

# EFFECTS OF INTERCELLULAR RESISTANCES ON ESTIMATES OF THE INTRACELLULAR RESISTANCE TO CO<sub>2</sub> UPTAKE BY PLANT LEAVES

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## Abstract

A model is presented for net CO<sub>2</sub> uptake by plant leaves, which is particularly relevant to those leaves having different upper and lower stomatal resistances. The model includes intercellular space resistances to CO<sub>2</sub> uptake via both the upper and lower surfaces. Experimental data obtained with *Pelargonium hortorum* Bailey show that the ratio of the rates of CO<sub>2</sub> exchange through the lower and upper surfaces respectively is in all cases greater than the ratio of the corresponding rates of water vapour exchange. This provides evidence for a substantial vapour phase resistance to CO<sub>2</sub> uptake via the upper epidermis, which is not accounted for by the usual water vapour analogue. The data also suggest that calculations of intracellular resistance  $r'_i$ , using conventional resistance models, may be subject to error. However, the magnitude of the error in estimates of the intracellular resistance is seldom likely to exceed 10%, so that for most purposes changes in the conventional models do not appear to be warranted.

## I. INTRODUCTION

The estimation of the "intracellular resistance" to CO<sub>2</sub> uptake by leaves is of wide interest to plant physiologists and ecologists. The intracellular resistance gives an indication, for any particular leaf, of its intrinsic photosynthetic efficiency with possible variations due to stomata eliminated. It is in fact a complex term including limitations to photosynthesis caused by the various diffusion and enzymic processes involved in CO<sub>2</sub> assimilation. The intracellular resistance used in this paper is equivalent to the "mesophyll resistance" of Troughton and Slatyer (1969), and is defined by the following equation

$$r'_i = (c_s - \Gamma)/P, \quad (1)$$

where  $r'_i$  is the intracellular resistance,  $c_s$  is the CO<sub>2</sub> concentration at the cell surface,  $\Gamma$  is the CO<sub>2</sub> compensation point, and  $P$  is the net photosynthetic rate. This relation only applies under conditions where CO<sub>2</sub> alone is limiting photosynthesis. This definition of  $r'_i$  is equivalent to that obtained from the reciprocal of the initial slope of a curve relating net photosynthesis to the CO<sub>2</sub> concentration at the surface of the cell wall.

The estimation of  $c_s$  is commonly from

$$c_s = c_a - Pr_w K, \quad (2)$$

where  $c_a$  is the ambient CO<sub>2</sub> concentration,  $r_w$  is the overall leaf resistance to water

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vapour transfer (comprising boundary layer, stomatal, cuticular, and intercellular components), and  $K$  is the ratio of the diffusivities of water vapour and  $\text{CO}_2$  in air. This derivation of  $c_s$  assumes that the vapour phase pathway for  $\text{CO}_2$  entry into the leaf has the same characteristics as the water vapour pathway in transpiration (see e.g. Koller 1970). (This may not be strictly true, as is suggested by the evidence presented in this paper.) Also the correction of the water vapour resistance to the  $\text{CO}_2$  resistance by means of the ratio  $K$  may not be valid, since some of the transfer in the boundary layer will be turbulent and not diffusive (reviewed by Jarvis 1971). Any errors in the estimation of  $c_s$  will lead to errors in  $r'_i$ , as can be seen from equation (1).

Another possible source of error in gas-exchange estimates of  $r'_i$  occurs in anisolateral leaves, in which the upper and lower surfaces have different vapour phase resistances to  $\text{CO}_2$  entry (Raschke 1958). This effect has been analysed for the water vapour pathway by Moreshet, Koller, and Stanhill (1968), and for the  $\text{CO}_2$  pathway by Gale and Poljakoff-Mayber (1968) and by Koller (1970).

In this paper an alternative model is presented for studying the effect of differing upper and lower stomatal resistances on leaf chamber estimates of  $r'_i$ . This model, and also that of Koller (1970), are applied to experimental data on the gas exchange of the individual surfaces of leaves of *Pelargonium hortorum* Bailey using a double leaf chamber (Jarvis and Slatyer 1966), and their predictions are compared. The applicability of both these models is restricted to conditions where there is efficient mixing of the air above and below the leaf. It can be shown that, for the effects of anisolaterality on estimates of  $r'_i$  to be detectable, both Koller's model and the present one predict that the ratio of upper to lower stomatal resistances must exceed 3 for most plants. Hence a cultivar of *P. hortorum* was selected which had a ratio of the upper to lower stomatal frequencies of about 6, in the expectation that significant effects on  $r'_i$  would be observed.

The results are most reasonably interpreted on the basis of the present model, and provide strong, though indirect, evidence for the existence of a large intercellular  $\text{CO}_2$  resistance not analogous to any portion of the water vapour resistance, at least for one surface of *P. hortorum* leaves.

## II. THEORY

The present model to estimate the effect of different resistances in the upper and lower stomatal pathways is similar to that used by Koller (1970). In our case the  $\text{CO}_2$  pathway may be represented by the analogue in Figure 1, where  $r'_{w_1}$  and  $r'_{w_2}$  are the upper and lower leaf resistances for  $\text{CO}_2$ , respectively. [Resistances to  $\text{CO}_2$  transfer are represented throughout by primes, and are obtained from the corresponding water vapour resistances by multiplication by  $K$  ( $= 1.56$ )]. The resistances  $r'_{x_1}$ ,  $r'_{x_2}$ , and  $r'_{x_3}$  are the additional intercellular space resistances which occur in the  $\text{CO}_2$  pathway as compared with the water vapour pathway. These resistances separate an upper "effective sink" for  $\text{CO}_2$  from the upper epidermis, a lower effective sink from the lower epidermis, and the two sinks, respectively.  $2r'_i$  is the effective intracellular resistance of each effective sink. The two effective sinks are defined as having equal values of  $2r'_i$ , which to a first approximation may be taken as having equal chlorophyll contents. Therefore the overall intracellular resistance is  $r'_i$ , which is the

parallel sum of the resistances of the upper and lower effective sinks. Figure 2 explains the derivation and meaning of these effective sinks. The complex three-dimensional CO<sub>2</sub>-absorbing surface in a leaf is replaced by two equivalent planar surfaces and the appropriate diffusive resistances.

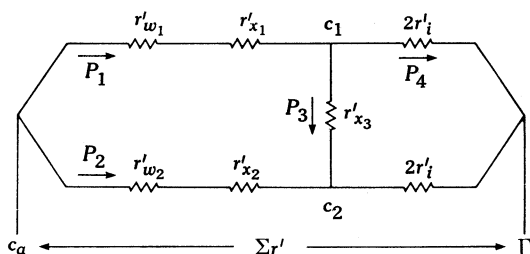


Fig. 1.—Electrical analogue of CO<sub>2</sub> uptake used in this paper. The symbols used are explained in the text.

This model differs from Koller's in that here the intracellular sink strengths of the upper and lower mesophyll layers, and hence their  $r'_i$  values, are defined as equal, with the consequent necessity for the inclusion of the two additional intercellular space resistances ( $r'_{x1}$  and  $r'_{x2}$ ). Using our model it is possible to estimate  $r'_i$  and  $r'_{x3}$  from double leaf chamber measurements (Jarvis and Slatyer 1966), without the need for an absolute estimate of  $r'_{x3}$ , if an estimate for the ratio of  $r'_{x1} : r'_{x2} : r'_{x3}$  is available. A first approximation to the positions of the effective sinks, and hence to the ratio of the intercellular resistances, could be obtained from the median positions of the upper and lower halves of the total number of chloroplasts, as indicated in Figure 2. For the present paper, however, the method adopted was to set  $r'_{x2}$  equal to

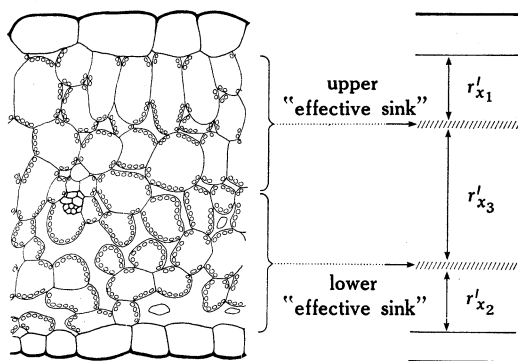


Fig. 2.—Line drawing of transverse section of a leaf of *P. hortorum*; the interpretation at the right-hand side of the drawing, shows the concepts of upper and lower "effective sinks" for CO<sub>2</sub>, with their associated intercellular space resistances  $r'_{x1}$ ,  $r'_{x2}$ , and  $r'_{x3}$ .

zero (which gives a minimum for  $r'_{x1}$ ), and calculate  $r'_{x1}$  and  $r'_i$  using estimated values for  $r'_{x3}$ . The values used for  $r'_{x3}$  were approximately half the corresponding values used in Koller's model, since, as can be seen from Figure 2, our  $r'_{x3}$  represents an intercellular space diffusion resistance through about half the mesophyll, while in Koller's model  $r'_{x3}$  represents the resistance through the whole mesophyll.

From the circuit in Figure 1,  $r'_i$  is given by

$$r'_i = (c_1 + c_2 - 2\Gamma) / 2(P_1 + P_2),$$

where  $P_1$  and  $P_2$  are the net CO<sub>2</sub> uptakes through the upper and lower leaf surfaces,

respectively, and  $c_1$  and  $c_2$  are the  $\text{CO}_2$  concentrations at the surfaces of the effective sinks, as shown in Figure 1. Therefore, using the usual transport equation and substituting for  $c_1$  and  $c_2$  this becomes

$$r'_i = [2c_a - P_1(r'_{w_1} + r'_{x_1}) - P_2(r'_{w_2} + r'_{x_2}) - 2\Gamma]/2(P_1 + P_2), \quad (3)$$

where  $c_a$  is the ambient  $\text{CO}_2$  concentration. Given  $r'_{x_2}$  and  $r'_{x_3}$ , one can also calculate  $r'_{x_1}$  since, in Figure 1,

$$P_3 = P_1 - P_4. \quad (4)$$

Therefore, again using the transport equation and substituting for  $P_3$  and  $P_4$ ,

$$(c_1 - c_2)/r'_{x_3} = P_1 - [(c_1 - \Gamma)/2r'_i],$$

and then substituting for  $c_1$  and  $c_2$ , and rearranging

$$r'_{x_1} = \{2r'_i[P_2(r'_{w_2} + r'_{x_2}) - P_1(r'_{w_1} + r'_{x_3})] + r'_{x_3}(c_a - P_1r'_{w_1} - \Gamma)\}/P_1(2r'_i + r'_{x_3}). \quad (5)$$

On substituting for  $r'_i$  from equation (3), this becomes a quadratic in  $r'_{x_1}$ :

$$\begin{aligned} & [r'_{x_1}]^2(P_1^2) - [r'_{x_1}]\{P_1(2c_a - 2\Gamma + P_2r'_{x_3} - 2P_1r'_{w_1})\} \\ & - \left( P_1(2r'_{w_1}c_a + r'_{x_3}c_a - P_1[r'_{w_1}]^2 - 2r'_{w_1}\Gamma - r'_{x_3}\Gamma) \right. \\ & \quad + P_2\{r'_{x_3}(\Gamma - c_a) + r'_{x_2}(2\Gamma + 2P_2r'_{w_2} - 2c_a + P_2r'_{x_2}) + 2r'_{w_2}\Gamma + P_2[r'_{w_2}]^2 - 2r'_{w_2}c_a\} \\ & \quad \left. - P_1P_2(r'_{w_2}r'_{x_3} + r'_{x_2}r'_{x_3} - r'_{w_1}r'_{x_3}) \right) = 0. \end{aligned} \quad (6)$$

This equation may be solved for  $r'_{x_1}$  in the usual way, and  $r'_i$  may then be obtained from equation (3). If, however, the ratio  $r'_{x_1} : r'_{x_2} : r'_{x_3}$  is known, then the absolute values of  $r'_i$  and the intercellular space resistances may be calculated by a similar procedure.

These results may be compared with estimates of the intracellular resistances from overall gas-exchange data and from Koller's model. For overall gas-exchange data, from equations (1) and (2),  $r'_i$  is given by

$$\begin{aligned} r'_i &= (c_s - \Gamma)/(P_1 + P_2) \\ &= [c_a - (P_1 + P_2)r_wK - \Gamma]/(P_1 + P_2). \end{aligned} \quad (7)$$

Using Koller's model the intracellular resistance is obtained (using our symbols) from

$$r'_i = r'_{i_1}r'_{i_2}/(r'_{i_1} + r'_{i_2}), \quad (8)$$

where  $r'_{i_1}$  and  $r'_{i_2}$  are the intracellular resistances of the upper and lower mesophyll layers respectively. These resistances may be calculated from

$$r'_{i_1} = r'_{x_3}(c_a - P_1r'_{w_1} - \Gamma)/[P_1(r'_{w_1} + r'_{x_3}) - P_2r'_{w_2}], \quad (9)$$

and

$$r'_{i_2} = r'_{x_3}(c_a - P_2r'_{w_2} - \Gamma)/[P_2(r'_{w_2} + r'_{x_3}) - P_1r'_{w_1}]. \quad (10)$$

Unlike Koller's original equations, these equations allow for photorespiratory effects since  $\Gamma$  is used instead of zero as the  $\text{CO}_2$  concentration at the final sink for  $\text{CO}_2$  (Koller 1970, p. 978, equation 9).

The approach adopted here of defining the upper and lower intracellular resistances as equal, and thus only obtaining an overall value for  $r'_i$ , is thought to be

more realistic, bearing in mind the present state of leaf chamber technology, than attempting to obtain the true values of each of these resistances. The present model overcomes, to some extent, problems associated with actual definition of the two layers and their resistances. It also provides for a more flexible interpretation of the intercellular space resistances, where it is recognized that the sinks and pathways for  $\text{CO}_2$  assimilation are not identical to the sources and pathways for water in transpiration. It must also be pointed out that the description of the transfer processes in a complex three-dimensional system such as a leaf, by the simple resistance analogues proposed, is a substantial simplification. Though leaf anatomy is too complex and variable to allow a rigorous three dimensional flux analysis, such a study on a simple leaf model can be shown to lead to small (i.e. second order) corrections in the resistance analogue results (H. G. Jones and R. O. Slatyer, unpublished data).

Another important point is that the model is not invalidated by the use of equation (1) under conditions where  $\text{CO}_2$  is not the sole factor limiting photosynthesis. Any conclusions regarding the intercellular space resistances are independent of the definition of  $r'_i$  which is employed. Any "apparent  $r'_i$ " calculated under conditions of non-limiting  $\text{CO}_2$  will overestimate the true  $r'_i$ .

### III. MATERIALS AND METHODS

#### (a) Plant Materials

The experiments were performed on an unknown cultivar of *P. hortorum* which had fairly large (15 cm diameter) leaves, and had about six times as many stomata per unit area on the abaxial as on the adaxial surface. The plants were grown in pots in the greenhouse for two months before the experiments. Newly expanded leaves were used throughout.

#### (b) Gas-exchange Measurements

The measurements of gaseous exchange from the two surfaces of the leaf were made in the double leaf chamber of Jarvis and Slatyer (1966). Water vapour measurements were made with differential thermocouple psychrometers (Slatyer and Bierhuizen 1964), which were checked against a Cambridge systems dew point hygrometer. The exposed leaf area was limited to a circle of 3.6 cm diameter ( $10.2 \text{ cm}^2$  area), owing to the difficulty of fully separating the two halves of the inner chamber. Because of the need to obtain adequate accuracy in gas-exchange measurements, and the associated need to avoid tissue desiccation, both relatively high  $\text{CO}_2$  concentrations, and relatively low light intensities were employed in some experiments. However,  $\text{CO}_2$  was the main factor limiting net photosynthesis in experiments 2-7. Measurements of net photosynthesis were made in both 21% oxygen and in 1% oxygen, the latter to suppress photorespiration (Björkman 1966; Tregunna, Krotkov, and Nelson 1966). For most measurements the  $\text{CO}_2$  concentrations in the two outgoing airstreams were maintained equal, this concentration being taken as the average concentration over the whole leaf surface. Slatyer (1971) discusses this assumption. Leaf temperatures were taken as the average of the readings of two fine wire copper-constantan thermocouples stuck to the lower (shaded) leaf surface by small (1 by 2 mm) pieces of trimmed surgical adhesive. It had previously been shown that this method gave comparable results to the actual insertion of the thermocouples in lateral veins. Light intensities are given in  $\text{Wm}^{-2}$  (300-700 nm).

### IV. RESULTS

The data collected from the double leaf chamber experiments were used, in conjunction with various estimates of the parameters  $r'_{x_1}$ ,  $r'_{x_2}$ , and  $r'_{x_3}$  to calculate  $r'_i$  on the basis of overall gas-exchange data, Koller's model, and the present model. The gas-exchange data from 12 separate experiments are given in Table 1.

TABLE 1  
GAS-EXCHANGE DATA FOR THE *P. HORTORUM* EXPERIMENTS

Parameter	Experiment No.											
	1	2	3	4	5	6	7*	8	9	10	11	12
$c_a(\mu\text{g l}^{-1})$	477	373	374	588	604	595	642	568	568	664	351	305
$P_1(\text{ng CO}_2 \text{ cm}^{-2}\text{s}^{-1})$	6.9	6.8	5.6	7.7	5.9	7.5	3.9	5.4	6.5	4.4	3.8	3.4
$P_2(\text{ng CO}_2 \text{ cm}^{-2}\text{s}^{-1})$	33.2	26.4	26.4	44.8	45.8	45.3	40.2	43.8	48.0	37.3	26.2	23.7
$r_{w_1}(\text{s cm}^{-1})$	4.2	4.9	5.1	13.3	12.7	11.9	41.2	11.4	13.1	16.6	11.4	11.7
$r_{w_2}(\text{s cm}^{-1})$	1.8	2.1	2.0	2.4	2.3	2.3	4.5	2.3	2.4	3.3	2.7	2.8
$r'_{w_1}(\text{s cm}^{-1})$	6.5	7.6	8.0	20.7	19.8	18.6	64.2	17.7	20.5	25.9	17.7	18.2
$r'_{w_2}(\text{s cm}^{-1})$	2.8	3.3	3.1	3.8	3.6	3.6	7.0	3.6	3.7	5.1	4.1	4.4
$P_2/P_1$	4.8	3.9	4.7	5.8	7.8	6.0	10.3	8.1	7.4	8.5	6.9	7.0
$r'_{w_1}/r'_{w_2}$	2.4	2.3	2.5	5.5	5.5	5.2	9.2	4.9	5.5	5.1	4.3	4.1
Light intensity ( $\text{W m}^{-2}$ )	106	108	230	132	210	285	170	60	90	90	90	90
Measurement mode	Air	Air	Air	Air	Air	Air	Air	1%O <sub>2</sub>	1%O <sub>2</sub>	Air	1%O <sub>2</sub>	1%O <sub>2</sub>

\* In experiment 7, the leaf was inserted upside down in the chamber.

Table 1 shows that the ratio of the rates of CO<sub>2</sub> exchange through the lower and upper surfaces respectively ( $P_2/P_1$ ) is in all cases greater than the ratio of the corresponding rates of water vapour exchange (which are given by  $r_{w_1}/r_{w_2}$ ). This observation suggests that there is a significant difference between the total resistances of the upper and lower internal pathways for CO<sub>2</sub> uptake.

Considering first Koller's model, estimates of his intercellular space diffusion resistance ( $r'_{x_3}$  in the terminology of the present paper) have been of the order of 1–3 s cm<sup>-1</sup>. Methods employed for estimating this intercellular space resistance have included the nitrous oxide diffusion porometer (Jarvis and Slatyer 1970), and estimates from leaf anatomy (Jarvis, Rose, and Begg 1967). For cotton, with leaves about 200 μm thick, these methods produce values of the average intercellular space CO<sub>2</sub> diffusion resistance of 2–3 s cm<sup>-1</sup>. Since the leaves used in these experiments were nearer 300 μm in thickness, somewhat higher values of  $r'_{x_3}$  should be assumed for Koller's model (where  $r'_{x_1}$  and  $r'_{x_2}$  are zero), and were taken as 3 and 6 s cm<sup>-1</sup>. In our model where  $r'_{x_3}$  does not account for the whole intercellular space resistance (see Fig. 2), values of 1 and 3 s cm<sup>-1</sup> were used.

The three methods for calculating  $r'_i$  are compared, for the *P. hortorum* data, in Table 2. In the first row,  $r'_i$  is calculated from overall gas-exchange measurements, using equation (7). The estimates of  $r'_{i_1}$ ,  $r'_{i_2}$ , and  $r'_i$  using Koller's model [equations (9), (10), and (8), respectively] and a value for  $r'_{x_3}$  of 3 s cm<sup>-1</sup> are given in rows 2–4, while the corresponding estimates using an  $r'_{x_3}$  of 6 s cm<sup>-1</sup> are given in rows 5–7. These estimates of  $r'_i$  are all similar to, but smaller than, the corresponding estimates based on an overall model. Table 2 also shows that the calculated values of  $r'_{i_1}$  are all negative or very large. Since negative intracellular resistances are physically improbable, this indicates that Koller's model does not adequately explain the data. Rows 8 and 9 give the values of  $r'_{x_1}$  and  $r'_i$ , calculated from equations (6) and (3), respectively, on the assumptions that  $r'_{x_2} = 0$  and  $r'_{x_3} = 1$ . Rows 10 and 11 give corresponding estimates of  $r'_{x_1}$  and  $r'_i$  on putting  $r'_{x_3} = 3$ . It can be seen that the present model also gives estimates of  $r'_i$  slightly smaller than those in row 1, but without introducing negative resistances. If larger estimates of  $r'_{x_2}$  are used, the values of  $r'_{x_1}$  which give feasible estimates of  $r'_{x_3}$  become even larger. The present model, therefore, suggests that for *P. hortorum*,  $r'_{x_1}$  is very much greater than  $r'_{x_2}$ , and greater than  $r'_{x_3}$ .

It must be pointed out that, since many of the experiments had to be conducted under conditions of low light or high CO<sub>2</sub> concentration, CO<sub>2</sub> was not the sole factor limiting photosynthesis in all experiments. Therefore the true values for  $r'_i$  will generally be less than those calculated. Even so, the comparisons between the various models, for any one experiment, are still valid.

## V. DISCUSSION

The estimates of  $r'_i$  obtained by the overall method are generally less than 10% higher than those obtained from the model developed in this paper. Consequently, for *P. hortorum*, and probably also for most other species commonly used in leaf chamber work, the error introduced by using the overall method, regarding the estimates from our model as accurate, are not great enough to warrant the

TABLE 2  
COMPARISON OF THE THREE METHODS FOR CALCULATING  $r'_i$

Parameter	Experiment No.											
	1	2	3	4	5	6	7	8	9	10	11	12
$r'_i$	7.0	5.3	5.7	5.7	6.3	6.0	5.5	8.6	7.3	8.8	8.2	7.7
$r'_{i_1}$	-37	40	-30	63	-37	$\infty$	-43	-31	-53	-21	-35	-23
$r'_{i_2}$ ( $r'_{x_3} = 3$ )	5.5	4.3	4.4	6.3	5.2	5.9	4.8	6.4	6.2	5.7	6.1	5.3
$r'_i$	6.4	4.9	5.1	5.7	6.0	5.9	5.4	8.0	7.2	7.8	7.4	6.9
$r'_{i_1}$	$\infty$	$\infty$	$\infty$	49	$\infty$	90	$\infty$	$\infty$	$\infty$	-52	-93	-67
$r'_{i_2}$ ( $r'_{x_3} = 6$ )	6.5	5.1	5.2	6.5	6.0	6.4	5.4	7.6	7.1	7.1	7.3	6.5
$r'_i$	6.5	5.2	5.2	5.7	6.2	5.9	5.5	8.2	7.1	8.2	8.0	7.2
Present model												
$r'_{x_1}$ ( $r'_{x_3} = 1$ )	4.5	2.7	3.6	1.8	6.5	3.0	6.2	15.0	9.9	12.5	13.7	15.3
$r'_i$	6.7	5.1	5.5	5.5	6.2	5.8	5.4	8.1	7.0	8.6	7.9	7.2
$r'_{x_1}$ ( $r'_{x_3} = 3$ )	6.3	3.9	5.1	4.2	10.0	5.6	10.3	21.4	15.5	16.7	19.1	20.1
$r'_i$	6.5	5.0	5.4	5.4	6.0	5.7	5.3	7.8	6.7	8.4	7.6	6.9



average user of gas-exchange equipment changing from conventional procedures. However, the results are of scientific interest and the validity of the relatively high values for  $r'_{x_1}$  warrant discussion.

Estimates of  $r'_i$  based on Koller's model are generally similar to those based on the present model, though negative values of  $r'_{i_1}$  are obtained in many cases. The negative or large values for  $r'_{i_1}$  imply that the upper mesophyll layers have a lower photosynthetic efficiency than the lower layers. It is possible that the values of  $r'_{x_3}$  used in Koller's model are too low, and that this leads to the apparent negative estimates for  $r'_{i_1}$ .

The existence of a difference between the internal paths for  $\text{CO}_2$  assimilation via the upper and lower epidermes may also be deduced from a comparison of the ratio of the lower to upper stomatal conductances with the ratio of net photosynthetic rates through the lower and upper surfaces. For *P. hortorum* the latter ratio is greater than the former, which suggests that there is a significant extra  $\text{CO}_2$  uptake resistance in the upper pathway for  $\text{CO}_2$  entry.

The present model suggests that these results are due to  $r'_{x_1}$  being much greater than  $r'_{x_2}$ . That means, that for the leaves used in these experiments, there is a large intercellular resistance to  $\text{CO}_2$  uptake via the upper leaf surface compared with that in the lower leaf surface, which is not accounted for by the water vapour analogue estimate of gas phase resistance to  $\text{CO}_2$  uptake. It is still possible, however, that there may be true differences between the photosynthetic efficiencies of the upper and lower mesophyll layers.

The apparently large intercellular gas phase resistance to  $\text{CO}_2$  uptake via the upper stomatal pathway ( $r'_{x_1}$ ), may be partly due to the thick upper epidermis of the leaves used. The major reason for a large  $r'_{x_1}$  in this plant is probably related to the large average interstomatal distance on the upper surface ( $360\text{ }\mu\text{m}$  as compared with  $140\text{ }\mu\text{m}$  on the lower surface). Since the average leaf thickness is  $300\text{ }\mu\text{m}$ , many cells of the upper mesophyll layers will be nearer the stomata of the lower surface than the upper, and hence will contribute more to the  $\text{CO}_2$  uptake through the lower surface. This effect may be enhanced by the fact that the structure of the upper layers of the mesophyll does not seem well adapted for lateral diffusion in the intercellular spaces, which is supported by the observations of Meidner (1955), using a double-bore capillary mercury-drop porometer, who showed that some leaves had very little lateral gas conductivity in the intercellular spaces of the mesophyll. These effects could explain why  $r'_{x_1}$  (the average upper intercellular space resistance to  $\text{CO}_2$  uptake) is apparently larger than  $r'_{x_3}$  in the plants used for these experiments.

The possibility that the results could be explained by an intracellular factor such as photorespiration lowering the apparent efficiency in the upper mesophyll layers was discounted, since some of the measurements were made under 1% oxygen, which should suppress photorespiration.

The possibility of high light inhibition of photosynthesis in the upper mesophyll layers was discounted, since similar ratios of the upper to lower gas-exchange rates (see Table 1) were obtained at low light intensities, and also when the leaf was inserted in the chamber upside down. This also ruled out the possibility of there being an error in the water vapour resistance calculation, due to temperature gradients

through the leaf lamina. Since the thermocouples which measure leaf temperature are placed on the shaded surface of the leaf, the estimate of the saturation vapour pressure for transpiration resistance estimates from the upper surface may be too low as the top surface temperature will be higher than the bottom surface temperature. However, this was not a significant source of error in our experiments.

Another feasible explanation of the results could be that the conversion from a water vapour to a  $\text{CO}_2$  resistance, based on the ratio  $K$ , is invalid, since the cuticular pathway is likely to provide a much higher resistance to  $\text{CO}_2$  than to water vapour (Heath 1969). This effect would be greatest when the lower cuticular resistance is infinite, and the  $\text{CO}_2$  transfer resistance of the upper cuticular pathway is also infinite. Using the water vapour resistances shown in Table 1, the magnitude of the error in estimation of the ratio of upper to lower  $\text{CO}_2$  resistances may be calculated. Using the above assumptions and a value for the upper cuticular resistance to water vapour of  $90 \text{ s cm}^{-1}$  (obtained from experiments in very low light), the error was found, in all cases, to be too small to explain the observed ratio of photosynthetic rates between the two leaf surfaces.

The sum of the intercellular space resistances  $r'_{x_1}$ ,  $r'_{x_2}$ , and  $r'_{x_3}$  used in our model is not always the same as the corresponding value of  $r'_{x_3}$  used in Koller's model. This is because our  $r'_{x_1}$  may also include a factor due to differences in the intracellular efficiencies of the upper and the lower layers of the mesophyll. The surprisingly high calculated values for  $r'_{x_1}$  suggest that at least some of the anisolaterality in the  $\text{CO}_2$  uptake pathways may be explicable in terms of such differences in intracellular efficiencies. It is not possible to distinguish between the two interpretations of the data presented in this paper, from the gas-exchange data alone. However, in conjunction with simple anatomical studies, which show that there are normal photosynthetic cells throughout the lamina in this variety of *P. hortorum* (see Fig. 2), and with the indirect evidence presented above, an explanation based on an increased intercellular diffusion resistance for  $\text{CO}_2$  in the upper mesophyll becomes more likely than an explanation based solely on a lower photosynthetic efficiency in the upper layers of the mesophyll.

The presence of an intercellular resistance to  $\text{CO}_2$  uptake, not included in the usual water vapour analogue correction for the gas phase portion of the pathway, may be a general phenomenon. This would mean that many present estimates of the value of the intracellular resistance ( $r'_i$ ), even in plants without different upper and lower stomatal resistances, might be overestimates. The existence of such an intercellular space resistance to  $\text{CO}_2$  uptake, not accounted for by the water vapour analogue, is usually neglected, or else implicitly included in the calculated intracellular resistance.

An interesting ecological implication of the results is that the present cultivar of *P. hortorum* should have a low water-use efficiency, since the stomata on the upper surface allow reasonable transpiration rates, yet are very inefficient at absorbing  $\text{CO}_2$ . It would thus appear likely that only those plants which have similar numbers of stomata on both surfaces of their leaves, or those which are truly hypostomatous, would occur naturally in situations where water supply is an important factor influencing natural selection.

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