

Short Communication**Analysis of Operation and Calibration of a Ventilated Diffusion Porometer**

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Porometers, for the study of stomatal aperture, have taken various forms. Many were impossible or inconvenient to use in the field until the development of a portable, viscous flow porometer (1, 12). Because this porometer measures viscous, not diffusive, resistance of leaves and also measures the two epidermes in series, not in parallel, its utility is limited (12).

A portable porometer, which measures the rate of diffusion of water vapor from the interior of a leaf into a dry chamber, was first described by Wallihan (13); the porometer is applied to each side of an amphistomatous leaf, in turn, and treats the two epidermes as parallel resistors. Modification and calibration of the instrument was undertaken by Van Bavel, Nakayama and Ehrler (11) also by Kanemasu, Thurtell and Tanner (4). To reduce the effective instrument resistance and inaccuracies due to thermal streaming from the leaf, the air in the chamber was rapidly recirculated with a fan (5, 8, 9); however, the ventilation prevented calibration with the use of tubes of various lengths, as were used by Van Bavel *et al.* (11). While our manuscript was under review, a ventilated diffusion porometer for use with leaves of small area was described by Byrne, Rose and Slatyer (3). Their porometer was calibrated by determining the volume of saturated air introduced into the chamber to obtain the required change in meter reading.

The ventilated porometer has proved invaluable in measuring relative stomatal apertures (9, 10); we now present a calibration procedure which gives absolute resistances in the conventional units of seconds per centimeter and, moreover, gives us an understanding of the physical parameters that enter into the functioning of the apparatus. Further, we show that temperature has a marked effect on the calibration of the porometer.

INSTRUMENT DESIGN

The porometer comprises two units: an acrylic chamber which can be clamped onto a leaf (Fig. 1), and a meter to record the changes in humidity. Inasmuch as the detailed construction and operation of the instrument has been described elsewhere (9), only the essential details are repeated here.

The acrylic chamber, which has a volume of 32 cm³, contains a lithium chloride humidity sensor (Hygrosensor 4-4832, Hygro-dynamics, Inc., Silver Spring, Md.), a fan (Ideal Toy Corp., Hollis, N.Y.) which circulates the air at approximately 85 cm sec⁻¹, and a plunger (Fig. 1, C) containing silica gel which can be drawn into the circuit to dry the air in the chamber. When a leaf is clamped between the two pieces of closed-cell sponge rubber (Fig. 1, G), 2.85 cm² of one epidermis is exposed to the chamber; alternatively, a bunch of pine needles can be inserted into the chamber through a portal by removing plug H (Fig. 1).

A circuit, basically similar to that described by Kanemasu *et al.* (4), and two 6.75 v mercury batteries, which are housed in the meter, generate the stable a-c supply required by the humidity sensor. The changes in electrical resistance produced by the changes in humidity within the chamber are then observed on a 0- to 10- μ amp meter (9).

In practice, the chamber is dried until the ammeter indicates 2 μ amp, and then the leaf is inserted and the time required for the amperage to rise from 4 to 9 μ amp is measured; this corresponds to a change in relative humidity from 20.5 to 28.8% at 28 C. When the stomata are closed sufficiently for the time to exceed 1 min, the time required for a smaller change in amperage, e.g., 4 to 8 μ amp, is measured. This decreases the time to less than 1 min, preventing stomatal closure during observation. Finally, the air temperature in the chamber is observed.

THEORY AND CALIBRATION

The diffusion rate of water vapor from within the leaf into the porometer is a function of the area of leaf exposed to the chamber (A), the volume of the chamber (V), the resistance to diffusion (r), and the change in concentration ($C_\infty - C$) between the saturation concentration at the given temperature and the concentration in the chamber, as given in the equation:

$$\frac{dC}{dt} = \frac{A(C_\infty - C)}{rV} \quad 1$$

The resistance (r) is the sum of the stomatal resistance (r_s) and an additional resistance, the porometer resistance (r_p), which includes the boundary layer resistance (r_a). Because of the rapid recirculation and mixing by the fan, the concentration (C) within the chamber is uniform.

Our procedure will be to evaluate theoretically the boundary layer resistance and to compare it to the measured porometer resistance. We shall then observe the effect of adsorption of water vapor by the sensor, and finally calculate the stomatal resistance. We shall then test our calculated resistances with perforated plates of varying resistances and finally show how temperature affects our calibration. The determination of stomatal resistance is our goal because it regulates both transpiration and photosynthesis.

Boundary Layer Resistance (r_a). The porometer can be considered as approximating a cylinder of radius R with a diffusion sector of area A . Assuming fully developed Poiseuille flow, the boundary layer resistance (r_a) can be shown to be given by the equation

$$Dr_a \approx 1.1 \left(\frac{DR\rho}{U} \right)^{1/2} \quad 2$$

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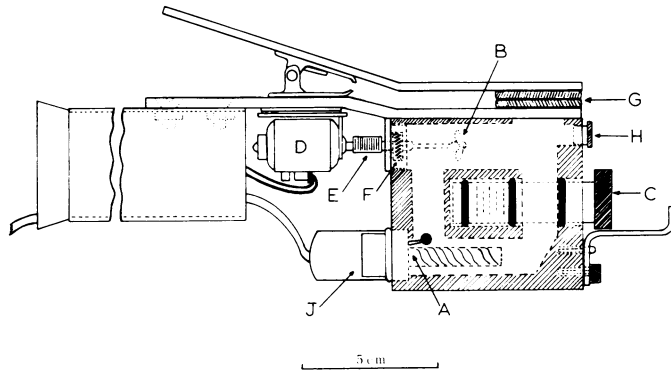


FIG. 1. Side view of porometer. A: Humidity sensor and bead thermistor; B: fan; C: plunger containing desiccant; D: motor; E: rubber coupling; F: sealed bearing; G: closed-cell sponge rubber; H: port; J: electrical connector.

where U is the maximal velocity in the center of the cylinder, D is the diffusivity of water vapor in air and ρ is the radius of the diffusion sector, *i.e.*, $A = \pi\rho^2$

For the present porometer $\rho = 0.96$ cm, $R \simeq 0.9$ cm, $U = 85$ cm sec⁻¹, and $D = 0.270$ cm² sec⁻¹ at 28 C; therefore, the boundary layer resistance (r_a) is approximately 0.6 sec cm⁻¹ at 28 C.

Chamber Capacity (V). As has been noted previously (Equation 1), the variation in time of the concentration in the chamber is governed by:

$$V \frac{dC}{dt} = A \frac{(C_\infty - C)}{r_s + r_p}$$

The chamber capacity (V) is composed of two parts; the first is the volume of the chamber itself ($V_c = 32$ cm³), and the second is the effective capacity of the sensor (V_s) as it adsorbs water. A third component of the chamber capacity, *viz.*, the adsorption and absorption of water by the walls of the acrylic chamber, can be safely neglected. Both adsorption and absorption can be estimated and shown to contribute less than 1% of the total effective volume. Furthermore, when the chamber walls were coated with a hydrophobic grease, no significant change in the measured times was observed.

The effective capacity of the sensor (V_s) should be a function of C , and therefore, we can define an average sensor capacity (\bar{V}_s) by:

$$\int_{C_0}^C \frac{V_s d\alpha}{C_\infty - \alpha} = -\bar{V}_s \ln \left[\frac{(C_\infty - C)}{(C_\infty - C_0)} \right] \quad 3$$

where α , the variable of integration, varies from C_0 , the concentration in the chamber at $t = 0$, to C , the concentration at t , and \bar{V}_s may be a function of C . From Equations 1 and 3 we derive:

$$\ln \left[\frac{(C_\infty - C_0)}{(C_\infty - C)} \right] = \frac{At}{(r_s + r_p)(\bar{V}_s + V_c)} \quad 4$$

If an additional half sensor is placed in the porometer (insufficient room exists for a whole sensor), \bar{V}_s becomes $1.5 \bar{V}_s$ and the time t becomes t' . By dividing Equation 4 into the same equation with $1.5 \bar{V}_s$ and t' , we eliminate r_s and r_p and obtain a convenient way of measuring \bar{V}_s :

$$\frac{t'}{t} = \frac{(1.5 \bar{V}_s + V_c)}{(\bar{V}_s + V_c)} \quad 5$$

A sensor similar to the one in the porometer was cut in half, and the ratio $t':t$ was then measured for each half sensor. A mean ratio of 1.3434 ± 0.0124 was obtained; the corresponding \bar{V}_s

is 74 ± 5 cm³, a volume considerably larger than that of the chamber ($V_c = 32$ cm³). Since small variations in $t':t$ lead to large changes in \bar{V}_s , the coefficient of variation of \bar{V}_s was 34%; this is an intrinsic error of the apparatus inasmuch as we did not need to introduce any external sources of error to obtain \bar{V}_s . The ratio of $t':t$ did not differ when measurements were made over different portions of the amperage scale, indicating that $t':t$ and \bar{V}_s were independent of the concentration (C), within the precision of the instrument.

Now that the volumes are known we can compare the porometer resistance (r_p) with the theoretical boundary layer resistance (r_a) when the stomatal resistance (r_s) is zero. By using saturated filter paper in the diffusion sector, the time for an amperage change from 4 to 9 μ amp was 4.14 ± 0.04 sec at 28 C. The r_p was, therefore, 1.05 ± 0.10 sec cm⁻¹; this is larger than the theoretical r_a of 0.60, but was expected because the filter paper is not directly in the line of flow and the aperture is near a corner. However, the close agreement between the calculated r_a and measured r_p shows us that r_p is largely comprised of r_a and gives us confidence that we have not omitted any basic component in the physical interpretation of r_p .

Stomatal Resistance (r_s). Since all other parameters are now known, we can calculate the stomatal resistance from the lapse times by means of Equation 4.

The observed r_s can be checked and all of our derivations confirmed if the resistance of a perforated plate is observed to be the same as that calculated from the dimensions of the plate. We used the plates described by Lee (6) to check the calibration. The resistance of the plates was calculated from the Brown and Escombe expression (2), which was recently validated by a 3-dimensional mathematical analysis (7). Since the source of water, wet filter paper, was placed a small distance (h) behind the perforated plate, an additional resistance, h/D , is involved. The resistance is given by:

$$r_s = \left[h + \frac{1}{n} \left(\frac{d}{\pi a^2} + \frac{1}{2a} \right) \right] / D \quad 6$$

where n is the number of holes, a is their radius, and d is the thickness of the plate. The first plate had 720 holes per cm², each hole having a depth (d) of 7.5×10^{-3} cm, and a radius of 0.0115 cm; the time for a 4 to 9 μ amp change in amperage was 9.07 ± 0.06 sec. According to Equation 4, this is equivalent to a stomatal resistance of 1.25 ± 0.20 sec cm⁻¹ at 28 C. The calculated resistance from Equation 6 was 1.49 sec cm⁻¹. Confirmation of the calibration was obtained with 10 other plates.

Effect of Temperature. All observations thus far were for a temperature of 28 C. We now need to establish how the calibration varies with temperature. First, it should be noted that the relative humidity within the chamber will vary with temperature; hence, the range of humidities over which the lapse times are observed will also vary (the manufacturer of the sensor supplies this information). We also found that both r_p and \bar{V}_s are dependent on temperature as shown in Table I; r_p varied with temperature as $D^{-2/3}$ which is the variation predicted by Equation 2. Now, therefore, the relationship between the observed lapse time and stomatal resistance for any temperature can be obtained; this relationship is shown in Fig. 2.

Table I. Effect of Temperature on the Porometer Resistance (r_p), and Effective Sensor Capacity (\bar{V}_s)

Temperature	15 C	20 C	25 C	30 C	35 C
r_p	1.14	1.10	1.07	1.04	1.01
\bar{V}_s	137	110	83	56	29

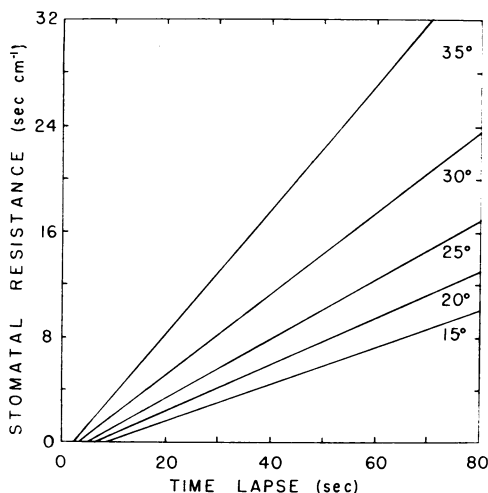


FIG. 2. Effect of temperature on the relationship between stomatal resistance (sec cm^{-1}) and lapse time (sec) for a ventilated diffusion porometer. Lapse times only apply for a 4 to 9 μamp change in amperage.

Below 10 C the mercury cells become very inefficient, causing the fan speed to decrease; even at 15 C, prolonged use will result in a decline in fan speed. We avoided this difficulty by using a d-c power source during calibrations.

CONCLUSIONS AND SUMMARY

A ventilated diffusion porometer has been calibrated to give a measure of the stomatal resistance. Our calibration, which was theoretically determined, introduced no additional errors into the system. The close agreement between the predicted boundary layer resistance (r_a) and empirically determined porometer resistance (r_p), and the agreement between the stomatal resistance (r_s) obtained from the theoretical calibration and that obtained experimentally with the perforated plates, show us that we understand the physical parameters that enter into the functioning of the instrument. Moreover, it gives us confidence in the calibration and illustrates the value of the porometer as an accurate instru-

ment for measuring stomatal resistance. The calibration showed that the r_p was largely governed by r_a and revealed that the lithium chloride sensor has a large capacity to adsorb water. Rearranging Equation 4, we see that for a given temperature, when the porometer is clamped onto a leaf, the stomatal resistance (r_s) is linearly related to the time (t) for a fixed increase in humidity.

$$r_s = At / \ln \left[\frac{(C_\infty - C_0)}{(C_\infty - C)} \right] (\bar{V}_s + V_c) - r_p$$

Furthermore, a wide range of stomatal resistances can quickly be measured by adjusting the humidity range over which the changes are timed. A Fortran program, "PORO," uses this calibration to convert lapse times to stomatal resistance.

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