

RESPIRATION OF LEAVES DURING PHOTOSYNTHESIS

II.* EFFECTS ON THE ESTIMATION OF MESOPHYLL RESISTANCE

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Summary

Neglect of respiration has led to errors in estimation of the mesophyll resistance to CO₂ transport. All current methods lead to overestimation if an increase in photosynthesis is accompanied by an immediate increase in respiration, but two methods are valid if the respiration rate remains constant. An improved method is proposed, in which air of known CO₂ content is made to pass through the leaf.

I. INTRODUCTION

In the light of recent studies of leaf respiration during photosynthesis (Lake 1967), it appears that the magnitude of the mesophyll resistance to CO₂ transport and effects of experimental treatments on this resistance have sometimes been overestimated.

Variations in mesophyll resistance contribute to genotypic differences in the maximum photosynthetic rates in normal air (Holmgren, Jarvis, and Jarvis 1965), the effects of transpiration suppressants (Slatyer and Bierhuizen 1964), and the effects of CO₂ enrichment of the air in glasshouses (Wittwer and Robb 1964); so it is very desirable to estimate it accurately.

Mesophyll resistance has been defined by Penman (1942) as the resistance to viscous flow of air in the intercellular spaces, but more recently it has come to mean the intracellular resistance, r_m , to diffusive transport between the mesophyll cell walls and the chloroplasts (Gaastra 1959). The "apparent mesophyll resistance" (Whiteman and Koller 1967) includes carboxylation and excitation resistances (Monteith 1963) defined as occurring within the chloroplasts; it equals r_m when photosynthesis is light-saturated and limited only by the rate of supply of CO₂.

II. METHODS OF ESTIMATING MESOPHYLL RESISTANCE

Most attempts to measure r_m have been based on Gaastra's (1959) method, in which the total resistance, r_{tc} , in the pathway of CO₂ between a transducer in the ambient air and the chloroplasts is estimated from measurements of CO₂ exchange during CO₂-limited photosynthesis. The path from the transducer to the walls of the mesophyll cells is taken to be the same for water vapour as for CO₂, so the resistance, r_{tw} , in this path can be estimated from measurements of transpiration (Gaastra 1959; Slatyer and Bierhuizen 1964). The resistance r_m is then taken to be given by $r_{tc} - r_{tw}$.

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The electrical analogue recently developed in connection with leaf respiration during CO₂-limited photosynthesis yielded an equation [equation (7) of Lake 1967] which can be written in the form

$$r_m = (c_t - \beta B r_m) / F - r_{tw}, \quad (1)$$

where c_t is the CO₂ concentration (dimensions ML⁻³) at the transducer, B is the flux density (dimensions ML⁻²T⁻¹) of respiratory CO₂ production, F is the flux density of CO₂ flowing into the leaf, and β is a non-dimensional constant. The dimensions of r are L⁻¹T. In calculating B and F the leaf area is taken to be the area circumscribed by the leaf outline.

The magnitude of β depends in part on the relative sizes of two resistances to CO₂ transport within the photosynthesizing cells; namely r_{rw} between the respiratory sites and the cell walls, and r_{rc} between the respiratory sites and the chloroplasts (Lake 1967). No estimates have been made of the magnitude of r_{rw} for leaves, but in experiments with *Chlorella pyrenoidosa*, Good and Brown (1961) found that the resistance to oxygen transport between the respiratory sites and the surface of the cells was negligible compared with the resistance encountered in the agitated solution in which the *Chlorella* was suspended. The corresponding intracellular resistance to CO₂ transport is unlikely to be larger.

If mesophyll cells are comparable in this respect with *Chlorella*, so that r_{rw} is small, and if in addition the resistance to CO₂ transport in the intercellular spaces is negligible compared with r_m , then respiratory CO₂ production can be considered to take place at the walls of the mesophyll cells, i.e. $\beta = 1$. This was the assumption made by Monteith (1963). However, he also assumed that for a crop of beans the rate of respiratory CO₂ production in darkness, B^* , provided a good estimate of B , whereas it now appears that for beans $B < B^*$ (Ozbun, Volk, and Jackson 1965) while for some other dicotyledons $B \gg B^*$ (Lake 1967). Accurate estimation of r_m by Monteith's (1963) method requires accurate information about B and this is not yet available for most plants.

Gaastra (1959) showed that, for turnip, r_{tw} was constant at high light intensities over a range of values of c_t from 0 to 740 $\mu\text{g/l}$ (400 p.p.m. by volume at 20°C and 760 mmHg). He also found that, as equation (1) predicts, F was linearly related to c_t over the same range and he took this as evidence that the CO₂ concentration at the chloroplasts was zero at high light intensities. The mesophyll resistance was then calculated from

$$r_m = c_t / F - r_{tw}, \quad (2)$$

but comparison with equation (1) shows that the term $\beta B r_m / F$ was thereby neglected. For some plants, $B \geq 0.25F$ during CO₂-limited photosynthesis in normal air (Lake 1967). If this was so in the case of Gaastra's (1959) turnip and sugar-beet and if $\beta \approx 1$, his estimate of 5–7 sec cm⁻¹ for r_m was too great by 25% or more.

Kuiper (1965) showed that at temperatures below 25°C treatment of bean leaves with decenyl succinic acid increased F at a given value of c_t but had little effect on r_{tw} . Using equation (2) to interpret this result he concluded that the treatment had reduced r_m . However, if equation (1) is used a different interpretation

is possible, for then the increase in F can be accounted for, at least in part, by a depressing effect of the treatment on B . Indeed, Kuiper (1965) states that the treatment was likely to cause such an inhibition of respiration, so he may well have overestimated the effect on r_m .

These examples illustrate that underlying the problem of estimating r_m is the problem of estimating B . Holmgren, Jarvis, and Jarvis (1965) appeared to overcome this difficulty by using the slope of the curve relating CO_2 -limited F and c_i to provide an estimate of $r_m + r_{tw}$ under conditions chosen such that r_{tw} did not vary. This is essentially similar to the method used by Bierhuizen and Slatyer (1964). Differentiation of equation (1) with respect to F then gives

$$r_m + r_{tw} = dc_i/dF, \quad (3)$$

provided that B and r_m do not depend on F . This was apparently the assumption made by Holmgren, Jarvis, and Jarvis (1965), but as B is often greater than B^* , dB/dF might be expected to have a positive value over at least part of the range of F and equation (3) then overestimates $r_m + r_{tw}$.

A similar difficulty is encountered in the method proposed by Whiteman and Koller (1967), who wrote

$$r_m = (c_i - \Gamma)/F - r_{tw}, \quad (4)$$

i.e. they replaced the term $\beta B r_m$ in equation (1) by Γ , the value of c_i at which $F = 0$. Putting $F = 0$ in equation (1) gives

$$\beta B r_m = \Gamma. \quad (5)$$

The method has a practical advantage over that of Holmgren, Jarvis, and Jarvis (1965), in that it is not necessary to hold r_{tw} constant in the course of determining Γ . However, if B increases with F , Γ underestimates the value of $B r_m$ appropriate to normal air and the use of equation (4) overestimates r_m .

III. RESPIRATION, ILLUMINATION, AND PHOTOSYNTHESIS

A proper estimation of r_m evidently depends on a knowledge of the relation between B and F . Isotopic studies of the gas exchange of bean leaves (Ozbun, Volk, and Jackson 1965) have begun to resolve this problem, but bean is apparently not one of the plants for which $B > B^*$. A possible explanation of the observation that for many other plants $B > B^*$ is that although illumination may inhibit the process of dark respiration (Forrester, Krotkov, and Nelson 1966), the rate of the respiratory process which takes place in the light increases with illumination flux density. It is usually assumed that the rate of photosynthetic uptake of CO_2 at the chloroplasts, A , can become light-saturated. If so, then at illumination flux densities great enough to saturate F , the respiration rate $B (= A - F)$ must also be independent of light, and estimation of r_m by the use of equations (3) or (4) is satisfactory.

Whiteman and Koller (unpublished data) found that for *Helianthus annuus* at 23–26°C, Γ was constant at about 145 $\mu\text{g/l}$ at illumination flux densities in the range 1000–5000 f.c. and only rose to 180 $\mu\text{g/l}$ at 500 f.c., so that for this plant at least any direct effect of light on B was confined to quite low intensities.

It is possible that B is not directly affected by light but increases with A , as suggested by Decker (1957). McCree and Troughton (1966) found a close correlation between the rate of dark respiration, B^* , and the value of F measured in the preceding light period, so it appears possible that short-term changes in A may be accompanied by changes in B . However, the estimation of r_m will only be affected if the time taken for B to respond appreciably to a change in A is less than the time taken to make the necessary measurements. If the response is rapid, it is likely to be the rate of respiration, B_r , of the photosynthetic tissues (Lake 1967) that varies with A and in the simple case of a linear relation,

$$\begin{aligned} B_r &= B_r(A=0) + \gamma A \\ &= [B_r(A=0) + \gamma F]/(1-\gamma), \end{aligned} \quad (6)$$

where γ is a constant. Equation (1) can be rewritten with B separated into contributions from photosynthetic and non-photosynthetic tissues [equation (6) of Lake 1967] and substituting for B_r from equation (6) then leads to the conclusion that

$$r_m(\text{est.}) \geq r_m \geq (1-\gamma)r_m(\text{est.}), \quad (7)$$

where $r_m(\text{est.})$ is the value estimated from equations (3) or (4). Little is known about the possible magnitude of γ , but one can take comfort from the fact that for bean it is apparently zero (Ozbun, Volk, and Jackson 1965).

IV. CONCLUSIONS

There remains a need for further information about the factors which influence the rate of respiratory CO_2 production in the light and about the rate at which the plant responds to these factors. Meanwhile, the best methods of estimating r_m appear to be those which rely on equations (3) or (4).

Both these equations contain r_{tw} , a resistance which is often comparable in magnitude with r_m (Holmgren, Jarvis, and Jarvis 1965) and which must be estimated by indirect means. The importance of an exact knowledge of r_{tw} would be reduced if air of known CO_2 content was passed through the leaf, so exercising more direct control over c_w , the concentration of CO_2 at the surface of the mesophyll cells. Equations (3) and (4) then reduce to

$$r_m = dc_w/dF, \quad (8)$$

and

$$r_m = (c_w - \Gamma)/F. \quad (9)$$

These equations appear to provide a useful basis for improving the accuracy of estimating mesophyll resistance and the leaf chamber described by Jarvis and Slatyer (1966) can be modified for this purpose by arranging for a pressure difference to be maintained between the abaxial and adaxial sides so that air flows through an amphistomatous leaf.

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VI. REFERENCES

- BIERHUIZEN, J. F., and SLATYER, R. O. (1964).—Photosynthesis of cotton leaves under a range of environmental conditions in relation to internal and external diffusive resistances. *Aust. J. biol. Sci.* **17**, 248.
- DECKER, J. P. (1957).—Further evidence of increased carbon dioxide production accompanying photosynthesis. *J. sol. Energy Sci. Engng* **1**, 30.
- FORRESTER, M. L., KROTKOV, G., and NELSON, C. D. (1966).—Effect of oxygen on photosynthesis, photorespiration and respiration in detached leaves. *Pl. Physiol., Lancaster* **41**, 422, 428.
- GAASTRA, P. (1959).—Photosynthesis of crop plants as influenced by light, carbon dioxide, temperature, and stomatal diffusion resistance. *Meded. LandbHoogeschool Wageningen* **59**, 1.
- GOOD, N. E., and BROWN, A. H. (1961).—The contribution of endogenous oxygen to the respiration of photosynthesising *Chlorella* cells. *Biochim. Biophys. Acta* **50**, 544.
- HOLMGREN, P., JARVIS, P. G., and JARVIS, M. S. (1965).—Resistances to carbon dioxide and water vapour transfer in leaves of different plant species. *Physiologia Pl.* **18**, 557.
- JARVIS, P. G., and SLATYER, R. O. (1966).—A controlled-environment chamber for studies of gas exchange by each surface of a leaf. CSIRO Aust. Div. Land Res. Tech. Pap. No. 29.
- KUIPER, P. J. C. (1965).—Temperature dependence of photosynthesis of bean plants as affected by decenylsuccinic acid. *Pl. Physiol., Lancaster* **40**, 915.
- LAKE, J. V. (1967).—Respiration of leaves during photosynthesis. I. Estimates from an electrical analogue. *Aust. J. biol. Sci.* **20**, 487.
- MCCREE, K. J., and TROUGHTON, J. H. (1966).—Prediction of growth rate at different light levels from measured photosynthesis and respiration rates. *Pl. Physiol., Lancaster* **41**, 559.
- MONTEITH, J. L. (1963).—Gas exchange in plant communities. In "Environmental Control of Plant Growth". (Ed. L. T. Evans.) p. 95. (Academic Press, Inc.: New York.)
- OZBUN, J. L., VOLK, R. J., and JACKSON, W. A. (1965).—Effects of potassium deficiency on photosynthesis, respiration and the utilization of photosynthetic reductant by mature bean leaves. *Crop Sci.* **5**, 497.
- PENMAN, H. L. (1942).—Theory of porometers used in the study of stomatal movement in leaves. *Proc. R. Soc. B* **130**, 416.
- SLATYER, R. O., and BIERHUIZEN, J. F. (1964).—The influence of several transpiration suppressants on transpiration, photosynthesis, and water-use efficiency of cotton leaves. *Aust. J. biol. Sci.* **17**, 131.
- WHITEMAN, P. C., and KOLLER, D. (1967).—Estimation of mesophyll resistance to diffusion of carbon dioxide and water vapour. In "Techniques and Procedures for Determining Productivity in the Field". (Ed. F. E. Eckardt.) (UNESCO: Paris.) (In press.)
- WITTWER, S. H., and ROBB, W. (1964).—Carbon dioxide enrichment of greenhouse atmospheres for food crop production. *Econ. Bot.* **18**, 34.

