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Relations between CO_2 Exchange Rate, CO_2 Compensation, and Mesophyll Resistance from a Simple Field Method[1]

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ABSTRACT

An equation is developed that relates apparent photosynthesis to CO_2 light compensation values, resistance to CO_2 diffusion in air, and the apparent mesophyll resistance to CO_2 transport. The equation also yields values for total photosynthesis and light respiration from measurements of apparent photosynthesis. A simple method for measuring leaf CO_2 exchange rates in the field with a hand-operated syringe is described. Results obtained with this device and data published in recent literature are used with the new equation to show that photosynthesis may be limited more by mesophyll resistance than by photorespiration.

Additional index words: **Stomatal resistance, Photorespiration, Gross photosynthesis, Chloroplast CO_2 concentrations.**

THE growth of any plant ultimately depends on its carbon balance. Leaf absorption of CO_2 is an important component of this balance and has been described by relations of the type

$$\mathrm{CER}\cdot\gamma = \frac{C_a - C_c}{r_a + r_s + r_M} = \frac{C_a - C_i}{r} = \frac{C_i - C_c}{r_M} \qquad [1]$$

where CER is the leaf CO_2 exchange rate, $\mathrm{nmol\cdot s^{-1}\cdot cm^{-2}}$, and C_a the concentration of CO_2 in $\mu\mathrm{l\cdot liter^{-1}}$, in the air around the leaf with C_i and C_c the respective concentrations in the substomatal cavities and at the chloroplast surfaces. The gas diffusion resistances to CO_2 transport, $\mathrm{s\cdot cm^{-1}}$, are r_a, indicating the viscous flow air boundary, and r_s, indicating the leaf surface including both the stomata and the cuticular layer. The symbol r represents the sum of r_a and r_s, while r_M is the total apparent mesophyll CO_2 transfer resis-
24 tance. The constant, $\gamma =$ ~~0.095,~~ makes the dimensions consistent (assuming standard pressure and 24.4 liters of gas/mole of CO_2 at 20 C). The variables CER, C_a, r, and r_s can be measured, but values for C_i, C_c, and r_M must be obtained indirectly.

Values for r_M have been calculated from equation 1 by estimating a value for C_c and measuring CER, r, and C_a. Other methods of obtaining r_M follow from assuming C_c constant, so that

$$r_M\gamma = \frac{dC_i}{d\mathrm{CER}} \simeq \frac{\Delta C_i}{\Delta \mathrm{CER}} \qquad [2]$$

which require two simultaneous measurements of CER and r under different levels of light intensity, CO_2 or O_2 concentrations, or water stress. Lake (6) has discussed the problems associated with these various methods of calculating mesophyll resistance.

The method reported here enables one to calculate r_M from a single set of measurements of CER, r_s, C_a, and Γ, where Γ is the CO_2 compensation point in light. These measurements can be made quickly on plant leaves in the field with simple equipment.

MATERIALS AND METHODS

Theory. The concentration of CO_2 in the substomatal cavities depends on the resistances to CO_2 diffusion through the air boundary layer, the leaf surface, and the mesophyll tissue. The CO_2 compensation point in light and the concentration of CO_2 at the chloroplasts will also affect C_i. As $r \rightarrow 0$, $C_i \rightarrow C_a$, and as r becomes large, $C_i \rightarrow \Gamma$, so the relation between r and C_i must be a curve with the general shape shown in Fig. 1. There are a number of functions one might choose to represent this curve, one of the simplest being

$$C_i = a \exp\,(-\,r/\beta) + b. \qquad [3]$$

The boundary conditions require that $a = C_a - \Gamma$ and $b = \Gamma$. The term β represents the sink strength of the mesophyll tissue for CO_2 and so is a function of r_M and C_c. The relationship between β and r_M will be investigated using experimental values of r_M.

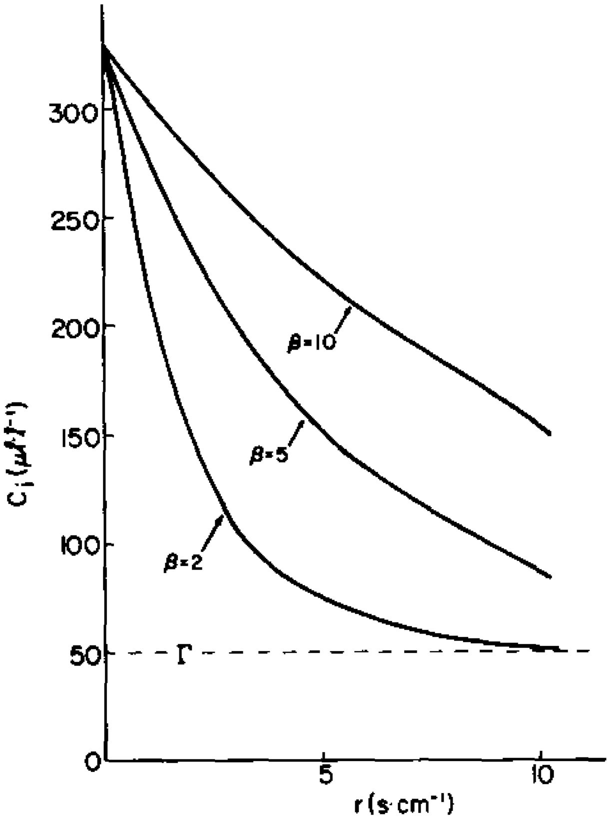

Fig. 1. The concentration of CO_2 in the substomatal cavities as a function of r and β, as given by equation 3 with $C_a = 330\ \mu_l\cdot_l^{-1}$ and $\Gamma = 50\ \mu_l\cdot_l^{-1}$.

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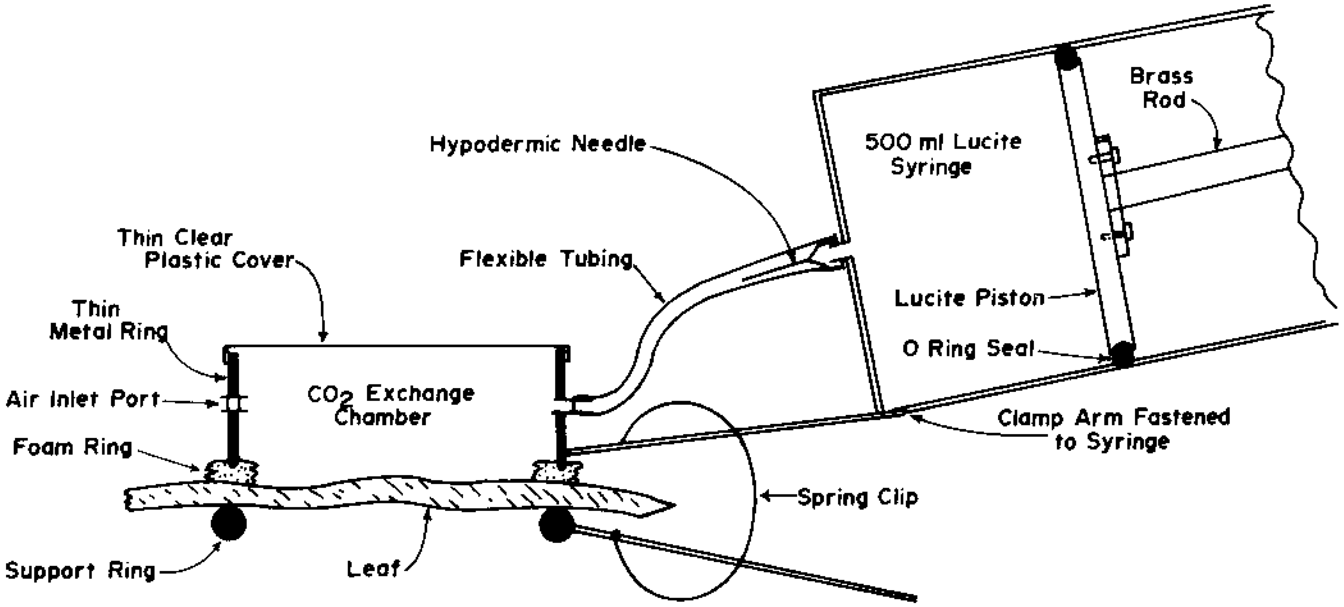

Fig. 2. A cross-sectional diagram of the apparatus used to measure net CO$_2$ uptake by leaves in the field. The cover is 9.8 cm in diameter and 2 cm high with r$'_a$ = 4 at 500 cm^3·min^{-1}.

Combining equations 1 and 3 leads to

$$\mathrm{CER}\cdot r\cdot\gamma = (C_a - \Gamma)\ (1 - \exp\frac{-r}{\beta}) \qquad [4]$$

which solved for β gives

$$\beta = r\left[\ln\left(\frac{C_a - \Gamma}{C_a - \Gamma - \gamma\cdot r\cdot \mathrm{CER}}\right)\right]^{-1} \qquad [5]$$

Once β has been evaluated from equation 5 using a single set of experimental measurements, equation 4 may be used to estimate the total or true photosynthetic rate by taking $\Gamma = 0$. The difference between this value and the measured CER is an estimate of the light respiration rate.

It is logical to suppose that values of C$_c$ in the immediate neighborhood of the chloroplasts will ordinarily fall between Γ and zero, depending on the mesophyll resistance and the concentration of CO$_2$ in the substomatal cavities. As a first approximation using logic similar to that forming equation 3, one may write

$$C_c = \Gamma \exp - [\beta/(C_i\ 10^{-2})] \qquad [6]$$

where 10^{-2} is a somewhat arbitrary constant used to weight the effect of C$_i$ on C$_c$ values. Using this relation in equation 1 gives

$$r_M = \frac{C_i - \Gamma\exp - [\beta/(C_i\ 10^{-2})]}{\mathrm{CER}\ \gamma} \qquad [7]$$

subject to the assumption that transport between the stomatal cavities and the chloroplasts of the carbon to be used in photosynthesis is directly proportional to the difference between C$_i$ and C$_c$. Values of r$_M$ calculated from equation 7 will fall between those calculated from equation 1, using the limits $C_c = 0$ or $C_c = \Gamma$. Generally, changing from one of these limiting values to the other leads to a change in the calculated values of r$_M$ that is less than the normal variation in experimental measurements of r under field conditions.

Measuring CER, r, and **Γ.** A number of methods for measuring CER have been proposed (8). In this case, the flux of CO$_2$ into the leaf was measured in the field with the simple apparatus shown in Fig. 2. The chamber was first clipped on the leaf and flushed by emptying the syringe, immediately followed by drawing 500 ml of air through the inlet port across the leaf surface and back into the syringe during a 1-min period. With a little practice and the use of a stopwatch, the operator can make the flow rate nearly constant. The air sample was then transferred from the syringe into an evacuated teflon bag through a septum port, taken into the laboratory, and its CO$_2$ concentration measured with an infrared analyzer. Teflon bags may be purchased through most specialty gas supply catalogs and are sufficiently impermeable to CO$_2$ to permit gas sample storage for several hours.

The rate of CO$_2$ exchange by the leaf in the chamber is

$$\mathrm{CER}' = 1.4 \times 10^{-8}\ \frac{q}{A}\ (C_a - C) \qquad [8]$$

where q is the air flow in cm^3·min^{-1}, A is the area covered on the leaf in cm^2, C is the concentration in μl·liter^{-1} of the CO$_2$ sample drawn off from the leaf, and 1.4×10^{-8} is a constant making the dimensions consistent (assuming 24.4 liters of gas/mole of CO$_2$, and including a factor of 2 because the exchange is measured on only one side of the leaf). The CO$_2$ uptake by the leaf is less in the chamber than under natural field conditions because the CO$_2$ concentration in the chamber is lower and the air boundry layer resistance higher. These effects can be accounted for by solving equation 1 simultaneously for r$_a$ + r$_M$ with both the natural and chamber conditions, giving

$$\mathrm{CER} = \mathrm{CER}'\ \frac{C_a}{0.5\,(C_a + C) - \gamma\,\mathrm{CER}'\,(r'_a - 0.5)} \qquad [9]$$

The prime marks indicate conditions in the chamber, 0.5 is the average boundary layer resistance in the field (11), C$'_a$ is taken as 0.5 (C$_a$ + C), and C$_c$ has been dropped as it is much less than C$_a$ and C$'_a$. There is, of course, some experimental variation associated with this simple procedure for measuring CO$_2$ exchange rates in the field. In general, values of CER measured over consecutive 1 min time periods on the same sugarbeet (*Beta vulgaris* L.) or corn (*Zea mays* L.) leaf agreed within ± 0.13 nmol·s^{-1}·cm^{-2} unless the light changed rapidly or the wind was strong and gusty.

Leaf resistance to water vapor transfer was measured with a commercially available diffusion porometer[3]. These values were converted to r$_a$, rounded to the next higher whole number and then taken as r. This was justified because of the normally low boundary layer resistance in the field (11), and the variation of one or more s·cm^{-1} often encountered in random measurements of r$_a$ on different parts of the same leaf under field conditions.

The CO$_2$ light compensation values were measured by bringing leaf samples with about 75 cm^2 of surface area into the lab-

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[3] Lambda Instrument Corporation, Lincoln, Nebr. Trade names and company names are included for the benefit of the reader and do not imply any endorsement or preferential treatment of the product listed by the USDA.

Table 1. Examples of measured and calculated gas exchange and transport parameters using data obtained in this study from recent literature.

Plant	CER measured	Total photosynthesis eq. 4, $\Gamma = 0$	Γ measured	β eq. 5	r measured	r_M eq. 7	Source
	$\text{nmol}\cdot\text{s}^{-1}\cdot\text{cm}^{-2}$		$\mu\ell\cdot\ell^{-1}$	$\text{s}\cdot\text{cm}^{-1}$			
Sugarbeet (*Beta vulgaris* L.)	1.64	1.96	52	6.6	1	7.3	†
	0.79	1.01	70	13.4	1	16.5	†
	0.98	1.20	60	9.5	4	10.2	†
	1.70	2.08	50‡	5.0	1‡	5.6	(9)
	2.15	2.65	50‡	3.3	2	3.2	(2)
Corn (*Zea mays* L.)	3.28	3.34	12	2.3	3	1.2	†
	0.82	0.88	16	12.3	7	9.9	†
	2.40	2.46	10‡	2.7	4.5	1.2	(5)
	3.66	3.79	10‡	1.7	3	0.7	(5)
Bean (*Phaseolus vulgaris* L.)	1.14	1.39	60‡	7.8	3	8.6	(1)
	0.63	0.76	60‡	13.2	7	14.0	(1)
Sunflower (*Helianthus annuus* L.)	2.37	2.78	40‡	3.4	1	3.5	(3)
Sorghum (*Sorghum vulgare* L.)	2.52	2.59	10‡	3.5	3	2.4	(4)
	2.84	2.90	10‡	3.5	2	2.7	(4)

† Observations from this study. ‡ Estimated value.

oratory and placing them in glass jars submerged in a water bath at 20 C. The samples were illuminated with 40-watt incandescent bulbs outside the jar, giving an average photosynthetic photon flux density of 42.5 $\text{nE}\cdot\text{cm}^{-2}\cdot\text{s}^{-1}$ on the leaf surface in the wavelength range of 400 to 700 nm. The jar was flushed with air low in CO_2 (bubbled through a KOH solution), and then equilibrated for 15 to 20 min before measuring the CO_2 level, which was taken as Γ. The leaf surface temperature in the jar was about 25 C. When necessary, the bath temperature can be increased so that the leaf in the jar is about the same temperature as in the field. The light intensity can also be reduced to match that of shaded leaves in the field that are not light saturated.

While the leaves of the plants studied here were large enough to accommodate the 75 cm² cover shown in Fig. 2, the same technique could be used on smaller leaves by completely enclosing them and measuring their surface area. A better approach for smaller leaves might be to reduce the cover size to fit the leaf of interest and use a smaller syringe. This would yield a smaller air sample that may require a gas chromatograph for analysis, but there is an advantage in covering only one side of the leaf because perturbations of its normal environment are less. In any case, the distance between the leaf and the top of the cover should be small to reduce the boundary layer resistance. This resistance can be measured for any shaped cover. Desiccant traps on the chamber's inlet and outlet may be used to get the steady state rate of evaporation from a wet filter paper at a known temperature and given air flow under the cover as

$$r'_a = \frac{C_s - 0.8\ C_o}{1.56\ Q} \qquad [10]$$

where Q is the evaporation rate in $\text{mg}\cdot\text{s}^{-1}\cdot\text{cm}^{-2}$, C_s is the concentration of water vapor at the wet surface in $\text{mg}\cdot\text{cm}^{-3}$ (known from the temperature of the filter paper and the vapor pressure of water at that temperature), C_o is the concentration of water vapor leaving the chamber $\text{mg}\cdot\text{cm}^{-3}$, 0.8 is a weighting factor with water-free air entering the chamber, and 1.56 accounts for the difference between the diffusion rates of CO_2 and H_2O in air. Other simple methods are also available for estimating r'_a (8, 11).

RESULTS AND DISCUSSION

Some representative values of measured and calculated photosynthetic parameters are presented in Table 1. The mesophyll resistance to CO_2 transport is a dynamic leaf property. Its normal range of values depends on plant species, environmental conditions, and probably other factors that are not yet recognized. Indeed, r_M and Γ are probably linked to some degree in most plants (7).

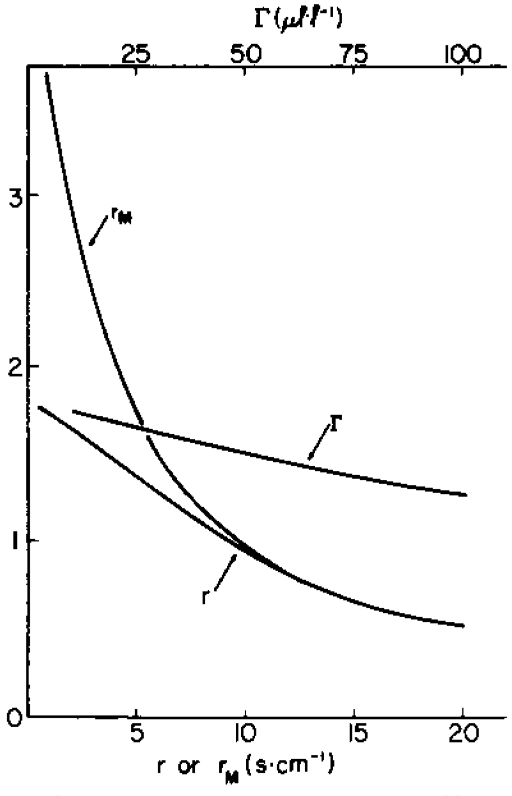

Fig. 3. The affects on CER caused by varying individually the values of r, r_M, or Γ given the conditions that $r = 3$, $r_M = 6$, $\Gamma = 60$, and $C_a = 330$.

The values of β and r_M in Table 1 are, for practical purposes, equal within the limits of random and experimental errors of field measurements. Because the model for r_M suggested by equation 1 is such a gross oversimplification of CO_2 internal transport pathways, and because of the uncertainty of C_c values (7, 11), it would probably be better to think of r_M as a parameter characterizing the internal sink strength or affinity of the leaf mesophyll for CO_2. This gives equation 1 the form

$$\mathrm{CER} = \frac{C_a - \Gamma}{\gamma\ r} \left(1 - \exp \frac{-r}{r_M}\right). \qquad [11]$$

It is interesting to compare this form to the assimilation equation proposed by van Bavel (10) because of the relationship that is implied between Γ, r_M, and photon flux density. It appears that changes in CER resulting from changes in light intensity may largely be affected through the mesophyll sink strength for CO_2, i.e., through β (r_M).

Equation 11 has been used to draw the curves in Fig. 3 illustrating the relative effects of Γ, r, and r_M on limiting CER. Reducing the mesophyll resistance would be an effective way to increase CO_2 assimilation. Reducing the CO_2 light compensation point would have a lesser effect on CO_2 exchange, as shown by the slope of the curves in Fig. 3 and by the small differences between CER and total photosynthesis in Table 1. These differences are, in fact, estimates of the light respiration rates. The low mesophyll resistances for corn and sorghum in Table 1 suggest, in conjunction with the curves in Fig. 3, that their potential for rapid growth results from small r_M values even more than from their low photorespiration rates.

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