Effect of Errors in Measuring Leaf Temperature and Ambient Gas Concentration on Calculated Resistances to CO_2 and Water Vapor Exchanges in Plant Leaves

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ABSTRACT

Errors as small as 1 C in the measurement of leaf temperature (T_{leaf}) are shown to cause significant changes in the estimated value of the stomatal resistance (expressed in terms of total resistance to water vapor transfer, Σr_{H_2O}). The effect increases as T_{leaf} increases and as ambient relative humidity increases, if other conditions are maintained constant. The effect on the key CO_2 exchange parameter, the intracellular (or mesophyll) resistance, r_{int}, tends to be small under open stomata conditions but increases rapidly as stomatal closure occurs, particularly if the true value of r_{int} is relatively small.

Errors in the determination of the ambient water vapor and CO_2 concentrations can also significantly affect the calculated value of Σr_{H_2O} and r_{int}. The effect on Σr_{H_2O} and Σr_{CO_2} increases as the ratio of the inlet/outlet concentration departs from unity and also increases as the assumed leaf-air concentration difference decreases. The combined effect on r_{int} tends to be less than the individual effects on Σr_{H_2O} and Σr_{CO_2} since both are in the same direction.

In recent years the study of water vapor and CO_2 exchanges in plant communities, individual plants, and single shoots and leaves has played an important role bridging the gap between traditional approaches to physiological and ecological aspects of plant performance and plant distribution. The main equipment used for this purpose is the leaf or branch chamber, with which net photosynthesis and transpiration are measured as the difference in CO_2 and water vapor content, respectively, of the air entering and leaving the chamber.

As these measurements have become more quantitative, it has been realized that the actual measurements of net photosynthesis and transpiration are of limited value unless supplemented by information regarding the environment in which they are conducted and, particularly, the resistances to transfer of CO_2 and water vapor between the sources and sinks in the leaves and in the ambient air (see, for example, 1, 2, 6, 10, 11).

Since resistance across the boundary layer external to the leaf can be made relatively constant by experimental technique, and since this resistance is not affected by the physiological condition of the plant, except to a small degree, the key parameters are the leaf resistance (r_{leaf}), which includes stomatal and cuticular resistances to transfer, and the intracellular resistance

(r_{int}). The latter is not a true diffusive resistance in the same sense as the gas phase resistances; rather, it includes all the biophysical and biochemical factors which are associated with the CO_2 concentration change between the external surface of the mesophyll and the effective carboxylation/decarboxylation "surface" within the leaf cells. These two parameters, combined with appropriate values of the external boundary layer resistance, can be used to predict rates of net photosynthesis and transpiration of the tissue studied under prescribed environmental conditions.

The present contribution deals with errors that can be introduced to these determinations when two of the key assumptions with regard to the sources and sinks are not completely valid.

THEORY

General. In a leaf chamber (or branch or whole plant chamber), the net fluxes of water vapor and CO_2, in relation to the differences in gas concentrations inducing flow and the resistances to transfer, are linked by simple linear equations of the form

$$E = \frac{FK(e_{out} - e_{in})}{a} = \frac{K(e_{leaf} - e_{amb})}{(r_{air} + r_{leaf})_{H_2O}} \tag{1}$$

and

$$A = \frac{F(c_{in} - c_{out})}{a} = \frac{(c_{amb} - c_{leaf})}{(r_{air} + r_{leaf} + r_{int})_{CO_2}} \tag{2}$$

where E and A represent the net transpirational and photosynthetic fluxes, respectively, in units such as $g/cm^2 \cdot sec$; F is the flow of air (cm^3/sec) and a, the area of the leaf surface (cm^2). The pressure of water vapor (mm Hg) entering and leaving the chamber is represented as e_{in}, e_{out}; e_{leaf}, e_{amb} represent the vapor pressure in the leaf and in the ambient air (g/cm^3). K is a constant to correct the e values from water vapor pressures to concentrations in g/cm^3 (see 9). The symbols c_{in}, c_{out}, c_{leaf}, and c_{amb} refer to corresponding concentrations of CO_2 (g/cm^3). The symbol r_{air} refers to the gas phase resistance (sec/cm) encountered by the water vapor and CO_2 fluxes between the bulk air and the natural surface of the leaf; r_{leaf} refers to the resistance between the natural surface of the leaf and the mesophyll wall surfaces; and r_{int} refers to the additional resistance in the CO_2 pathway to account for "resistance" encountered by CO_2 within the liquid phase of the leaf. In subsequent equations the notation $\Sigma r_{H_2O} = (r_{air} + r_{leaf})_{H_2O}$ and $\Sigma r_{CO_2} = (r_{air} + r_{leaf} + r_{int})_{CO_2}$ is used.

In using these equations the following assumptions are generally made:

1. That e_{leaf} is the saturation vapor concentration at the leaf temperature and can therefore be directly obtained from leaf temperature measurement (see, for example, equation 8).

2. That c_{leaf} is the CO_2 concentration at the "effective" carboxylation/decarboxylation surface within the leaf. Under conditions where CO_2 is limiting the rate of photosynthesis (a requirement for unequivocal estimation of r_{int}), it is equal to the CO_2 compensation point, Γ (1, 10).

3. That c_{amb} is given by the outlet concentration (c_{out}) of the CO_2 and water vapor passing through the chamber. That is, the stirring within the chamber or by way of a bypass circulation system is so effective that the outlet vent effectively samples the ambient air within the chamber. (It is recognized that some chambers are constructed for linear flow across a leaf so that this assumption would not be expected to apply. The parts of the paper concerned with this particular assumption therefore do not apply to special chambers of this type.)

4. That the ratio of $(r_{air} + r_{leaf})_{CO_2}$ to $(r_{air} + r_{leaf})_{H_2O}$ is given by the reciprocal ratio of the diffusion coefficients of CO_2 and water vapor in air and that the value of this ratio, (D_{H_2O} D_{CO_2}), is 1.56 (Gale and Poljakoff-Mayber [3], H. G. McPherson and R. O. Slatyer [unpublished data]).

5. For the purposes of this paper, it is also assumed that in a leaf chamber leaf geometry can be maintained constant, and ventilation characteristics can be made sufficiently effective so that r_{air} is constant and of relatively low value compared to r_{leaf}.

With these assumptions, equations 1 and 2 can be rewritten as follows:

$$E = \frac{K(e_{leaf} - e_{amb})}{\Sigma r_{H_2O}} = \frac{K(e_{sat(Tleaf)} - e_{amb})}{\Sigma r_{H_2O}} \quad (3)$$

$$A = \frac{c_{amb} - \Gamma}{\Sigma r_{CO_2}} = \frac{c_{amb} - \Gamma}{(1.56 \; \Sigma r_{H_2O} + r_{int})} \quad (4)$$

It follows that the primary parameters (Σr_{H_2O}, Σr_{CO_2} and r_{int}) linking the observed fluxes with the concentration differences, are given by

$$\Sigma r_{H_2O} = \frac{K(e_{sat(Tleaf)} - e_{amb})}{E} \quad (5)$$

$$\Sigma r_{CO_2} = \frac{c_{amb} - \Gamma}{A} \quad (6)$$

and

$$r_{int} = \frac{c_{amb} - \Gamma}{A} - 1.56 \; \Sigma r_{H_2O} \quad (7)$$

$$= \Sigma r_{CO_2} - 1.56 \; \Sigma r_{H_2O} \quad (7a)$$

Effect of Error in T_{leaf}. From equations 3 to 7, it is apparent that an error in leaf temperature measurement will directly affect Σr_{H_2O} and indirectly affect r_{int}. The effect of such errors can readily be seen from the following analysis.

If an asterisk is used to denote a true value, equation 5 indicates the assumed value of Σr_{H_2O}, when T_{leaf} indicates the measured leaf temperature. The true value of Σr_{H_2O} is then given by

$$\Sigma r_{H_2O}^* = \frac{K(e_{sat(T_{leaf}^*)} - e_{amb})}{E} \quad (8)$$

so that the absolute error incurred is given by

$$\Delta \Sigma r_{H_2O} = \Sigma r_{H_2O} - \Sigma r_{H_2O}^* = \frac{K(e_{sat(Tleaf)} - e_{sat(T_{leaf}^*)})}{E} \quad (9)$$

and the relative error is given by

$$\left[\frac{\Delta \Sigma r}{\Sigma r^*}\right]_{H_2O} = \left[\frac{\Sigma r - \Sigma r^*}{\Sigma r^*}\right]_{H_2O} = \frac{e_{sat(Tleaf)} - e_{sat(T_{leaf}^*)}}{e_{sat(T_{leaf}^*)} - e_{amb}} \quad (10)$$

Similarly, the true value of r_{int} will be given by

$$r_{int}^* = \Sigma r_{CO_2} - 1.56 \; \Sigma r_{H_2O}^* \quad (11)$$

so that the absolute error incurred is given by

$$\Delta r_{int} = r_{int} - r_{int}^* = 1.56 \; (\Sigma r_{H_2O}^* - \Sigma r_{H_2O}) \quad (12)$$

and the relative error is given by

$$\frac{\Delta r_{int}}{r_{int}^*} = \frac{r_{int} - r_{int}^*}{r_{int}^*} = \frac{1.56 \; (\Sigma r_{H_2O}^* - \Sigma r_{H_2O})}{\Sigma r_{CO_2} - 1.56 \; \Sigma r_{H_2O}^*} \quad (13)$$

Effect of Error in the Assumption that Ambient Concentration is Equal to Outlet Concentration. From equations 3 to 7, it can be appreciated that if the assumption that $c_{amb} = c_{out}$ (or $e_{amb} = e_{out}$) is not valid, Σr_{H_2O} and Σr_{CO_2} will be directly affected and r_{int} indirectly affected.

If, as before, an asterisk is used to denote a true value, equation 5 indicates the value of Σr_{H_2O} when it is assumed that the ambient concentration is equal to the outlet concentration. It can be rewritten in the form

$$\Sigma r_{H_2O} = \frac{K(e_{leaf} - e_{out})}{E} \quad (14)$$

If the assumption is in error, the true value of Σr_{H_2O} is given by

$$\Sigma r_{H_2O}^* = \frac{K(e_{leaf} - e_{amb}^*)}{E} \quad (15)$$

so that the relative error introduced is given by

$$\left[\frac{\Delta \Sigma r}{\Sigma r^*}\right]_{H_2O} = \left[\frac{\Sigma r - \Sigma r^*}{\Sigma r^*}\right]_{H_2O} = \frac{e_{amb}^* - e_{out}}{e_{leaf} - e_{amb}^*} \quad (16)$$

The effect of errors in these assumptions on Σr_{CO_2} can be evaluated by a similar set of equations. Taking, as before, the estimated value of Σr_{CO_2}, as being given by equation 6 written in the form

$$\Sigma r_{CO_2} = \frac{c_{out} - \Gamma}{A} \quad (6a)$$

the true value of Σr_{CO_2} is then given by

$$\Sigma r_{CO_2}^* = \frac{c_{amb}^* - \Gamma}{A} \quad (17)$$

and the relative error is then given by

$$\left[\frac{\Delta \Sigma r}{\Sigma r^*}\right]_{CO_2} = \left[\frac{\Sigma r - \Sigma r^*}{\Sigma r^*}\right]_{CO_2} = \frac{c_{out} - c_{amb}^*}{c_{amb}^* - \Gamma} \quad (18)$$

The effect of these errors on r_{int} can be discerned in a similar manner. Taking equation 7a as indicating the apparent value of r_{int}, the true value of r_{int} is then given by

$$r_{int}^* = \Sigma r_{CO_2}^* - 1.56 \; \Sigma r_{H_2O}^* \quad (19)$$

so that the relative error in r_{int}^* is given by

$$\frac{\Delta r_{int}}{r_{int}^*} = \frac{(\Sigma r_{CO_2} - \Sigma r_{CO_2}^*) - 1.56 \; (\Sigma r_{H_2O} - \Sigma r_{H_2O}^*)}{\Sigma r_{CO_2}^* - 1.56 \; \Sigma r_{H_2O}} \quad (20)$$

RESULTS

Effect of Error in T_{leaf}. Figure 1 shows the effect of errors in T_{leaf}, for $T_{leaf} - T_{leaf}^*$ values up to 3 C, on the relative error in
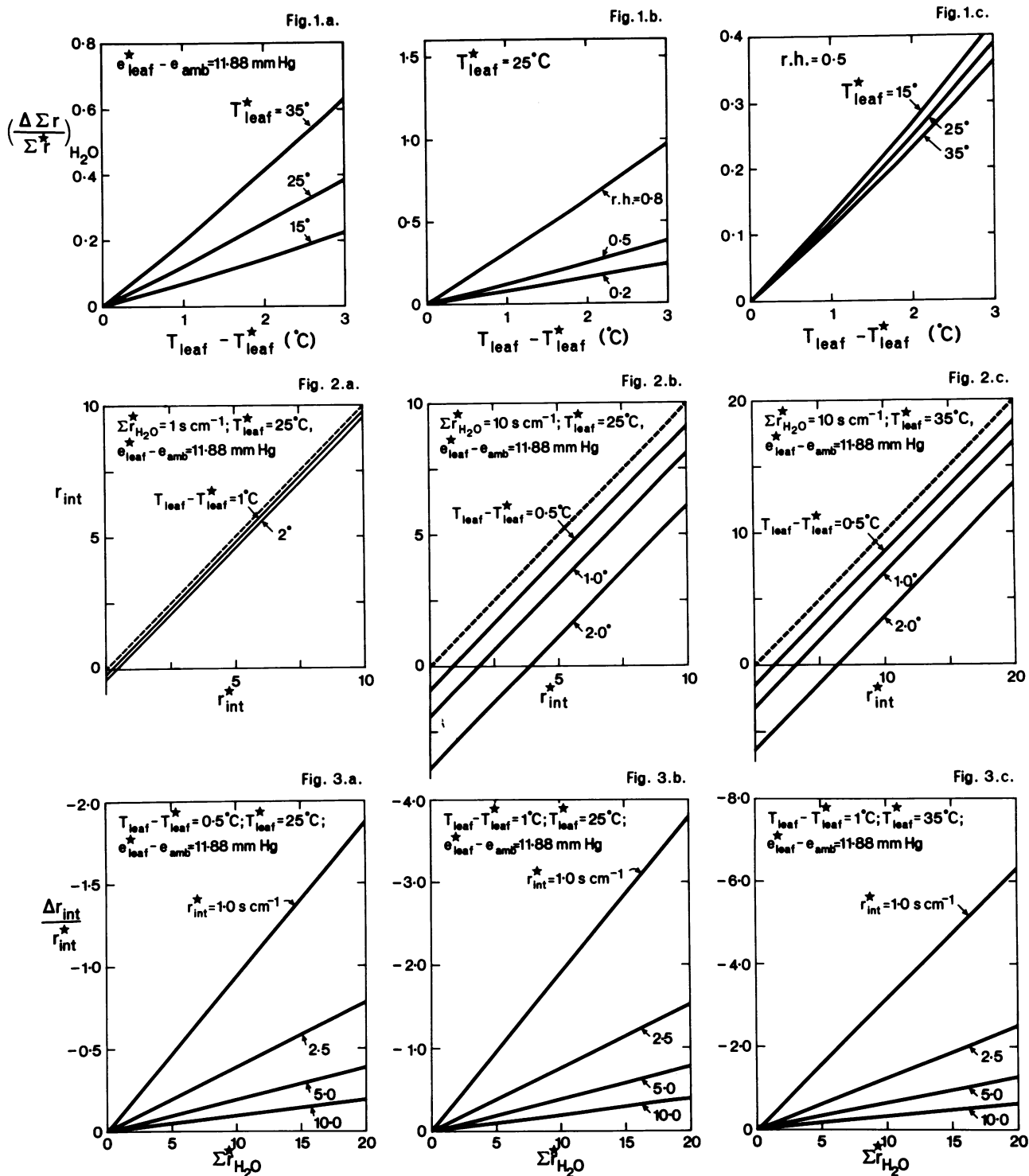
FIG. 1. Effect of error in measurement of leaf temperature $(T_{leaf} - T^*_{leaf})$ on the relative error induced in the estimated total diffusive resistance to water vapor transport $(\Delta\Sigma r/\Sigma r^*_{H_2O})$. a: Effect at three levels of leaf temperature when $e^*_{leaf} - e_{amb} = 11.88$ mm Hg; b: effect at three levels of ambient humidity when $T^*_{leaf} = 25$ C; c: effect at three levels of leaf temperature when ambient relative humidity = 0.50.

FIG. 2. Effect of errors in measurement of leaf temperature $(T_{leaf} - T^*_{leaf})$ on the estimated intracellular resistance (r_{int}). a: Effect of $T_{leaf} - T^*_{leaf} = 1.0$ and 2.0 C at $T^*_{leaf} = 25$ C, $e^*_{leaf} - e_{amb} = 11.88$ mm Hg and $\Sigma r^*_{H_2O} = 1$ sec/cm; b: as for Figure 2a except that $T_{leaf} - T^*_{leaf} = 0.5$, 1.0 and 2.0 C, $\Sigma r^*_{H_2O} = 10$ sec/cm; c: as for Figure 2b except that $T^*_{leaf} = 35$ C.

FIG. 3. Effect of total leaf diffusive resistance to water vapor transport $(\Sigma r^*_{H_2O})$ on the relative error induced in the estimated intracellular resistance $(\Delta r_{int}/r^*_{int})$ at various levels of r^*_{int}. a: Effect observed when $T_{leaf} - T^*_{leaf} = 0.5$ C, $T^*_{leaf} = 25$ C, $e^*_{leaf} - e_{amb} = 11.88$ mm Hg; b: as for Figure 3a except that $T_{leaf} - T^*_{leaf} = 1.0$ C; c: as for Figure 3b except that $T^*_{leaf} = 35$ C.

the sum of the diffusive resistance to water vapor transport $(\Delta\Sigma r/\Sigma r^*)_{H_2O}$. The data indicate that, for the range of leaf temperatures and ambient humidities generally encountered out of doors, values of $T_{leaf} - T^*_{leaf} = 1$ C are likely to cause effects on $(\Delta\Sigma r/\Sigma r^*)_{H_2O}$ of the order of 10% or more, and if $T_{leaf} - T^*_{leaf} \geq 3$ C substantial errors will occur under all conditions tested. With increasing leaf temperature at constant levels of $e^*_{leaf} - e^*_{amb}$ (Fig. 1a) or increasing ambient humidity at constant T_{leaf} (Fig. 1b), the magnitude of the error increases. For a given ambient humidity level (R.H. = 0.50), there is relatively little effect of leaf temperature on the observed results (Fig. 1c) and such effect as does exist becomes less as T^*_{leaf} increases (because the increase in the denominator of equation 10 with increase in T^*_{leaf} is slightly greater than the increase in the numerator).

Figure 2 shows how errors in T_{leaf} measurement cause errors in the calculated value of r_{int} for different initial values of r^*_{int}. Since Σr_{CO_2} is unchanged by error in T_{leaf}, the effect on r_{int} is due entirely to change in Σr_{H_2O} (see equation 12), and is therefore the same for any initial value of r_{int} and any given value of $(T_{leaf} - T^*_{leaf})$. When Σr_{H_2O} is very low (i.e., wide open stomata) as in Figure 2a, only small absolute effects are introduced. As the stomata close, the absolute effect is much greater (Fig. 2b), and at higher leaf temperatures (e.g., 35 C, as in Fig. 2c), the effect is increased further when other conditions are unchanged. However, $(e_{leaf} - e_{amb})$ values usually increase with increasing T_{leaf}, so under natural conditions the effect may tend to be reduced at higher temperatures as is evident from Figure 1.

It is apparent from Figure 2 that when the stomata are even partly closed overestimation of T^*_{leaf} (i.e., $T_{leaf} - T^*_{leaf} \geq 0$) will often indicate apparently negative values of r_{int}, an absurd result.

In Figure 3, the effects of various levels of Σr_{H_2O} on the relative error in r_{int}, $(\Delta r_{int}/r^*_{int})$ is shown for various values of r^*_{int}.

The diagrams show clearly that if r^*_{int} is low (≤ 2.5 sec/cm) significant relative errors develop in $\Delta r_{int}/r^*_{int}$, even for $T_{leaf} - T^*_{leaf} = 0.5$ C, whenever Σr_{H_2O} rises above values characteristic of open stomata (Fig. 3c). The reason for this is seen from equation 13. Since Σr_{CO_2} is constant for any one r^*_{int} value, the denominator of equation 13 decreases as Σr_{H_2O} increases and the rate of decrease is greater, the lower the value of r^*_{leaf}, and hence Σr_{CO_2}. With r^*_{int} values of the order of $r_{int} = 5 - 10$ sec/cm, values of $\Delta r_{int}/r^*_{int}$ do not exceed about 10 to 20% unless stomata are partly closed ($\Sigma r_{H_2O} = 10$ sec/cm). With temperature errors of the order of $T_{leaf} - T^*_{leaf} \geq 1$ C, however, the effects on $\Delta r_{int}/r^*_{int}$ are approximately twice as great (Fig. 3b) and increase with T^*_{leaf}, when $(e^*_{leaf} - e_{amb})$ is constant (Fig. 3c). This is because the effect of temperature on Σr_{H_2O} operates on the numerator of equation 13. As $(T_{leaf} - T^*_{leaf})$ increases, the relative error in $\Delta r_{int}/r^*_{int}$ increases with Σr_{CO_2} or r^*_{int} constant.

It is apparent that whenever $\Sigma r_{H_2O} > 10$ sec/cm, if $T_{leaf} - T^*_{leaf} > 1$ C, minimum errors of estimation of r_{int} of the order of 100% will tend to occur if r^*_{int} values themselves are low.

Effect of Error in Ambient Concentration Estimates. In Figure 4 the effect of errors in the assumption that $e^*_{amb} = e_{out}$, on the relative error in Σr_{H_2O}, is shown. Across the abscissa of each diagram, the error in estimation of ambient concentration is depicted to change from zero, where $e^*_{amb} = e_{out}$, to a situation where $e^*_{amb} = (e_{out} + e_{in})/2$. This is likely to be the "worst" situation encountered, for example in poorly ventilated chambers.

As can be appreciated from equation 16, the error tends to be proportional to the change in concentration across the chamber and inversely proportional to assumed leaf-air concentration difference. The error always leads to underestimation of the true (Σr_{H_2O}) resistance since e_{out} is higher than e^*_{amb}. With the range of conditions depicted, only small ($<10\%$) errors in $\Delta\Sigma r_{H_2O}/\Sigma r^*_{H_2O}$ tend to arise, as long as $e_{in}/e_{out} \geq 0.9$. At any one level of e_{in}/e_{out}, the error is reduced by conditions which increase $(e_{leaf} - e_{out})$, either by an increase of T_{leaf} at any one

outlet humidity level (Fig. 4b compared with 4a), or by a reduction in e_{out} at any one T_{leaf} value (Fig. 4a compared with 4c). Clearly, the converse also applies.

In Figure 5, the corresponding changes in $\Delta\Sigma r_{CO_2}/\Sigma r^*_{CO_2}$ are shown. The Γ values selected are generally typical of high ($\Gamma = 100$ ng/cm^3) and low ($\Gamma = 0$) compensation point plants respectively (see, for example, 4, 10).

The data show that the error in $(\Delta\Sigma r/\Sigma r^*)_{CO_2}$ increases rapidly as the assumption $c^*_{amb} = c_{out}$ becomes less valid and as the ratio c_{out}/c_{in} drops. As long as the $c_{out}/c_{in} \geq 0.9$, errors of $<10\%$ arise, but where $c_{out}/c_{in} < 0.8$, substantial effects tend to occur. There is relatively little effect of the level of c_{out} or Γ on the pattern of the results. Since Γ is relatively small compared with c^*_{amb}, the denominator of equation 18 is rather insensitive to changes in Γ (Fig. 5a compared with 5c) although the error is greater for the higher value of Γ chosen. As c_{out} decreases, the error also increases slightly. This is because c^*_{amb} is linked with c_{out} in such a way that decreasing c_{out} increases the numerator of equation 18 to a greater degree than the denominator.

Figure 6 shows the integrated effect of the errors induced in Σr_{H_2O} and Σr_{CO_2} on the estimated values of r_{int}. In all cases it is assumed that $c^*_{amb} = (c_{out} + c_{in})/2$ and $e^*_{amb} = (e_{out} + e_{in})/2$. Three different measurement modes are depicted. Figure 6a refers to the mode in which a relatively unaltered ambient airstream is passed through the chamber. Under these conditions the relative change in CO_2 concentration (c_{out}/c_{in}) is generally much less than the corresponding change in water vapor concentration (associated with the different absolute concentrations of each gas and the normal ratio of photosynthesis to transpiration). Typically, in experiments air flow rates are adjusted so that c_{out}/c_{in} is maintained at a value of about 0.9; the corresponding value of e_{in}/e_{out} is generally about 0.5 or lower. Accordingly, values of $c_{out}/c_{in} = 0.9$ and $e_{in}/e_{out} = 0.5$, respectively, were used for Figure 6a.

Figure 6a shows that as stomatal aperture is reduced and Σr_{H_2O} rises, a progressively greater error is introduced. This effect operates both on the numerator and, particularly, the denominator of equation 20. The error is reduced at higher values of r^*_{int}; this effect operating also directly on the denominator of equation 20 (since $\Sigma r_{CO_2} - \Sigma r_{H_2O} = r^*_{int}$) and indirectly on the numerator. It can be appreciated that, had a value of say $e_{in}/e_{out} = 0.25$ been used, more pronounced changes would have occurred, since the lower the ratio of e_{in}/e_{out} the greater the relative change in $\Delta r_{int}/r^*_{int}$.

In Figure 6b an alternative measurement mode is depicted. The initial conditions are the same as in Figure 6a ($c_{out}/c_{in} = 0.9$; $e_{in}/e_{out} = 0.5$, at $r^*_{int} = 2.5$ sec/cm, $\Sigma r_{H_2O} = 1.0$ sec/cm). However, it is assumed that as stomatal aperture decreases and Σr_{H_2O} and Σr_{CO_2} rise, the inlet/outlet gas concentration differential changes in inverse proportion to the sum of the resistances. In other words, total gas flow rate is maintained unchanged so that, with stomatal closure and the concomitant reduction in rates of net photosynthesis and transpiration, the inlet/outlet ratios would approach unity. Clearly, as this occurs, the noise/signal ratio increases so that instrument resolution problems arise. Even so, this mode is widely used in practice, the flow rate only being reduced to improve measurement resolution when the noise/signal ratio becomes dominating.

Figure 6b shows that this procedure leads to negligible errors; even at low r^*_{int} and high Σr_{H_2O} values, errors of only about 6% arise.

In Figure 6c, the effects of maintaining both gas ratios at a value of 0.9 is shown, for the same range of r^*_{int} and Σr_{H_2O} values as used for Figures 6a and 6b. The effect of errors in the basic assumption that $e^*_{amb} = e_{out}$ and $c^*_{amb} = c_{out}$ is to reduce both Σr_{H_2O} and Σr_{CO_2} below the true values, but when c_{out}/c_{in} and e_{in}/e_{out} are both 0.9 the relative change in Σr_{H_2O} is less than that in Σr_{CO_2}, so the effect on $\Delta r_{int}/r^*_{int}$ is negative. In the
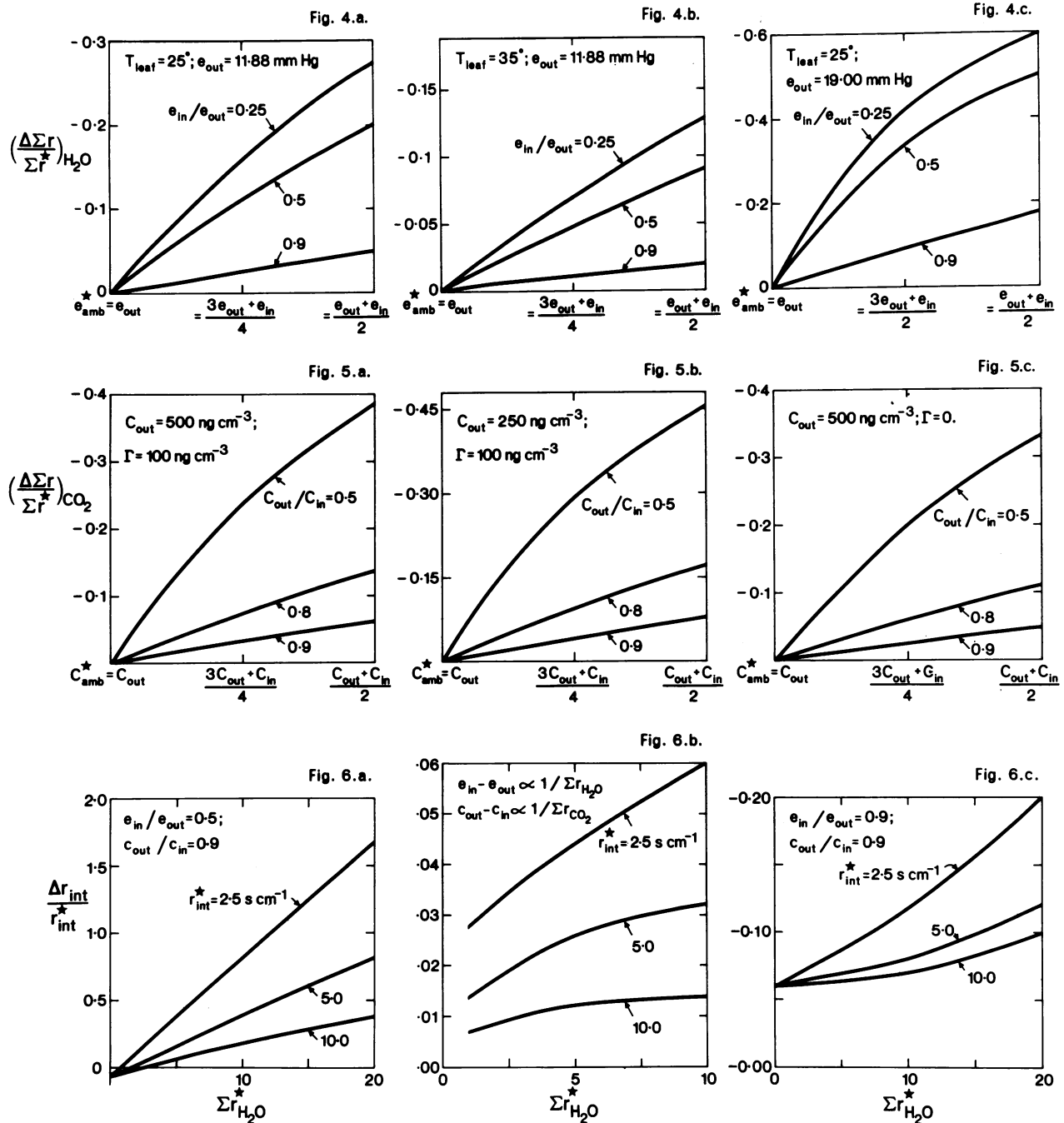
FIG. 4. Effect of error in the assumption that $e^{*}_{amb} = e_{out}$ on the relative error induced in the estimated total diffusive resistance to water vapor transport $(\Delta\Sigma r/\Sigma r^{*})_{H_2O}$ at different levels of e_{in}/e_{out}. a: Effect at $T_{leaf} = 25$ C, $e_{out} = 11.88$ mm Hg; b: same as Figure 4a except that $T_{leaf} = 35$ C; c: same as Figure 4a except that $e_{out} = 19.00$ mm Hg.

FIG. 5. Effect of error in the assumption that $c^{*}_{amb} = c_{out}$ on the relative error induced in the estimated total diffusive resistance to CO₂ transport $(\Delta\Sigma r/\Sigma r^{*})_{CO_2}$ at different levels of c_{out}/c_{in}. a: Effect at $c_{out} = 500$ ng/cm³, $\Gamma = 100$ ng/cm³; b: same as Figure 5a except that $c_{out} = 250$ ng/cm³; c: same as Figure 5a except that $\Gamma = 0$.

FIG. 6. Effect of total diffusive resistance to water vapor transport (Σr_{H_2O}) on the relative error induced in the estimated intracellular resistance $(\Delta r_{int}/r^{*}_{int})$ at different levels of r^{*}_{int}. In all cases $T_{leaf} = 25$ C, $e_{leaf} - e_{amb} = 11.88$ mm Hg; $c_{out} = 500$ ng/cm³, $\Gamma = 100$ ng/cm³. Also, it is assumed that $e^{*}_{amb} = (e_{in} + e_{out})/2$, $c^{*}_{amb} = (c_{in} + c_{out})/2$. a: Effect when $e_{in}/e_{out} = 0.5$, $c_{out}/c_{in} = 0.9$; b: effect when $(e_{in}/e_{out}) \propto 1/\Sigma r_{H_2O}$, $(c_{out} - c_{in}) \propto 1/\Sigma r_{CO_2}$, c: effect when $e_{in}/e_{out} = 0.9$; $c_{out}/c_{in} = 0.9$.

hypothetical case where $\Sigma r^{*}_{H_2O} = 0$, the error is entirely due to error in Σr_{CO_2} and then the value of $\Delta r_{int}/r^{*}_{int}$ is -0.06 (as also seen in Fig. 5a). As Σr_{H_2O} increases, the error becomes progressively more negative, the increase being slower at higher values of r^{*}_{int}. Even so, the relative error does not exceed -20% for the conditions used here, suggesting that this mode is also not likely to introduce major errors, particularly under open stomata conditions.

DISCUSSION

The data presented in this paper indicate that substantial errors can arise in the determination of stomatal resistance (indicated here as total gas phase resistance to water vapor transport, Σr_{H_2O}) and intracellular resistance, r_{int}.

The effect of an error in leaf temperature measurement affects Σr_{H_2O} directly, and the data indicate that even errors of the order

of 1 C can cause significant effects on the calculated value of Σr_{H_2O} under typical leaf temperature and ambient humidity conditions. The effect on r_{int} is indirect, since the calculation of Σr_{CO_2} is unaffected by errors in leaf temperature measurement. In general, under open stomata conditions, the effects are not large, particularly if r^*_{int} is relatively high. However, when the stomatal resistance is high and r^*_{int} is low, the calculated value of r_{int} can be affected to a substantial degree; differences of more than 100% arise under many conditions experienced out of doors. It can be appreciated that errors of several degrees in leaf temperature measurement cause proportionally larger effects.

The effect of errors in the assumption that the ambient concentration of CO_2 and water vapor inside the chamber equals the outlet concentration can also cause substantial effects. These errors affect both Σr_{H_2O} and Σr_{CO_2} directly, and r_{int} indirectly through the differential effect on Σr_{H_2O} and Σr_{CO_2}. Fortunately, this differential effect is generally less pronounced since the effect on both Σr_{H_2O} or Σr_{CO_2} is in the same direction. It can be negative or positive depending on the relative magnitude of the direct effects. The effect on Σr_{H_2O} is greater at higher ambient humidity levels and at lower temperatures, that is, at lower values of $e_{leaf} - e_{amb}$. Similarly, the effect on Σr_{CO_2} is greater at lower values of $c_{out} - \Gamma$, caused either by relatively low values of c_{out} or relatively high values of Γ.

Of the measurement modes examined, the one which caused the greatest effect on r_{int} was that in which c_{out}/c_{in} and e_{in}/e_{out} are maintained constant (by changing the rate of air flow more or less proportionally with change in net photosynthesis and transpiration) at values significantly different from unity (Fig. 6a). The mode which caused the least effect was that in which air flow was maintained constant as net photosynthesis and transpiration changed (Fig. 6b). However, this procedure poses difficult instrument problems since the noise/signal ratio increases rapidly as c_{out}/c_{in} and e_{in}/e_{out} approach unity. The third mode, in which the inlet-outlet differential was assumed to be maintained at the smallest levels satisfactory for instrument performance (Fig. 6c) provided an intermediate result.

In order to minimize the sources of error considered in this paper, several procedures appear to be desirable. Firstly, with regard to errors in temperature measurement: these can be reduced by a combination of ventilation conditions, leaf exposure, and sensor characteristics. Rapid ventilation, by reducing the thickness of the boundary layer, tends to increase both sensible and latent heat exchange between leaf and air. Even in closed stomata situations, where elevated leaf temperatures can be expected, the real leaf-air temperature difference can often be maintained at less than 1 C by this procedure. Since, in the absence of a direct radiation error in the sensor, the measured value of leaf temperature is frequently intermediate between the true leaf temperature and the air temperature, this is an important precautionary step. Rapid ventilation also minimizes variability in boundary layer thickness from point to point on the leaf so that leaf exposure becomes less critical. Even so, it is desirable to have leaves mounted in leaf chambers so that all parts of the leaf receive similar radiation loads. If part of a leaf is normal to the light source and part is parallel to it, it can be appreciated that the measurement of an average leaf temperature raises difficult sampling problems.

With regard to the assumption that the ambient concentration

of water vapor and CO_2 is indicated by the outlet concentration, the degree to which this assumption is met in any particular measurement system can be checked by reducing the air flow rate and the degree of ventilation and comparing the results obtained with those from standard conditions. If Σr_{H_2O} and Σr_{CO_2} do not change significantly, this is the best possible evidence that the assumption $c^*_{amb} = c_{out}$ (or $e^*_{amb} = e_{out}$) is valid in the system being used.

If this simple check leads to changes in the values obtained for Σr_{H_2O} and Σr_{CO_2}, the system can be improved by improving the ventilation system (which will also reduce leaf-air temperature differences, as already discussed) and by increasing the flow rate so that the inlet-outlet ratios are maintained as close to unity as the resolution characteristics of the water vapor and CO_2 detectors will permit with acceptable noise/signal ratios.

If the primary requirement of a measurement system is to estimate r_{int} rather than both Σr_{H_2O} and r_{int}, an alternative procedure for measuring r_{int}, by flowing air through a leaf, exists (7). This method is limited to amphistomatous leaves but, as well as providing an alternative procedure, it also enables a comparison to be made of the two methods for r_{int} estimation. Unpublished data of the author indicate good agreement between the methods under open stomata conditions.

This paper has not considered other sources of error in leaf chamber measurements of CO_2 and water vapor exchange. Several of these require independent investigation. They include the validity of using leaf temperature as an indicator of e_{leaf} (5), the validity of using a fixed ratio of D_{CO_2}/D_{H_2O} in converting diffusion resistances to water vapor transfer to those for CO_2 transfer (McPherson and Slatyer, unpublished data), and the problems introduced by differential closing of upper and lower stomata in amphistomatous leaves (3). Also, the reliability of the techniques and instruments used to measure rates of air flow, CO_2 concentration, water vapor concentration, and leaf temperature has not been questioned.

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