Why Is the Substomatal Chamber as Large as It Is?

Received for publication April 13, 1981 and in revised form December 9, 1981

William F. Pickard
Department of Electrical Engineering, Washington University, Saint Louis, Missouri 63130

Abstract

The rate of CO2 uptake by the mesophyll is examined as a function of the size of the substomatal chamber. Using the techniques of classical electric circuit analysis and a model in which the uptake is linear in the ambient CO2 concentration, it is shown that the optimal chamber radius is several times larger than the pore radius. This is somewhat larger than necessary for the reduction of transpirational water loss, and it offers an explanation for the otherwise inexplicably large size of the chamber.

In papers of a biophysical or biomathematical nature (e.g., Refs. 13 and 18), the substomatal chamber is frequently represented by a hemisphere or hemielipsoid (the bounding mesophyll) set upon an equatorial plane (the epidermis) with a circular or elliptical hole (the stomatal pore) at its origin; the hemispherical case is shown in Figure 1. In these representations, the characteristic dimensions of the chamber are usually shown as being much larger than the width of the pore; that is, for Figure 1, $\beta \gg \alpha$. This difference in scale between chamber and pore is not as pronounced in more anatomically attuned representations (3, 6, 12, 15, 16), but it is still pronounced. Inasmuch as the substomatal chamber represents apparently unused volume which could otherwise be occupied by photosynthetic tissue, we may ask what benefits, if any, a plant might derive from the elaboration of a chamber. Bearing in mind the principal functions of a stoma, two such benefits can be suggested: first, it might lead to decreased water loss; second, it might lead to increased CO2 uptake.

Water loss from five different mathematical models of the chamber was recently considered by Pickard (20), who showed that increasing chamber size did indeed reduce water loss but that, once the chamber dimension exceeded twice the pore width, little further decrease could be obtained by additional increases in the chamber size. Much the same conclusion can be inferred from the numerical studies of Tyree and Yianouli (25) on peristomatal evaporation. Thus, while a certain amount of chamber volume is useful in lowering transpirational loss, it would be difficult on the basis of water relations, alone, to account for the large size of the typical chamber.

Therefore, it seems reasonable to advance as an explanation of chamber size the hypothesis that increasing the chamber dimensions leads somehow to an increase in total CO2 uptake, despite the fact that these size increases can be accomplished only by reducing the quantity of photosynthetic tissue within the leaf. It will be shown below that this initially counterintuitive conclusion can, in fact, be accorded a fair degree of theoretical substantiation.

This consideration of the optimization of stomatal morphology should be viewed as complementary to recent works on the optimization of stomatal behavior (e.g., Ref. 7) to minimize transpirational loss while achieving desired CO2 uptake.

Lumped Circuit Model

The technique used in treating this problem is that of the classical resistive network analogy in which each portion of the stomatal complex will have its diffusion resistance for CO2 separately evaluated, and the total CO2 flux will then be estimated by the methods of lumped network analysis. This well-known procedure dates back at least as far as the classical studies of Brown and Escombe (4) and, over the years, has been applied successfully to a wide variety of mass transfer problems (cf. Ref. 5) and has been shown to be relatively robust (cf. Ref. 19). It yields qualitatively correct predictions under almost all circumstances and quantitatively correct ones if the transition zones between regions being lumped are geometrically small compared to the regions themselves.

Within the most commonly considered $C_3$ leaves, the primary photosynthetic tissue is the mesophyll, and the plastids of epidermal cells tend to be deficient in Chl. Therefore, it will be assumed that the principal path of CO2 flux is radially outward from the pore to the mesophyll; that this flux displays no significant angular dependence; and that the several resistances (or conductances) which depend upon chamber size can be evaluated from a suitable steady-state diffusion equation in which $r$ is the only independent variable.1

Let $J$ (mol/s) be the inward flux of CO2, $c_o$ (mol/m3) the concentration of CO2 in the ambient atmosphere, $c_m$ (mol/m3) the concentration of CO2 over the hemisphere $r = \alpha$, and $c_p$ (mol/m3) the concentration of CO2 over the hemisphere $r = \beta$. Then, by conservation of matter, (i.e., Kirchhoff's current law):

$$J = (c_o - c_m)/R_p = (c_o - c_p)/R_e = (c_p)/R_m$$

then, $J = (c_o - c_m)/R_p = (c_o - c_p)/R_e = (c_p)/R_m$ eqn 1

where the $R$ values (s/m) are the diffusion resistance of external atmosphere and pore up to $r = \alpha$ (subscript $p$) or of the chamber from $r = \alpha$ to $r = \beta$ (subscript $c$) or of the mesophyll (subscript $m$).

The first two relations of eqn 1 are Ohm's law analogs for diffusive mass transport in regions where no assimilation is occurring (cf. Ref. 19). The third relation of eqn 1, which seems to assert that the mesophyll-chamber boundary is supplying a drain of zero concentration, is, in fact, the result of distributed assimilation within the mesophyll and will be justified later in connection with eqn 11.

Eqn 1 can be solved for $c_p$ and $c_m$, and the net inward flux is shown to be:

$$J = \frac{c_o}{R_p + R_c + R_m}$$

Since $R_p$ will be independent of the chamber size $\beta$, the inward flux can be maximized merely by varying $\beta$ until a minimum of the sum ($R_p + R_m$) is achieved.

Steady-state diffusion within the chamber can be described

1 The units of the radii $\alpha$, $\beta$, and $\gamma$, are meters. The units of the other principal quantities will be indicated inside parentheses immediately after the symbols for the quantities are introduced.
The solution of eqn 3, subject to the prescribed boundary conditions, is:
\[ c = (c_a - c_p) \frac{\alpha \beta}{\beta - \alpha} + \frac{\beta c_p - a c_a}{\beta - \alpha}, \alpha \leq r \leq \beta \] eqn 4

However, the total inward flux over any radial hemisphere is:
\[ (2\pi r^2) \left( -D_c \frac{dc}{dr} \right) \]
which, by eqn 4, yields:
\[ J = 2\pi D_c - \frac{\alpha \beta}{\beta - \alpha} (c_a - c_p) \] eqn 5

where \( D_c \) (m²/s) is the diffusion coefficient for \( CO_2 \) in the chamber. And, by eqn 1 and eqn 5:
\[ R_e = \frac{\beta - \alpha}{\alpha \beta} \frac{1}{2\pi D_c} \] eqn 6

Clearly, \( R_e \) is monotone-increasing in \( \beta \).

Diffusion within the mesophyll is a more complicated matter, since the effects of path tortuosity within the intercellular spaces and of carbon fixation by the chloroplasts should also be taken into account. However, diffusion in a porous medium is often treated (2, 8) by assuming that the medium can be described simply by an effective diffusion coefficient. And, if the medium is, in addition, assimilating the diffusing substance at a rate \( f(c) \) (mol/m³-s), the governing equation becomes simply (cf. Ref. 7) the Poisson relationship:
\[ D_m \frac{1}{r^2} \frac{\partial}{\partial r} \left( r^2 \frac{dc}{dr} \right) - f(c) = 0 \] eqn 7

where \( D_m \) (m²/s) is the effective diffusion coefficient of the mesophyll and \( c(\beta) = c_p \). One additional boundary condition is needed to obtain a unique solution of eqn 7; it is most conveniently taken to be that, at some radius \( r = \gamma > \beta \), the zone of influence of the stomata under consideration has a boundary with the zones of the stomata adjacent to it and that no \( CO_2 \) diffuses across this boundary. That is:
\[ \frac{dc}{dr} = 0, r = \gamma \] eqn 8

The form of \( f(c) \) is less certain in view of the well-known saturation kinetics of \( CO_2 \) uptake (11, 21, 22) and the existence of metabolic \( CO_2 \) release which leads to compensation (21). However, there is a fair amount of evidence (9, 17, 21) to indicate that it should be roughly linear at physiological \( CO_2 \) concentrations, especially at high light intensities (11). Because of this and for the sake of mathematical simplicity, it will be assumed (cf. Ref. 9) that, in bright light,
\[ f(c) = \kappa^2 D_m c \] eqn 9

where \( \kappa^2 (m^{-2}) \) is a constant which is independent of the irradiance.

The solution of eqn 7 subject to these boundary conditions is:
\[ c = \beta c_p e^{iy - \gamma (i\gamma - 1)} + e^{-i\gamma + \gamma (i\gamma + 1)} \]
\[ \frac{r}{r} e^{iy - \gamma (i\gamma - 1)} + e^{-i\gamma + \gamma (i\gamma + 1)} \] eqn 10

The inward flux across \( r = \beta \) is then:
\[ 2\pi \beta \left[ -D_m \frac{dc}{dr} \right] \]
\[ J = 2\pi \beta c_p \frac{e^{iy - \gamma (i\gamma - 1)} - e^{-i\gamma + \gamma (i\gamma + 1)}}{e^{iy - \gamma (i\gamma - 1)} + e^{-i\gamma + \gamma (i\gamma + 1)}} \] eqn 11

which justifies the third part of eqn 1. Thus, by eqn 1 and eqn 11, the mesophyll conductance becomes:
\[ R_m = \frac{1}{2\pi \beta D_m} \left[ 1 + \kappa \frac{\tan h(\gamma - \beta) - 1}{\gamma - \tan h(\gamma - \beta)} \right] \] eqn 12

By differentiating eqn 12 with respect to \( \kappa \), it is readily shown that \( R_m (\kappa) \) is monotonically decreasing.

**MAXIMIZING THE RATE OF PHOTOSYNTHESIS**

Since there is no obvious reason that plants could not have evolved larger stomatal pores, it would seem permissible to assume that \( (R_e + R_s) \), the net diffusion resistance of the open stoma and its chamber, is small compared to \( R_m \), the effective diffusion resistance of the mesophyll. In fact, experimental studies show that, as the stomata open fully, the net photosynthetic \( CO_2 \) uptake \( F \) (mol/s) of leaves does tend to approach from beneath an asymptotic level (23). It should be observed that the situation \( R_m \gg (R_e + R_s) \) does, by eqn 1 and eqn 2, imply not only that \( J \) is a function of chamber size but also that \( c_p \) and \( c_a \) are sensibly independent of chamber size. This possibly surprising conclusion is, in fact, a reasonable consequence of making mesophyll transport the rate-limiting step in \( CO_2 \) uptake. 

\( (R_e + R_s) \) can be approximated by noting that \( R_s < 1/2\pi a D_c \) and that, for a pore of length \( \lambda \) (m), \( R_e \) cannot markedly exceed the resistance between a hemispherical cap and infinity \( (1/2\pi a D_c) \) plus the resistance of a tube with hemispherical caps at its ends \( (1 + 2\alpha)/\pi a D_c \); that is:
\[ R_p + R_e \approx \frac{1}{\pi a D_c} \left( 1 + \frac{\lambda + 2\alpha}{\alpha} \right) \] eqn 13

Since \( \lambda \) will seldom be more than \( 6\alpha \) (14), the condition that \( F \) be
Eqn 14 can, in turn, be converted from an inequality to an equality as

\[ \frac{9}{\pi \alpha D_c} \ll R_m \]  \hspace{1cm} \text{eqn 14}

where \( 0 < \epsilon \ll 1 \) is a dimensionless constant which remains to be estimated.

To compute an effective mesophyll resistance for use in both eqn 14, it is necessary to know typical values of \( \kappa \beta \) and \( \kappa \gamma \), and these can be estimated as follows. Suppose that the volume \( V \) (m\(^3\)) of a typical C\(_3\) leaf can be divided into hemispherical regions centered about its stomata, so that there are in all \( V/(2/3 \pi \gamma^2) \) stomata; since \( F = J V/(2/3 \pi \gamma^2) \) and eqn 2, eqn 11, and eqn 14 imply \( c_\beta \approx c_\alpha \), then

\[ \frac{F}{V k^2 D_m c_\alpha} = \frac{\beta}{\gamma} \left[ \frac{3}{\gamma (\kappa \gamma)^3} \left( 1 + \kappa \beta \frac{\kappa \gamma \tanh(\gamma - \beta)}{\kappa \gamma - \tanh(\gamma - \beta)} \right) \right] \]  \hspace{1cm} \text{eqn 15}

A contour diagram of this normalized flux is given in Figure 2.

Further, series expansion about \( \kappa \gamma = 0 \) indicates that, for a given fixed \( \kappa \gamma \), the maximum normalized flux occurs when \( \beta/\gamma \) has been optimized to:

\[ \left( \frac{\beta}{\gamma} \right)_{\text{opt}} = \sqrt{\frac{\kappa \gamma}{3}} \left[ 1 + c(\kappa \gamma) \right] \]  \hspace{1cm} \text{eqn 16}

It seems clear from Figure 2 that evolving a relatively small value of \( \kappa \gamma \) is advantageous to a species. However, eqn 15 implies that, for any fixed values of \( \kappa \gamma \) and \( \beta/\gamma \), it is also advantageous to evolve large values of \( \kappa \). Therefore, evolutionary development should favor the reduction of \( \gamma \) until either the marginal gain in normalized flux becomes small or \( \gamma \) becomes small compared to leaf thickness. In the first instance, further reductions will supply little additional CO\(_2\) but, by opening additional pores in the leaf's surface, should aggravate water loss. In the second instance, the hemispherical region assumption underlying eqn 15 will fail.

For a given plant, \( \beta/\gamma \) is fixed; presumably, \( (\kappa \gamma)_{\text{max}} \) will have been set so that the plant always operates within a contour of relatively high normalized flux and moves toward even higher contours with decreasing illumination. If (as will be necessary to operate within a high contour) both \( \beta/\gamma \ll 1 \) and \( \kappa \gamma \ll 1 \), then

\[ R_m = \frac{1}{2 \pi \beta D_m (\kappa \gamma)^2} \left[ \frac{\beta}{\gamma} \left( 1 - \frac{\beta}{\gamma} \right) + c(\kappa \gamma) \right] \]  \hspace{1cm} \text{eqn 17}

Thus, if equations 14 are taken to apply, it follows that

\[ \frac{\beta}{\alpha} \approx \frac{\epsilon D_c}{6 D_m} \left( \frac{\beta(\gamma)}{\gamma} \right) \left( \frac{\gamma}{\kappa \gamma} \right)^2 \]  \hspace{1cm} \text{eqn 18}

However, near optimal conditions, \( \beta/\gamma \) and \( \kappa \gamma \) are related by eqn 16, so that

\[ \frac{\beta}{\alpha} \approx \frac{\epsilon D_c}{54 D_m} (\beta/\gamma)^3 \]  \hspace{1cm} \text{eqn 19}

It would appear that values of neither \( D_c/D_m \) nor \( \beta/\gamma \) are available in the literature, and \( \epsilon \) is unknown. Fortunately, all can be estimated. For the diffusivity ratio (cf. Refs. 2 and 8), \( D_c/D_m \approx (\text{fraction of hemispherical region about the substomatal chamber which is air space}) \); as such, it is probably less than \( 1/4 \) and will here be taken to be \( 1/6 \). For \( \beta/\gamma \) an equally crude approximation can be found as follows. Inasmuch as the entire guard cell complex is often significantly smaller than an epidermal cell, \( 2\beta \) may be as small as one-half of an epidermal cell characteristic dimension. The interstomatal spacing (i.e., \( \gamma \)) is clearly several epidermal cells in extent. Thus, \( \beta/\gamma \) ought not to exceed \( 1/4 \) and will here be taken to be \( 1/6 \). \( \epsilon \) must be small but not too small, for reducing \( \epsilon \) necessarily increases \( \alpha \) and the transpirational water loss; it will here be taken to be \( 1/6 \). Thus, eqn 19 implies \( \beta/\alpha \approx 4 \).

The import of these calculations is that the experimentally observed relationships \( \beta/\gamma \ll 1 \) and \( \beta/\alpha \gg 1 \) can be explained as an evolutionary adaptation to maximize photosynthetic carbon fixation while minimizing transpirational water loss.

**DISCUSSION**

The development of the previous section can certainly be questioned on a number of grounds, such as the linear uptake model embodied in eqn 9, the hemispherical geometry used, and the values selected for \( \beta/\gamma \), \( \kappa \gamma \), and \( D_c/D_m \) and \( \epsilon \).

First, despite the observation that CO\(_2\) uptake can saturate at \( c_\alpha \) is raised, it does appear that, at physiologically realistic CO\(_2\) levels and under sufficiently high levels of illumination, the uptake should increase with \( c_\alpha \) and be at least quasilinear (9, 11, 17) in it. Moreover, the only form of increase with \( c_\alpha \), which could reasonably have been treated analytically was the linear one selected.

Second, to model as hemispheres the substomatal chamber and the region of parenchyma to which it feeds CO\(_2\) is obviously a considerable idealization. However, there appears to be no other geometry which is any less obviously a departure from complex reality. And, once again, the analytic advantages of the choice are large; even the choice of a cylindrical geometry would have complicated the development egregiously.

Third, the parameter values selected were, indeed, somewhat arbitrary. \( \beta/\gamma \) is small; but just how small is not clear, especially since the substomatal chamber and its surrounding are not ideal hemispheres to which rays can conveniently be assigned. Presumably, however, this ratio will lie somewhere between \( 1/10 \) (yields \( \beta/\alpha \gg 1 \)) and \( 1/4 \) (yields \( \beta/\alpha \approx 1 \)); \( 1/6 \) seemed a reasonable compromise. \( \kappa \gamma \) should be small. But, given the admittedly approximate nature of the model, inferences by way of eqn 16 from an 'observed' value of \( \beta/\gamma \) to a precise value of \( \kappa \gamma \) are unwarranted; the value chosen could be off by as much as a factor of two. Similarly, there is no good way of arriving at a value for \( D_c/D_m \), since it will vary strongly with the local structure of the parenchyma; the value of \( 1/6 \) was picked merely because it seemed plausible for a fairly dense tissue with high carbon fixation per
unit volume. And, while 1/6 is suitably much less than one in an applied mathematics sense, its choice for $\epsilon$ was likewise arbitrary.

Given these uncertainties, what then has the theory of this paper demonstrated? It has shown that there are grounds for believing that the size of the substomatal chamber is an adaptation to promote CO$_2$ uptake. Virtually any variation on the specific model employed here would yield a net CO$_2$ uptake which peaks for some intermediate value of $\beta/\gamma$ (or its homolog), since $\beta/\gamma \rightarrow 0$ eliminates the stoma, and $\beta/\gamma \rightarrow 1$ eliminates the photosynthetic tissue. Virtually any model would require $\kappa$ (or its homolog) to be small, since, otherwise (cf. eqn (1)), there will be CO$_2$-starved regions in the parenchyma. And virtually any model with a dense parenchyma would force $\beta/\alpha$ (or its homolog) to a large value, since $\beta/\alpha \gg 1$ is a consequence of $D_1/D_s < 1$ and $\kappa < 1$. That is, while almost any individual detail of this development can be faulted in one fashion or another, the overall trends which the development reveals seem inescapable. And, since the reduction of transpirational loss appears not to be a tenable explanation for large chamber size (20), the CO$_2$ uptake hypothesis must be provisionally accepted in the absence of any other serious contender.

The nature of the water loss problem confronted by land plants as they evolved their leaves has been stated by Raschke (22): 'Land plants are in a dilemma throughout their lives: assimilation of CO$_2$ from the atmosphere requires intensive gas exchange; the prevention of excessive water loss demands that gas exchange be kept low.' They solved this problem by developing a relatively impermeable cuticle punctured by numerous stomatal pores which could be opened or shut as the need arose. But, in so doing, they raised the additional problem of designing the stoma to minimize water loss while maximizing CO$_2$ uptake. And this they apparently solved by elaborating a large substomatal chamber, as explained above. However, it is of interest to inquire as to whether the plant somehow could not have developed a cuticle which is impermeable to water but permeable to CO$_2$ and, thereby, have saved itself the costs of stomatal generation and operation.

Much the same question was asked by Raschke (22), who noted that: 'The plant's dilemma could be solved if a substance existed whose permeability for CO$_2$ was several times greater than that for water vapor. Such a material has not appeared during the hundreds of million years of plant evolution, and neither has man been able to synthesize such a material.' One can, of course, quibble over the precise ratio of permeabilities which would make a solid cuticle desirable, but his point is well taken. And it is reasonable to ask whether such a material is, in principle, possible. The answer very probably is 'no.' For, while it is not yet possible to predict exactly and in advance the permeability of a given film to a given gas, there is a crude rule of thumb known as the Stannett-Szwarc relation (24), which states that the ratio of the permeability of gas-A to that of gas-B will be constant to within perhaps a factor of three, independent of the material constituting the polymer film. While ratios which involve noble gases (1, 10) or water vapor (1) are known to vary by a much larger factor, for the substances thus far studied (cf. Ref. 1), the permeability to CO$_2$ has always been consistently less than that to H$_2$O. This, of course, demonstrates nothing rigorously; however, it does strongly suggest not only that it proved impossible to evolve a film of the desired properties when plants colonized the land but also that it is highly unlikely one could ever evolve.

**CONCLUSIONS**

The two principal functions of the stoma, reduction of water loss and promotion of CO$_2$ uptake by the parenchyma, are both served by the existence of a substomatal chamber. To minimize the water vapor efflux, the chamber's characteristic dimension should be at least twice the pore width (20, 25). To maximize CO$_2$ influx, the ratio of chamber size to pore size should be somewhat larger.

**LITERATURE CITED**

2. ARMSTRONG W 1979 Aeration in higher plants. Adv Bot Res 7: 225–332
7. FAULANCE GD, ED SCHULZE, F KÜPPERS 1980 Responses to humidity by stomata of Nicotiana glauca L. and Corylus avellana L. are consistent with the optimization of carbon dioxide uptake with respect to water loss. Aust J Plant Physiol 7: 315–327