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On the Resistance to Transpiration of the Sites of Evaporation within the Leaf[1]

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ABSTRACT

The rates of transpiration from the upper and lower surfaces of leaves of *Gossypium hirsutum*, *Xanthium strumarium*, and *Zea mays* were compared with the rates at which helium diffused across those leaves. There was no evidence for effects of CO_2 concentration or rate of evaporation on the resistance to water loss from the evaporating surface ("resistance of the mesophyll wall to transpiration") and no evidence for any significant wall resistance in turgid tissues. The possible existence of a wall resistance was also tested in leaves of *Commelina communis* and *Tulipa gesneriana* whose epidermis could be easily peeled. Only when an epidermis was removed from a leaf, evaporation from the mesophyll tissue declined. We conclude that under conditions relevant to studies of stomatal behavior, the water vapor pressure at the sites of evaporation is equal to the saturation vapor pressure.

The stomata are considered to be the main sites of resistance to transpiration within the leaf. The bulk of this water is thought to evaporate from the mesophyll tissue, but an appreciable amount may also come from the inner walls of epidermal cells bounding the substomatal cavities (*e.g.* 17). The proportioning between the two currents may depend on magnitude and distribution of small temperature gradients within the leaf (6, 23). The possibility that the mesophyll may also offer a resistance to evaporation of appreciable magnitude has been debated for more than 70 years (16, 23). Various authors (*e.g.* 3) have concluded that the characteristics of the liquid flow system do not directly affect the rates of evaporation from leaves, but have ignored the effects of an internal cuticle which is reported to line the mesophyll cell walls (24). The hydraulic permeability of this cuticle is unknown but a value of 10^{-11} msec^{-1} bar^{-1}, which has been determined for storage tissue (1), is equivalent to a resistance to diffusion of water vapor of 2 sec cm^{-1} when the area of the mesophyll surface is 10 times that of the leaf (13). Several authors (10, 13, 15, 26) have recently presented evidence for the existence of such an additional barrier. Some of the evidence is equivocal since the measured magnitude of the wall resistance was small in relation to the accuracy of the measuring systems used. Nevertheless, if the published values should turn out to be correct then the resistance of the mesophyll walls to transpiration could be large enough to account for as much as one-half of the total resistance to transpiration located in the leaf when the stomata are wide open. If so, stomatal resistances computed from measurements of transpiration and water vapor pressure difference between leaf and air could be overestimated by a factor of up to 2. The computed drop in CO_2 concentration from the outside air across the epidermis to the intercellular spaces (18) would be overestimated to the same degree. As a result of this, the intercellular CO_2 concentration would be underestimated. Kaplan (14) found that the wall resistance to transpiration in *Atriplex halimus* in air containing 300 μl CO_2/l air was much higher than at 0 μl l^{-1}. This raises the possibility that earlier measurements of wall resistance, which were made in air kept free of CO_2 in order to reduce stomatal resistance, were underestimates. Jarvis and Slatyer (13) found that the wall resistance increased with increasing transpiration rate and with decreasing leaf water content.

This study was initiated because the quantitative basis of conventional studies of gas exchange of leaves seemed in doubt. Stomatal resistances may have been overestimated, particularly when stomata were open, calculated intercellular CO_2 concentrations were possibly too low, and the shapes of saturation curves of photosynthesis with respect to CO_2 were possibly distorted. The purpose of the present work was to determine the wall resistance to evaporation and the dependence of this resistance on transpiration rate and CO_2 concentration. Since wall resistance is determined as a residual term (see next section) from measurements of the diffusion of water vapor and another gas through the leaf, the accuracy of these measurements has to be high and known. We therefore describe the methods we applied in greater detail than usual.

THEORY

The principle involved was developed by Slatyer and Jarvis (26) and used by Gale *et al.* (10) and Jarvis and Slatyer (13): the resistance to diffusion of a physiologically inert gas (helium, in our case) through a leaf is subtracted from the series sum of the resistances to water vapor transfer of the two leaf surfaces, measured separately. The resistance to the diffusion of water vapor includes wall resistance, the resistance to the diffusion of helium does not.

The rate of evaporation from the upper (adaxial) surface of a leaf may be expressed by

$$J^u_{H_2O} = \frac{M_{H_2O}\,(e_w - e_a)}{RT\,\Sigma r^u_{H_2O}} \qquad (1)$$

where $J^u_{H_2O}$ is the rate of evaporation (g cm^{-2} sec^{-1}), M_{H_2O} is the mol wt of water, $(e_w - e_a)$ mm Hg is the vapor pressure difference between the sites of evaporation in the cell walls in the leaf, e_w, and the bulk air, e_a, R is the gas constant, T the absolute temperature of the leaf, and $\Sigma r^u_{H_2O}$ is the algebraic sum of the resistances encountered by the diffusing vapor (sec cm^{-1}). This formulation differs from that of Jarvis and Slatyer (13), which uses a gradient of vapor concentration (g cm^{-3}) and should, in principle, be used only when leaf and air temperature are equal. In the present case slight errors may arise using the leaf temperature for T, but these are smaller than those that arise when differences in temperature (and therefore density) are absorbed into the difference term

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($c_w - c_a$). The formulation of equation 1, used for instance by Gaastra (9), largely overcomes the objection by Hall *et al.* (11) that the units sec cm^{-1} for stomatal resistance are inherently in error in nonisothermal conditions. There are, nevertheless, effects of temperature and pressure (P) on diffusivity which make the units recently suggested by Cowan (6) attractive to us. In the present study the variation of P/T over the range of experiments was only 1%. If there is no cuticular transpiration the sum of the resistances may be expanded as

$$\Sigma r^u_{H_2O} = r^u_a + r^u_s + r^u_w \quad (2)$$

the superscript u identifying upper surface, and subscripts a, s, and w identifying the resistances offered by the boundary layer in the air, by the stomata, and by the cell wall.[3] When cuticular transpiration occurs through a resistance, r^u_c, equation 2 must be modified.

$$\Sigma r^u_{H_2O} = r^u_a + \left\{\frac{(r^u_s + r^u_w)r^u_c}{r^u_s + r^u_w + r^u_c}\right\} \quad (3)$$

A corresponding expression may be written for the lower surface.

$$\Sigma r^l_{H_2O} = r^l_a + \left\{\frac{(r^l_s + r^l_w)r^l_c}{r^l_s + r^l_w + r^l_c}\right\} \quad (3)$$

The flux of helium through a leaf may be described by

$$J_{He} = \frac{M_{He}(h^l_a - h^u_a)}{RT\,\Sigma r_{He}} \quad (5)$$

where J_{He} (g cm^{-2} sec^{-1}) is the flux, M_{He} is the mol wt of helium, $(h^l_a - h^u_a)$ mm Hg is the difference in partial pressures of helium between the air flowing under the leaf, h^l_a, and over the leaf, h^u_a, and Σr_{He} is the sum of the resistances encountered by the diffusing helium (sec cm^{-1}). If air containing helium is passed by the underside of a leaf, and air containing no helium passes above the leaf, the flux of helium through the leaf may be determined from

$$a\,J_{He} = \frac{M_{He}\,v\,h^u}{P} \quad (6)$$

where a (cm^2) is the area of leaf, v(mol sec^{-1}) is the rate at which air passes over the leaf, h^u (mm Hg) is the partial pressure of helium in the air after passing across the upper surface, and P (mm Hg) is the pressure of the air above the leaf.

From equations 5 and 6

$$\Sigma r_{He} = \frac{aP(h^l_a - h^u_a)}{vRTh^u} \quad (7)$$

This sum may be expressed in terms of the resistances to diffusion of water vapor by multiplying them by the ratio of diffusion coefficients of helium and water vapor in air.

$$\Sigma r_{He} = \frac{D_{H_2O}}{D_{He}}(r^l_s + r_i + r^u_s) + \left(\frac{D_{H_2O}}{D_{He}}\right)^{2/3}(r^l_a + r^u_a) \quad (8)$$

where r_i is the resistance of the additional path of helium through the intercellular spaces in the leaf. It arises because the sites of evaporation in the leaf, although not well defined, are effectively displaced from the center plane of the mesophyll. The ratios of the boundary layer resistances are inversely proportional to the two-thirds power of the diffusivities (19).

[3] Theoretically, the resistance of the intercellular air spaces between the sites of evaporation and the stomatal pore should be included in the sum of resistances. The magnitude of this resistance will depend on the location of the sites of evaporation in the leaf (17). We estimate this resistance to be smaller by 2 orders of magnitude than the resistances listed in equation 2. The resistance of the diffusion path across a whole leaf will be larger than that of the substomatal space and will be introduced later as r_i in equation 8.

We eliminate the effects of the differing diffusivities by rearranging equation 8 and defining R_{He} as

$$R_{He} = \frac{D_{He}}{D_{H_2O}}\Sigma r_{He} + \left(1 - \left(\frac{D_{He}}{D_{H_2O}}\right)^{1/3}\right)(r^l_a + r^u_a) = r^l_a + r^l_s + r_i + r^u_s + r^u_a \quad (9)$$

A similar expression for water vapor is found by adding equations 3 and 4. If there is no cuticular transpiration we define R_{H_2O} by

$$R_{H_2O} = \Sigma r^l_{H_2O} + \Sigma r^u_{H_2O} = r^l_a + r^l_s + r^l_w + r^u_w + r^u_s + r^u_a \quad (10)$$

If significant cuticular transpiration occurs the expression is modified by rearrangement of equation 4

$$r^l_a + r^l_s + r^l_w = \frac{\Sigma r^l_{H_2O}(r^l_c - r^l_a) + r^{l^2}_a}{r^l_c + r^l_a - \Sigma r^l_{H_2O}} = (\Sigma r^l_{H_2O})' \approx \frac{r^l_c\,\Sigma r^l_{H_2O}}{r^l_c - \Sigma r^l_{H_2O}} \quad (11)$$

since $r^l_a \ll r^l_c$. A similar expression can be derived for the upper surface:

$$R_{H_2O} = (\Sigma r^l_{H_2O})' + (\Sigma r^u_{H_2O})' = r^l_a + r^l_s + r^l_w + r^u_w + r^u_s + r^u_a \quad (12)$$

from equations 9 and 12

$$R_{H_2O} - R_{He} = r^l_w + r^u_w - r_i \quad (13)$$

MATERIALS AND METHODS

Leaf Chambers. In each experiment a leaf chamber made of anodized aluminum was clamped to the leaf. Separate streams of gas were passed over 2.44 cm^2 of each leaf side at flow rates equivalent to 37.8 l hr^{-1} at standard pressure and temperature. Pressure differences between top and bottom chambers were less than 0.5 mm H_2O which prevented bulk flow of gas through the leaf. To control leaf temperature, water was passed through the windows and walls of the leaf chamber. Copper-constantan thermocouples (0.1 mm) ran along the underside of the leaf. We estimate that the measurement of leaf temperature was accurate to better than 0.1 C at the point of measurement.

Air flow through the chamber was laminar. If a gas was taken up or given off by the enclosed leaf, the concentration of the gas exchanged with the leaf increased or decreased with the square root of the distance from the entrance into the chamber. The effective gas concentration k_{eff} in the chamber was obtained by integration: $k_{eff} = 0.33\,k_{in} + 0.67\,k_{out}$. This procedure of weighing k_{in} and k_{out} is based on the assumption that gas exchange is dominated by mass exchange. We know, however, that transpiration represents also a transfer of latent heat, which in turn affects temperature and vapor pressure at the sites of evaporation; heat transfer also depends on the development of the boundary layer. If the concentration of vapor at the leaf surface is assumed constant, and the flux is allowed to vary, $k_{eff} = 0.5\,(k_{in} + k_{out})$. The actual situation is somewhere in between and a detailed treatment is given by Cowan (4). The possible errors are very small in our system because $|k_{out} - k_{in}| \ll k_{in}$.

Boundary layer resistances (r_a) in the top and bottom sections of the chamber were measured by substituting thick moistened chromatography paper for the leaves and measuring water vapor pressure differences and evaporation rates. The resistance values obtained were between 0.25 and 0.30 sec cm^{-1}.

A water-jacketed xenon arc lamp (Osram XBF 6000 W/1) was the light source with heat-absorbing (Corning 4600) and neutral density filters (Plexiglas 800, Röhm und Haas, Darmstadt, Germany). Irradiance at the leaf level was 235 w m^{-2} of photosynthetically usable light; this corresponded to a quantum flux of

about 1.1 mE m^{-2} sec^{-1}.

Gas Analysis for H_2O and CO_2. The CO_2 concentration in the air as well as the differences in the contents of water vapor and CO_2 between air entering and leaving the leaf chambers were measured with differential IR gas analyzers (URAS 2, Hartmann und Braun, Frankfurt a. M., Germany). The analyzer for the absolute CO_2 content of the air was calibrated with gas mixtures accurate to within 0.5% of the desired values. The differential analyzers for CO_2 were calibrated for several base values between 0 and 1000 μl CO_2 l^{-1} air, using a Wösthoff mixing pump to give concentration differences between the reference and measuring lines equal to 1, 2, or 3% of the CO_2 concentration in the reference line. All gas streams entering the CO_2 analyzers, including the calibration gases, were brought to a dew point of 0 C to eliminate cross-sensitivity caused by spectral overlap of the absorption bands of gases with dipole moment, water vapor and CO_2, in the IR wavelength range from 2.5 to 12 μm.

Air with the required concentration of CO_2 was humidified and then brought to a dew point of 18.0 C in a controlled temperature bath (Lauda circulator, Brinkmann Instruments, Westbury, N.Y.). In addition, there was an extra stream of air passing through a condenser held at 1 to 3 C higher than the other condenser. This additional air stream served for the calibration of the differential analyzers for water vapor. IR gas analyzers for H_2O are slightly sensitive to CO_2 (again caused by overlap of absorption bands). This cross-sensitivity has little effect on the measurement of the water vapor content of air provided the CO_2 contents of the measuring gas and the reference gas are equal. If, however, the CO_2 content in one of the gas streams is altered (as happens, for example, when CO_2 is being assimilated by the leaf) an error is introduced into the measurement of humidity. This cross-sensitivity was removed by interposing filter cells in the sample and reference beams of the analyzers, and continuously flushing these cells with CO_2, at 1 liter hr^{-1}. The CO_2 was dry, having come from under compression.

Measurement of Helium Diffusion through the Leaf. Helium[4] was added at 1% (v/v) to the air passing through the bottom halves of the leaf chambers and to the reference lines used for the differential analysis of the gas exchange of the abaxial leaf surface. This was achieved using Wösthoff (Bochum, Germany) gas-mixing pumps fitted with supplementary delivery pistons (SA 18/3F).

Helium appearing in the gas stream passing over the upper side of the leaf was detected by a Varian MAT mass spectrometer, model GD150, operated at a pressure of 10^{-5} mm Hg. The spectrometer was calibrated for readings between zero and 200 μl helium/liter air, with gas mixtures prepared with cascaded Wösthoff mixing pumps. Repeated calibrations often remained constant within ±2% over a period of 2 weeks. Even when changes occurred, the relationship between output and concentration remained linear. From equation 7 it may be seen that only the ratio of the concentration of helium entering the lower chamber to that leaving the upper chamber is needed for calculation of Σr_{He}. Thus, measurements of the concentration of helium entering the lower chamber were made approximately twice/hr during an experiment.

Plants. Five species were chosen for the investigation: *Xanthium strumarium*, because its stomata can be insensitive to CO_2 (20) and in a state facilitating an investigation into the reported effect of CO_2 on wall resistance (21) without interference by stomatal movement; *Zea mays*, because it was among the species for which an apparent CO_2 dependence of the wall resistance had been reported (21), also because it is a C_4 grass with low transpiration ratio; *Gossypium hirsutum*, because dependencies of wall resistance on the rate of evaporation and on leaf water potential had been reported for this species (13); *Commelina communis* and *Tulipa gesneriana*, because the epidermis could be peeled easily from their leaves for a direct exposure of their mesophyll to dry air.

Plants of *X. strumarium* L. were cultivated in a greenhouse in soil. The natural light period was extended to the time from 4:30 in the morning to midnight by supplementary illumination with 0.3 mw m^{-2} from Sylvania Gro-lux fluorescent tubes (F40/GRO/WS). Air temperature maxima were about 23 C on cloudy and 27 to 29 C on sunny days, the RH was between 70 and 80%. Fully developed leaves on plants 3 to 4 weeks old were used in the experiments. The plants were kept pruned to the top five or six leaves and did not flower.

Z. mays L. (cv. Michigan 500) was grown in a sand-Vermiculite mixture for 3 weeks in growth chambers with a daily light period of 14 hr. Light intensity increased in three steps from 80 to 400 w m^{-2} within 5 hr; after 7 hr at light the intensity decreased again in steps. Light sources were General Electric Deluxe White Mercury H400DX33-1 and Lucalox LU400 lamps. The air temperature was 27 C during the day and 17 C at night, the RH was about 50%, day and night. The fifth or sixth leaves (in the sequence of emergence) were used for the experiments. The leaf part within the leaf chamber was about 30 cm from the leaf tip.

Plants of *G. hirsutum* L. (cv. Acala SJ-1; seeds from C. A. Beasley, University of California, Riverside) were cultivated in a growth chamber. The plants were illuminated for a 12-hr day^{-1} (60 w m^{-2} of photosynthetically usable light) from fluorescent lamps followed by 1 hr from incandescent lamps. The temperatures were 32 C during the day and 22 C during the night. The RH was 60%. The fifth leaf from the apex of 2-month-old plants was used.

C. communis L. (seeds from T. A. Mansfield, University of Lancaster, U.K.) was grown in a soil-Perlite mixture in a growth chamber under 85 w m^{-2} of light from fluorescent lamps for 16 hr daily, 85% RH, and a temperature of 27 C during the light period and 23 C during the dark period.

T. gesneriana L. leaves were from forced plants (7) and were obtained on the day of an experiment from A. De Hertogh of the Horticulture Department at Michigan State University.

RESULTS AND DISCUSSION

Significant resistances to transpiration at the sites of evaporation have been reported to occur and to increase with increasing CO_2 concentration (21), with increasing evaporation rates (13), and with decreasing water potential (13). The results of our experiments are discussed accordingly.

Wall Resistances, When Summed, Are Smaller than the Internal Resistance to Diffusion of Helium. The first experiment was designed to examine the time course, during stomatal opening, of the sum, R_{H_2O}, of the resistances to diffusion of water vapor from the two epidermes and of the resistance, R_{He}, to diffusion of helium through the leaf. In Figure 1a it is shown that as stomata of *X. strumarium* open in response to illumination of the leaf the resistance R_{H_2O}, calculated from equation 10 assuming no cuticular transpiration, and R_{He}, calculated from equation 9, both decline. At 14:50, illumination ceased and the resistances increased. The difference $R_{H_2O} - R_{He}$ is negative as the sum of the wall resistances, if they exist, is smaller than the resistance to diffusion of helium through the intercellular spaces of the leaf.

The change in value of $R_{H_2O} - R_{He}$ is partly caused by the changing proportion of transpiration contributed by the cuticle. Values of 0.02 and 0.03 cm sec^{-1} have been used for the cuticular conductances of the upper and lower epidermes of *Xanthium* (22). We observe that a plot of net assimilation *versus* $[CO_2]$ inside the leaf, obtained during stomatal openings, is only consistent with steady-state experiments with open stomata in which the ambient $[CO_2]$ was varied, when cuticular conductances for water vapor of

[4] Neon, which would have been preferable to helium in these experiments because its mol wt is close to that of water, is obscured in the mass spectrometer by doubly charged argon. Argon is a natural component of air and so highly ionizable that neon cannot be used as a tracer.

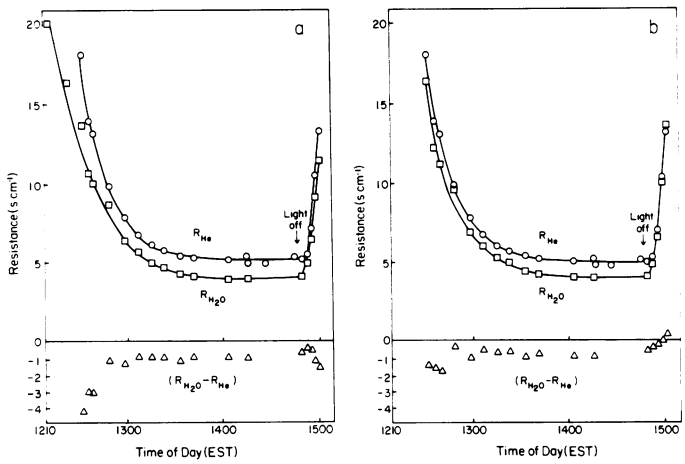

FIG. 1. a: Effects of stomatal opening on the time course of the sum, R_{H_2O}, of the resistances to diffusion of water vapor through the upper and lower epidermes of a detached leaf of *X. strumarium* and the resistance, R_{He}, to diffusion of helium through leaf. These resistances are calculated from equations 10 and 9, respectively, and ignore cuticular transpiration. $R_{H_2O} - R_{He}$ is the algebraic difference between the two. (The difference, $R_{H_2O} - R_{He}$, is negative, *i.e.* the internal resistance to the diffusion of helium is greater than the sum of the wall resistances.) b: Same time course is replotted assuming cuticular conductances of the upper and lower epidermes of 0.02 and 0.03 cm sec^{-1}, respectively. The resistance, R_{H_2O}, is here derived from equation 12. The disparity in $R_{H_2O} - R_{He}$ occurring in Figure 1a at resistances greater than 10 sec cm^{-1} is reduced when cuticular conductance is taken into account. The effect of cuticular conductance on the determination of $R_{H_2O} - R_{He}$ is small if $R_{H_2O} < 10$ sec cm^{-1}.

the indicated magnitude are assumed; CO_2 probably does not move through the cuticle (12). In Figure 1b the data from Figure 1a are replotted with R_{H_2O} calculated from equation 12 using the above cuticular conductances. Some disparity still exists, caused by the difficulty of obtaining simultaneity of measurement of R_{H_2O} and R_{He} during rapid stomatal responses. The effect of cuticular resistances on the determination of $R_{H_2O} - R_{He}$ is only significant when $R_{H_2O} > 10$ sec cm^{-1}.

We found the difference $R_{H_2O} - R_{He}$ to be negative in all cases in all species examined. In *X. strumarium* the difference was of the order of -1 sec cm^{-1}; in *Z. mays* it was larger, between -4 and -5.5 sec cm^{-1}. This is probably caused by the close packing of cells in *Zea*. The resistance to diffusion of CO_2 inside the leaf may, therefore, be appreciable when the leaf is assimilating rapidly because cells deeper in the leaf become involved in assimilation.

Gale *et al.* (10) thought that r_i increased with increasing transpiration rate and decreasing leaf water content. Our results do not provide evidence that such a change occurred under our experimental conditions; $R_{H_2O} - R_{He}$ remained constant when transpiration changed (Figs. 1b, 2 and 3).

Lack of Evidence for a CO_2 Dependence of Wall Resistance in Detached Leaves of *X. strumarium*. *X. strumarium* was used as the experimental plant to avoid interference of stomatal responses to CO_2 with the determination of a possible dependence of the wall resistance on CO_2. It has been found that stomata of leaves of *X. strumarium* are insensitive to CO_2 if the leaves contain little ABA (20). The use of *Xanthium* 'flags' (blades of detached leaves, trimmed to the area covered by the gas exchange chamber in order to reduce formation of endogenous ABA) eliminated stomatal interference in our examination of the response of ($R_{H_2O} - R_{He}$) to various CO_2 concentrations in the air.

Once the stomata were open, R_{H_2O} and R_{He} did not change when the CO_2 concentration was varied between 0 and 560 $\mu l\ l^{-1}$ (Fig. 2). Again, the difference $R_{H_2O} - R_{He}$ was always negative. No response of wall resistance to CO_2 was apparent. The rapid reductions in the apparent rate of transpiration when the ambient CO_2 concentration was raised, reported by Raschke and Gale (21), were artefacts caused by the cross-sensitivity to CO_2 of the water vapor analyzers. It is not known if this was also the case in the experiments of Kaplan (14).

Lack of Evidence for Effects of the Rate of Evaporation on Wall Resistance in *G. hirsutum*. We decided to examine the wall resistance of *G. hirsutum* as it was in this species that Jarvis and Slatyer (13) reported effects of transpiration rates on wall resistance. We decided to vary the transpiration rate through open stomata by manipulating leaf temperature in the range 25 to 30 C. In all seven leaves tested R_{H_2O} was less than R_{He}. The difference ($R_{H_2O} - R_{He}$), related to $[(r_w^u + r_w^l) - r_i]$, varied between leaves

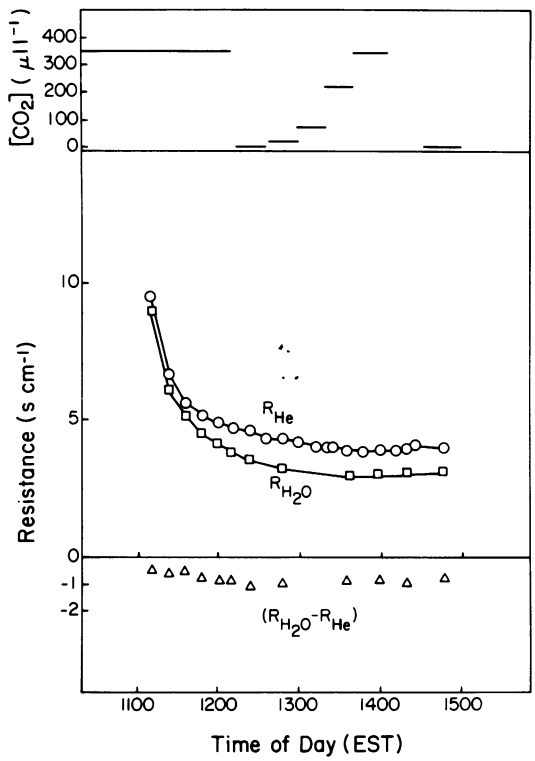

FIG. 2. Effects of stomatal opening and subsequent changes in ambient concentration of CO_2 on the time course of R_{H_2O}, R_{He}, and the difference, $R_{H_2O} - R_{He}$ for a detached leaf of *X. strumarium*. Calculations, based on equations 9 and 10, ignore cuticular transpiration, because $R_{H_2O} < 10$ sec cm^{-1} (see legend for Fig. 1).

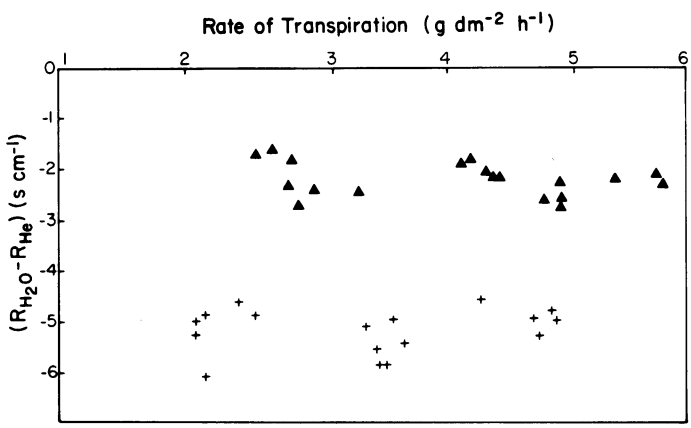

FIG. 3. Relationship between the difference, $R_{H_2O} - R_{He}$, and the rate of transpiration, E, from two leaves of *G. hirsutum*. Transpiration rate was varied by changing the temperature of the air stream passing over the leaves from 25 to 30 C. Cuticular transpiration is not taken into account. Data from five other leaves are not shown, and these fell between the two groups presented.

but the results were between those shown in Figure 3, which were collected over periods of approximately 1 hr at each temperature. No effect of transpiration rate is apparent. Jarvis and Slatyer (13) observed a decline in transpiration rate at large values of leaf-air vapor concentration differences. This phenomenon is likely to be caused by feedforward responses of stomata rather than by increased wall resistance (6). Using combinations of lowered humidity as well as increased leaf temperatures, Jarvis and Slatyer (13) observed an increase of r_w of 2 sec cm^{-1} over a similar range of transpiration rates. Since r_w is common to both sides this would be equivalent to an increase of 4 sec cm^{-1} ($R_{H_2O} - R_{He}$) in our notation. A partial explanation for this disparity comes from their statement that errors of up to 0.5 C may have occurred in their measurements of leaf temperature and that this could have led to errors of up to 5% in the measurement of stomatal resistance. Their values of mesophyll resistance increase with transpiration rate, and their greatest wall resistances were found when the total resistance ($r_w^l + r_s^l + r_s^u + r_w^u$) was about 16 sec cm^{-1}. Such an overestimate could occur if a leaf were cooler than the air and, from the data, this appears to have been the case.

An interesting feature of the data of Jarvis and Slatyer is that there appears to be a strong positive correlation between wall resistance and stomatal resistance. This was not observed in our studies. When R_{He} is plotted against R_{H_2O}, combining results from six leaves and assuming cuticular conductance of 0.03 cm sec^{-1} for the upper and lower epidermes, a linear relationship results with a slope close to unity (Fig. 4). The positive intercept corresponds to the intracellular resistance to the diffusion of helium. The measurements are necessarily less accurate at high resistances partly because of uncertainty about the correct value of cuticular conductance. The value of 0.030 cm sec^{-1} was the mean conductance from the upper epidermes of nine darkened leaves of *G. hirsutum*, which were also fed 0.1 mM ± ABA for 1 hr. However, one of these leaves, which had wilted, had a conductance of only 0.011 cm sec^{-1}. If a lower cuticular conductance were assumed, the slope of R_{He} *versus* R_{H_2O} would increase slightly and the apparent wall resistance decline accordingly with increasing stomatal resistance, a result opposite to that of Jarvis and Slatyer (13). We see no effects of rate of evaporation on the wall resistance of *G. hirsutum* and can only speculate about the reasons for the differences between our data and those of Jarvis and Slatyer.

Effects of Leaf Water Potential on Wall Resistance. Jarvis and Slatyer (13) reported that the wall resistance increased as leaf water potential declined. The simultaneous measurements of fluxes of water vapor and helium are only accurate when the stomata on both sides of the leaf are open and the technique does not work well in stressed leaves. We did, however, make an interesting observation while examining the fluxes of water from, and helium across, an intact leaf of *G. hirsutum*. Oscillations of stomatal conductance with a period of 30 min were observed and the resistance to diffusion of helium was synchronous with the sum of the resistances to vapor diffusion from both sides (dominated by the large resistance of the upper surface). It is known that changes in water content of the leaf are out of phase with the changes in conductance in these conditions (5). We saw no evidence for the presence of significant wall resistance in cotton over the physiological range of water potential occurring during the oscillations.

Because of the difficulty of making steady-state measurements of wall resistances we resorted to peeling the epidermis from the leaf and examining the flux of water vapor from the mesophyll tissue (8). The results are relevant only to the degree that the mesophyll tissue is the site of evaporation in intact leaves (17).

The rates of evaporation and the resistances to diffusion of vapor from pieces of wet filter paper were measured in four separate chambers and then the filter paper was replaced by sections from turgid leaves of *T. gesneriana* with an epidermis removed. The results (Table I) indicated that there was no significant wall resistance in the mesophyll of turgid *T. gesneriana* leaves. We obtained similar results with *C. communis* leaves, as did Fischer (8) with *Allium porrum*.

We next examined the evaporation from mesophyll tissue over longer time intervals. We first measured the evaporation in the dark from wet filter paper with Parafilm over the upper surface. Leaving the Parafilm in place we then replaced the filter paper with a *C. communis* leaf with its abaxial (lower) epidermis removed and found that the evaporation from the stripped surface of these leaves declined with time dropping to as low as 10% of the initial rate (Fig. 5). Sometimes the rate of evaporation from the mesophyll appeared lower than that from a filter paper as soon as the leaf was inserted into the leaf chamber. The experiments were repeated with stripped tulip leaves and the evaporation rates also declined, with typically 30% reduction in 1 hr.

As the water potential, ψ, of a leaf falls, the vapor pressure, e, inside the leaf, if it is intact, declines as

$$e = e_0 \exp(\psi \bar{V}_w / RT) \quad (14)$$

where $\bar{V}_w$ is the molar volume of liquid water and e_0 is the saturation vapor pressure at the temperature. A reduction of water potential by one bar would cause a 0.07% reduction in vapor pressure. This is not enough to account for the observed reductions in the rate of evaporation from stripped *Commelina* leaves. For instance, after a 50-min evaporation from exposed mesophyll the water potential of the tissue had only declined to −35 bars, measured in a thermocouple psychrometer using the isopiestic technique (2), but the rate of evaporation had declined to 19% of its initial value. This decline was not caused by restriction of intercellular pathways since when both epidermes of a *Commelina* leaf were stripped the evaporation rates declined but the flux of helium across the leaf did not. The change in properties of the surface of the mesophyll of *Commelina* was irreversible over the

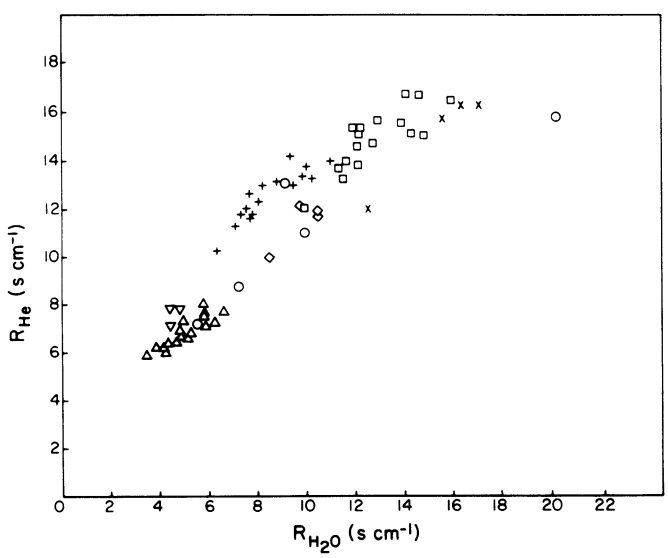

FIG. 4. Relationship between R_{He} and R_{H_2O} for seven different detached leaves of *G. hirsutum*, each of which is represented by a different symbol. R_{H_2O} is calculated from equation 12, assuming cuticular conductances of the upper and lower epidermes of 0.03 cm sec^{-1}.

Table I. Comparison of resistances to diffusion of vapor, from wet filter paper and from the mesophyll tissue of tulip in four leaf chambers

The temperature of the chambers was 23 C; the dewpoint of the air was 18.1 C; air flow 50 l/hr.

Chamber No.	Resistance to the diffusion of water vapor	
	filter paper	mesophyll
	sec cm^{-1}	
1	1.21	1.14
2	0.59	0.58
3	0.32	0.52
4	0.86	0.84

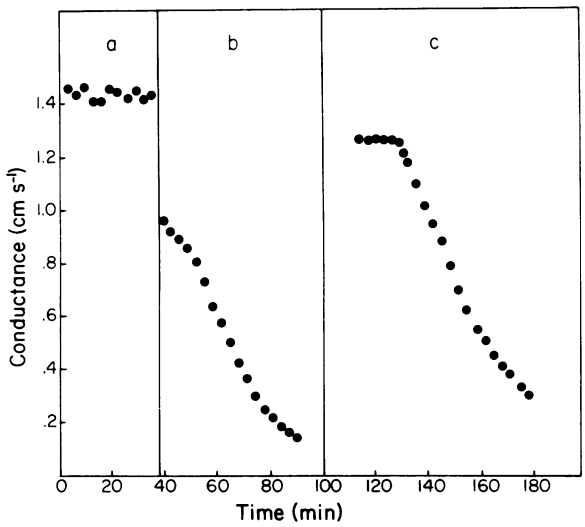

FIG. 5. Time course of the conductance to diffusion of water vapor from the lower surface of (a) a wet filter paper; (b) a *Commelina* leaf from which the lower epidermis had been removed, and (c) a second *Commelina* leaf as in (b). Upper epidermes were intact and in all cases Parafilm covered the upper surface. Air passing over the surfaces had a dew point of 18 C and a temperature of 23 C.

time scale in question. This became apparent in an experiment in which leaves were stripped and allowed to lose water by evaporation. After 35 min, Parafilm was placed on them for 150 min. When the Parafilm was removed again, the evaporation rates were lower than the initial ones. The cell walls were not "rewetted" from inside. These results appear to support the notion of an increased wall resistance in stressed leaves. However, when wet filter paper was replaced by leaves that were dried *prior* to stripping, no large immediate reduction in evaporation rate occurred (Table II). The phenomenon of declining evaporation from mesophyll after stripping may then be an artefact associated with removal of the epidermis, caused either by damage to the mesophyll or by a reduced capacity for hydraulic transport along epidermal cell walls (25).

CONCLUSION

Like Fischer (8), we saw no evidence for the presence of a wall resistance to transpiration under physiological conditions. Thus, we feel justified in continuing to assume that the water vapor pressure at the sites of evaporation in the leaf is equal to that over free water at the same temperature. There is no evidence that low leaf water potential, high rate of evaporation, or a variation in the intercellular CO_2 concentration makes the conventional determination of stomatal conductance and intercellular CO_2 concentration from transpiration rate and leaf temperature erroneous. However, we hesitate to include arid zone species in this generalization because we saw a decline in evaporation when epidermis was removed from leaves of *C. communis* and the mesophyll was fully exposed to a stream of air.

Table II. Comparison of measured resistances to diffusion of vapor from wet filter paper and from the mesophyll tissue of stressed leaves of Commelina communis

The dewpoint of the air was 18 C and the temperature of the leaf chambers was 23 C. The leaves were not illuminated. Water potentials were measured isopiestically after determination of the diffusion resistances.

Chamber	Filter paper	Mesophyll	
	Diffusion resistance	Diffusion resistance	Water potential
No.	sec cm^{-1}		bar
1	0.69	0.97	-26.7
2	0.67	0.76	-27.3

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