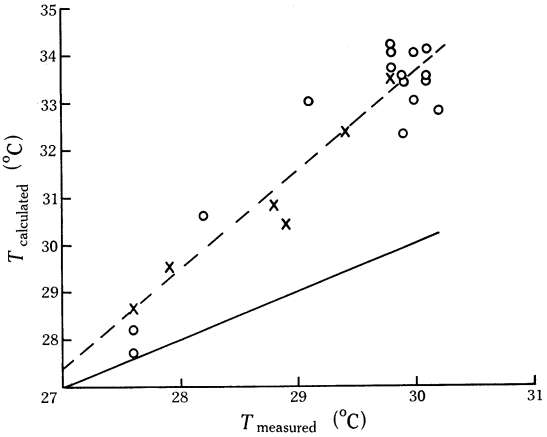

Fig. 2.—Relation between calculated and measured temperatures of a cotton leaf, illuminated at 270 W m^{-2} (○) and 150 W m^{-2} (×) (0·4–0·7 μm). The solid line represents $T_{calculated} = T_{measured}$; the broken line represents the regression, with $T_{calculated}$ as the dependent variable.

IV. Discussion

We shall take it that the resistance between the sites of respiratory carbon dioxide production and the cell walls is small compared with r_m (i.e. $\beta = 1$); if this is not so, we underestimate respiration rates. Provided that the carboxylation resistance is negligible compared with r_m, then for measurements in bright light in nitrogen the value of a in equation (3) corresponds to $1/r_m$, the mesophyll conductance, and the value of b represents the rate, B_1, of release of carbon dioxide into the intercellular spaces by a respiratory process which is independent of oxygen concentration.

The difference between the values of the slope a for measurements in air and nitrogen may indicate an effect of oxygen concentration on the mesophyll or carboxylation resistance (Samish and Koller 1968). Alternatively, it may be evidence of an inhibition of photosynthesis by oxygen (Björkman 1966): this was how Heath and Orchard (1968) interpreted somewhat similar measurements made by Forrester, Krotkov, and Nelson (1966) and by Tregunna, Krotkov, and Nelson (1966). However, respiration during photosynthesis is thought to use recent products of photosynthesis as substrates (e.g. Downton and Tregunna 1968) and we have therefore assumed that the rate of respiratory carbon dioxide release in air has a component $B_2+\gamma A$, where A is the gross assimilation rate at the chloroplasts (Lake 1967*b*, eqn 6), and both B_2 and γ depend on oxygen concentration. The values of r_m and A are taken to be independent of oxygen concentration. Then

$$F = (1-\gamma)(c_w/r_m)-B_1-B_2. \qquad (4)$$

To satisfy the values for a and b in Table 1, the quantitative form of equation (4) appropriate to the measurements in air is

$$F = (1-0{\cdot}38)(c_w/3{\cdot}0)-4{\cdot}6-11{\cdot}7 \text{ ng cm}^{-2} \text{ s}^{-1}. \quad (5)$$

As mentioned earlier, the derivation of equation (4) was based on the assumption that $\beta = 1$, i.e. there was negligible reassimilation at the chloroplasts; if this is not so, the actual rate of respiratory carbon dioxide production, when $c_w = 0$, may substantially exceed the measured rate of release into the intercellular spaces ($16{\cdot}3$ ng cm^{-2} s^{-1}). With $c_w > 0$, additional respiration dissipated carbon dioxide at a rate equivalent to 38% of the gross rate of assimilation.

The mesophyll resistance of $3{\cdot}0$ s cm^{-1} compares with the value of $4{\cdot}8$ s cm^{-1} which would have been inferred from the measurements in air without taking respiration into account.

The difference between the results for 1% oxygen and those for nitrogen, although not statistically significant, is in the expected direction. The value of Γ in nitrogen was 14 μg l^{-1} and if Γ depended linearly on oxygen concentration (Forrester, Krotkov, and Nelson 1966), the expected value in 1% oxygen would be 17 μg l^{-1}, compared with the measured 15 μg l^{-1}. Heath and Orchard (1968) found values of Γ for *Pelargonium zonale* and *Hydrangea* species which were between 37 and 55 μg l^{-1} in nitrogen and contrasted this with the observations of Forrester, Krotkov, and Nelson (1966) and Tregunna, Krotkov, and Nelson (1966) who found that Γ for several species was close to zero under such conditions. Either result seems possible, depending partly on whether there is a significant proportion of non-green tissue in the leaf, contributing to B_1, and also on the relative magnitude of the resistance, in the green tissue, between the respiratory sites and the chloroplasts compared with that between the respiratory sites and the cell walls.

Both Holmgren and Jarvis (1967) and Heath and Orchard (1968) found that under some circumstances the curve relating F to the ambient carbon dioxide concentration was non-linear near the origin. With control by the stomata eliminated, our results, which included many negative values of F, showed no evidence of this curvature, although the scatter (Table 1) was such that the test was imprecise.

Jolliffe and Tregunna (1968) have plotted (their fig. 3) measurements of the carbon dioxide assimilation rate of wheat at various temperatures against the ambient concentration of carbon dioxide in air or in 3% oxygen in nitrogen. The effect of oxygen concentration on the slope of the curves near the origin was very small, but the observations may have been affected by changes in stomatal resistance, which was not measured, so that the results are not strictly comparable with our own (Fig. 1) and the apparent contrast between wheat and cotton may be illusory.

Although the correlation coefficients in Table 1 are all about $0{\cdot}9$, the scatter of results they imply requires comment. Some of this variability may be due to differences in plant age and leaf position, but it is also possible that the gas flow rate was not the same through all parts of the leaf, especially when the stomatal resistance was large, when much of the flow may have been through a few pores. This would have the effect of changing the area basis of F, a possibility supported by the observation that F tended to be smaller than expected on occasions when the resistance to viscous flow through the leaf was unusually large.

Turning to the leaf temperature results (Fig. 2), the measured values, which were for a thermocouple embedded in a large vein beneath the leaf, were all less than the calculated values, which apply strictly to the stomatal cavities in the upper surface of the leaf. Although the leaf is far too thin for the whole of one side to be 4°C warmer than the other with the small energy fluxes occurring in our experiments, the temperature of a large vein might differ from that of the lamina as a whole, and it seems possible that vein temperature was influenced by the temperature of the transpiration stream, especially as the laboratory air temperature equalled the temperature towards which the calculated and measured temperatures converged. Other more commonplace explanations such as incipient drying of the tissue or conductance of heat along the thermocouple wires predict calculated leaf temperatures cooler than measured ones. It seems to be both more convenient and more accurate to calculate leaf temperature from measurements of the vapour pressure of air passed through the leaf, rather than to make a direct measurement with an embedded thermocouple.

V. Acknowledgments

Mr. O. R. Johnson and Miss J. Sheaffe gave valuable assistance and Dr. D. A. Thomas suggested the use of psychrometry to estimate leaf temperature.

The experimental work was done while both authors were located at the Division of Land Research, CSIRO, Canberra. One of us (J.V.L.) was on leave of absence from the National Institute of Agricultural Engineering, Silsoe, Bedford, England, and holding a Queen Elizabeth II Fellowship awarded by the Commonwealth of Australia, the other (R.O.S.) was on the staff of the Division.

VI. References

Begg, J. E., and Lake, J. V. (1968).—*Agric. Meteorol.* **5**, 283.
Björkman, O. (1966).—*Physiologia Pl.* **19**, 618.
Bravdo, Ben-Ami (1968).—*Pl. Physiol., Lancaster* **43**, 479.
Decker, J. P. (1957).—*J. sol. Energy Sci. Engng* **1**, 30.
Downton, W. J. S., and Tregunna, E. B. (1968).—*Pl. Physiol., Lancaster* **43**, 923.
Fock, H., and Egle, K. (1966).—*Beitr. Biol. Pfl.* **42**, 213.
Forrester, M. L., Krotkov, G., and Nelson, C. D. (1966).—*Pl. Physiol., Lancaster* **41**, 422.
Heath, O. V. S. (1951).—*Symp. Soc. exp. Biol.* **5**, 94.
Heath, O. V. S., and Orchard, B. (1968).—*J. exp. Bot.* **19**, 176.
Hesketh, J. D. (1967).—*Planta* **76**, 371.
Holmgren, P., and Jarvis, P. G. (1967).—*Physiologia Pl.* **20**, 1045.
Jarvis, P. G., and Slatyer, R. O. (1966).—Tech. Pap. CSIRO Aust. Div. Land Res. No. 29.
Jolliffe, P. A., and Tregunna, E. B. (1968).—*Pl. Physiol., Lancaster* **43**, 902.
Krotkov, G. (1963).—Natn. Acad. Sci. Publ. No. 1145. p. 452.
Lake, J. V. (1967*a*).—*Aust. J. biol. Sci.* **20**, 487.
Lake, J. V. (1967*b*).—*Aust. J. biol. Sci.* **20**, 495.
Moss, D. N. (1966).—*Crop Sci.* **6**, 351.
Poskuta, G., Nelson, C. D., and Krotkov, G. (1967).—*Pl. Physiol., Lancaster* **42**, 1187.
Samish, Y., and Koller, D. (1968).—*Ann. Bot.* (N.S.) **32**, 687.
Slatyer, R. O., and Bierhuizen, F. (1964).—*Pl. Physiol., Lancaster* **39**, 1051.
Tregunna, E. B., Krotkov, G., and Nelson, C. D. (1966).—*Physiologia Pl.* **19**, 723.
Zelawski, W. (1967).—*Bull. Acad. pol. Sci. Cl. II Sér. Sci. biol.* **15**, 565.