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Ann. Rev. Plant Physiol. 1982. 33:317–45

STOMATAL CONDUCTANCE AND PHOTOSYNTHESIS

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0066-4294/82/0601-0317$02.00

INTRODUCTION

Our objective is to examine the integration of stomatal functioning with leaf metabolism and leaf environment and to indicate how this integration helps optimize the use of water and the use of the machinery for photosynthesis, while only marginally limiting the photosynthetic process.

Reviewing the literature we find that the three following statements are often taken as axiomatic: 1. Stomata impose a large limitation on the rate of CO_2 assimilation. 2. The stomatal limitation is more severe when a plant is stressed than when it is not. 3. Stomata limit CO_2 assimilation of C_4 species more than that of C_3 species. We find little experimental support for these ideas.

We discuss how known mechanisms of stomatal response contribute to the integrated functioning outlined in the first sentence. This section is necessarily brief, and for more thorough treatments of such topics as ionic relations, biochemistry, and elastic properties of guard cells, the interested reader is referred to other reviews (40, 84).

HOW STOMATA AFFECT PHOTOSYNTHESIS

Introduction

Stomatal movements provide the leaf with the opportunity to change both the partial pressure of CO_2 at the sites of carboxylation [carboxylase $p(CO_2)$] and the rate of transpiration. In turn, changes in transpiration rate can cause changes in the temperature and water potential of the leaf. Fundamental to any examination of stomatal functioning is the understanding of how rate of assimilation of CO_2 responds to changes in the carboxylase $p(CO_2)$, leaf temperature, and transpiration rate.

Model of Leaf Photosynthesis

The nature of the above responses of rate of assimilation in C_3 and C_4 species depends on the factors that limit this rate. This often gives rise, for example, to a situation where the assimilation rate is more sensitive to light, temperature, and water status in one region [high $p(CO_2)$] than in another [low $p(CO_2)$] (98, 99). We believe that the nature of these responses is most easily described by reference to recent mechanistic models of photosynthesis (5, 28, 30, 34, 55, 77). We concentrate on the assimilation rate characteristics of C_3 species and for convenience refer to the model of Farquhar et al (30) as modified by Farquhar & von Caemmerer (28). We use their description of the nature of factors limiting assimilation rate under various conditions while recognizing the tentative nature of its biochemical basis. Nevertheless, we emphasize that there is a large amount of experimental data supporting the model (11, 98, 99; J. A. Berry and J. R. Seemann, unpublished).

RESPONSES OF ASSIMILATION RATE TO $p(CO_2)$ We now examine the modeled response of CO_2 assimilation rate to carboxylase $p(CO_2)$ as illustrated in Figure 1. At low $p(CO_2)$ the enzyme ribulose bisphosphate (RuP_2) carboxylase-oxygenase (Rubisco) is saturated with respect to the substrate RuP_2. The initial effect of increasing the $p(CO_2)$ is the activation of the enzyme. Subsequently, there is an almost linear response of assimilation rate to carboxylase $p(CO_2)$, C_c, the slope being proportional to the amount (maximum activity) of Rubisco in the leaf. In this region, assimilation rate A is approximated by

$$A = k(C_c - \Gamma) \qquad 1.$$

where Γ is the compensation point and k is the "carboxylation efficiency" (53). We have chosen not to call the slope the mesophyll conductance; we reserve that term for the conductance to diffusion in the mesophyll, as originally defined by Gaastra (32). At still higher $p(CO_2)$, if the rate of RuP_2 carboxylation is increased sufficiently, the capacity to regenerate RuP_2 becomes limiting. This capacity depends *inter alia* on the capacity for electron transport, which in turn depends on absorbed irradiance. The rates of electron transport, and of regeneration of ATP, then become independent of $p(CO_2)$. In this region the rates of regeneration of NADPH and of RuP_2 are also virtually independent of $p(CO_2)$. However, assimilation rate still increases somewhat with $p(CO_2)$ as RuP_2 is increasingly diverted from oxygenation to carboxylation.

RESPONSES OF ASSIMILATION RATE TO LEAF TEMPERATURE AND WATER POTENTIAL In the model, there is an optimum temperature for CO_2 assimilation which depends on $p(CO_2)$ and irradiance. Low temperatures reduce assimilation rate because of reduced activity of the Rubisco and of the capacity for electron transport. High temperatures also reduce electron transport capacity and increase the rates of CO_2 evolution from photorespiration and other sources, again causing assimilation rate to decline. Effects of reduced water potential are not considered in the model, but could be appropriately incorporated as a reduced capacity for RuP_2

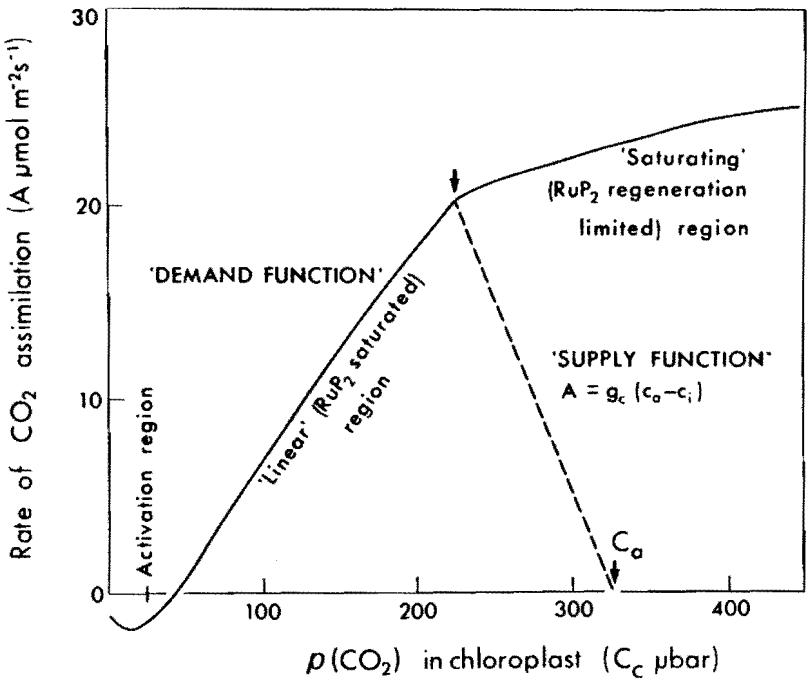

Figure 1 Rate of CO_2 assimilation versus CO_2 partial pressure in the chloroplast. The solid line indicates the "demand function," the dependence of rate of CO_2 assimilation on the partial pressure of CO_2 at the sites of carboxylation, according to the model of Farquhar et al (30), as recently modified (28). The dashed line indicates the "supply function," the equation describing the gaseous diffusion of CO_2 from the atmosphere to the intercellular spaces. The $p(CO_2)$ at the site of carboxylation is here assumed to be equal to the $p(CO_2)$ in the intercellular spaces. The transition from RuP_2 saturation to RuP_2 limitation is indicated by one arrow and the atmospheric $p(CO_2)$ by another.

regeneration (50, 98; T. D. Sharkey, M. R. Badger, and S. C. Wong, unpublished).

EXPERIMENTAL SUPPORT FOR THE MODEL Observed responses of assimilation rate, when it is "RuP_2 regeneration limited," to changes in $p(CO_2)$, leaf temperature, and water potential are different from those when it is "RuP_2 saturated" (11, 98, 99; J. A. Berry and J. R. Seemann, unpublished). Collatz (11) has shown that the concentrations of RuP_2 change in the predicted manner. Measurements of assimilation rate in the "RuP_2 saturated" region, and of the amounts and properties of Rubisco, have been shown to be consistent with the model (98, 99; J. A. Berry and J. R. Seemann, unpublished). Similarly, von Caemmerer (98, 99) has demonstrated the gas exchange measurements of assimilation rate in *Phaseolus*

vulgaris in the "RuP_2 limited" region are consistent with biochemical measurements of the capacity to regenerate RuP_2, generally limited or co-limited by the capacity for electron transport.

Stomatal Effects on Water Potential and Temperature

STOMATAL CONDUCTANCE Given that changes in leaf water potential, leaf temperature, and carboxylase $p(CO_2)$ can affect assimilation rate, we now discuss how these variables are affected by stomatal movements. As a measure of stomatal opening we use the conductance to diffusion, a parameter dominated by the areal density and apertures of stomata. Laisk et al (56) found that this lumped parameter may conceal significant variation in the properties of individual stomata, although Saxe (87) observed that individual stomata were well entrained.

EFFECTS ON LEAF WATER POTENTIAL Changes in stomatal conductance cause changes in leaf water potential by changing the transpiration rate. The reported effects of transpiration rate on leaf water potential are quite variable (76). Using wheat seedlings, Passioura (75) imposed changes in transpiration rate and found a linear response in the gradient of water potential between the soil solution and the stem system. The hydraulic resistance was such that a transpiration rate of 10 mmol $m^{-2}s^{-1}$ caused a drop in potential of 0.4 to 1 MPa. The water potential in the mesophyll was presumably even more negative, due to hydraulic resistances in the leaf.

Ion accumulation in the roots and cellular expansion in the leaves may complicate the relationship between water potential and transpiration rate (76). More information about this relationship is needed, especially when the hydraulic resistance in the plant is high, as may be the case when roots experience low temperature or anaerobiosis.

EFFECTS ON LEAF TEMPERATURE The dependence of leaf temperature on conductance is also via transpiration rate, but this time through the energy budget of the leaf (16, 49, 78). Although the physics of the energy budget are well understood, few laboratory studies compare actual and predicted changes in leaf temperature because of the difficulty in characterizing the nature of heat exchange. Particular attention should be paid to heat balance in assessing the effects of transpiration rate on assimilation rate at high temperatures and low wind speeds, as electron transport and photophosphorylation are sensitive at high temperatures. Advances in infrared thermometry may aid in this direction.

EFFECTS ON TRANSPIRATION RATE The dependence of transpiration rate E on conductance to diffusion of water vapor g is simply expressed using the new measure for conductance introduced by Cowan (13). This

measure (mol $m^{-2}s^{-1}$) has several features to recommend it (24, 35). Cowan related E, measured as a molar flux (mol $m^{-2}s^{-1}$), to g using

$$E = g\,(w_i - w_a) = g\,(e_i - e_a)/P \qquad 2.$$

where e_i and e_a are the vapor pressures of water inside the leaf and in the air, respectively, w_i and w_a are corresponding mole fractions of water vapor in air, and P is the atmospheric pressure.[1] The conductance can be subdivided into cuticular, stomatal, and boundary layer components in the standard manner (43).

Parkinson & Penman (74) have pointed out that transpiration causes convection effects in the stomatal pores. Equations describing these effects, rigorously derived by Jarman (42), have recently been combined with the new measure of conductance (99) to yield the following:

$$E = \frac{g\,(w_i - w_a)}{1 - (w_i + w_a)/2} = \frac{g\,(e_i - e_a)}{P - (e_i + e_a)/2} \qquad 3.$$

The vapor pressure e_i inside the leaf is usually taken to be the saturation pressure at the leaf temperature (26), but effects of reduced water potential can be incorporated simply (92). Thus the dependence of transpiration rate (and leaf temperature) on conductance is, in a mathematical sense, the simultaneous solution of Equation 3 and another equation representing the energy balance of the leaf.

Stomatal Effects on $p(CO_2)$ at Sites of Carboxylation

The dependence of carboxylase $p(CO_2)$ on conductance is conceptually similar to that of evaporation, but we deal with it in more detail. We first discuss the estimation of intercellular $p(CO_2)$, which is an important topic in its own right.

ESTIMATION OF INTERCELLULAR PARTIAL PRESSURE OF CO_2 Penman & Schofield (79) placed the diffusion of CO_2 and water vapor through stomata on a firm physical basis. Their notation has been supplanted, equivalent lengths becoming resistances and conductances and volume fluxes

[1]Mole or volume fractions are convenient to use when describing diffusion, but partial pressures are more appropriate when considering saturation vapor pressures and the effects of CO_2 and O_2 on Rubisco functioning. They are simply related via the atmospheric pressure, which unfortunately has an awkward value in the SI unit of pressure (Pascal).

becoming molar fluxes. Using contemporary notation, their equations are identical to those of Cowan (13), given by Equation 2 and the following:

$$A = g_c\,(c_a - c_i) = g_c\,(C_a - C_i)/P \qquad 4.$$

and so

$$c_i = c_a - A/g_c \qquad 5.$$

where C_a and C_i are the partial pressures of CO_2 in the air and inside the leaf, respectively, and c_a and c_i are the corresponding mole or volume fractions, and P is the total pressure. The conductance to diffusion of CO_2, g_c, was taken to be that to water vapor, divided by the ratio of the binary diffusivities of water vapor/air and CO_2/air. They took this factor to be 1.7, but the accepted value[2] is now 1.6 (1, 43). As the transfer processes change from molecular diffusion to turbulent mixing, the ratio of the conductances approaches 1 (84). The ratio of conductances to water vapor and CO_2 in the boundary layer is taken as 1.37, since the transfer coefficients in a laminar boundary layer are proportional to the ratio of binary diffusivities raised to the two-thirds power (93).

From measurements of the rain falling on, and dry matter accumulated by, a sugar beet crop, Penman & Schofield (79) estimated the $p(CO_2)$ inside the leaves to be 10–20% less than that in the atmosphere. Monteith (63) made micrometeorological measurements above bean crops and estimated a diurnal course of intercellular $p(CO_2)$, declining from about 250 μbar (25 Pa) in the morning to about 190 μbar at noon and increasing again in the afternoon. Gaastra (32) used the concept of c_i in several of his equations, but the first published values of c_i calculated from the gas exchange of single leaves appear to be those of Moss & Rawlins (66) in 1963.

Parkinson & Penman (74) pointed out that as water evaporates from the surfaces inside the leaf, mass flow out of the leaf occurs. This tends to carry some CO_2 molecules with it. The $p(CO_2)$ inside a leaf that has no net flux of CO_2, but a large transpiration rate, should be below the ambient $p(CO_2)$. In this situation, the amount of CO_2 carried out by the mass flow is balanced by the amount of CO_2 diffusing along the gradient thus created. Jarman (42) showed that this analysis was incorrect and treated the diffu-

[2]The ratio is often taken as 1.56 for the sole reason that this is the inverse ratio of the square root of the molecular weights. However, this is incorrect as the appropriate measures are binary diffusion coefficients CO_2/air and H_2O/air, the latter referring equally to the diffusion of air through water vapor and to water vapor through air. It is fortuitous that 1.56 is approximately correct.

sion of gases into and out of leaves as a ternary system involving the diffusion of CO_2, water vapor, and air. Fortuitously, the binary diffusion coefficient involving CO_2 and water vapor is approximately equal to that involving CO_2 and air, which simplifies the result to that derived by Parkinson and Penman. When the effect of transpiration on CO_2 flux is considered, assimilation rate is described by the following equation (99):

$$A = g_c (c_a - c_i) - \frac{(c_i + c_a)}{2} E \qquad 6.$$

Rearranging, the equation for c_i becomes

$$c_i = \frac{(g_c - E/2)\, c_a - A}{g_c + E/2} \qquad 7.$$

At normal CO_2 concentrations (340 μl l^{-1} in 1981) in a leaf with a transpiration rate of 5 mmol m^{-2} s^{-1}, an assimilation rate of 20 μmol m^{-2} s^{-1}, and a conductance to CO_2 of 0.2 mol m^{-2} s^{-1}, the effect of transpiration is to lower c_i by 7 μl l^{-1} below that calculated from Equation 5.

The intercellular CO_2 concentration so calculated, c_i, is actually the concentration at the sites of evaporation within the leaf. Based on experimental observations (61), an electrical analog of evaporation (13), and calculation (82, 96), it has been concluded that most evaporation takes place from sites in close proximity to the stomata. CO_2 has to diffuse further, through the intercellular air spaces, and this led Meidner (61) to suggest that the calculated c_i is an overestimate of the mean concentration around the mesophyll cells. However, the validity of the calculated c_i has been verified recently by Laisk (55) and by Sharkey et al (89a). The latter group used a double-sided gas exchange chamber with one side of the leaf for measurement of ordinary gas exchange from which C_i was calculated. The other side formed part of a closed loop in which a peristaltic pump pumped the air to a CO_2 gas analyzer and then back to the leaf. The $p(CO_2)$ in the air equilibrated with that inside the leaf and was generally 5 to 10 μbar below the calculated value for the other side of the leaf, as would be expected because of intercellular air space resistance (45). Under conditions where gas exchange occurs symmetrically through both sides, the difference in $p(CO_2)$ between the sites of evaporation and the center of the leaf would be about a quarter of the above.

RELATIONSHIP BETWEEN INTERCELLULAR AND CARBOXYLASE PARTIAL PRESSURE OF CO_2 Equation 7 in the previous section describes how stomatal conductance affects the intercellular mole fraction of CO_2, c_i. To determine how stomatal conductance affects the carboxylase $p(CO_2)$, C_c, we examine the relationship between C_i and C_c (c_i and c_c when expressed as mole fractions).

Gaastra (32) called the resistance to diffusion in the liquid phase (the cell wall, cytoplasm, and chloroplast stroma), the mesophyll resistance r_m. It is important that the terms "liquid phase resistance" or "mesophyll resistance" as used here be clearly distinguished from the "carboxylation resistance" discussed in the next section. Gaastra wrote the following equation:

$$A = \frac{c_a - c_c}{r_a + r_s + r_m} \qquad 8.$$

where r_a and r_s are the boundary layer and stomatal resistances to diffusion of CO_2. Equation 8 ignores the distributed nature of the sources and sinks for CO_2. However, the resistance to diffusion of CO_2 from the mitochondria to the chloroplast is not likely to be large, since these organelles are in close proximity. Further, the rate of evolution of CO_2 by the mitochondria is probably only a small fraction of the rate of RuP_2 carboxylation in most conditions. Apart from this possible complication, Equation 8 is a simple, valid extension of Equation 4, since Equation 4 may be rewritten as

$$A = \frac{c_a - c_i}{r_a + r_s} \qquad 9.$$

Confusion has arisen because Gaastra thought the $p(CO_2)$ inside the chloroplast was near zero. He made this assumption because he found that under normal conditions the assimilation rate was somewhat independent of temperature (33) and because he found that under normal conditions the assimilation rate was linearly related to $p(CO_2)$ at low $p(CO_2)$ (32). From considerations of the temperature dependence of electron transport and photorespiration, we now understand why the rate of assimilation does not continue to accelerate with increasing temperature. From considerations of Rubisco kinetics we know why assimilation rate initially varies linearly with $p(CO_2)$.

From the above, $c_c = c_i - A\, r_m$. Farquhar & von Caemmerer (28) recently concluded that c_c is much nearer c_i than Gaastra thought, and that r_m could be neglected for most purposes in modeling photosynthesis. In support, they noted that significant carbon isotope discrimination, which occurs in C_3 leaves, is incompatible with a substantial liquid phase resistance (25).

Integration of the Effects of Stomatal Conductance on Photosynthesis

We now bring together the various effects of stomatal conductance on CO_2 assimilation rate that we discussed in previous sections. We first consider the special case where carboxylase $p(CO_2)$ is in the linear region of Figure 1. Ignoring the effects of transpiration rate on leaf temperature, water potential, and diffusion of CO_2, assimilation rate is given approximately by Equation 1, and combining this with Equation 8 we obtain

$$A = \frac{C_a - \Gamma}{[r_a + r_s + r_m + (Pk)^{-1}]\,P} \qquad 10.$$

where P is the atmospheric pressure and k is the carboxylation efficiency. In other words, $(Pk)^{-1}$ may be treated as some sort of "carboxylation resistance," although there is no real analogy between resistance and enzyme kinetics. At light saturation, normal atmospheric $p(CO_2)$ and near the temperature optimum, the carboxylase $p(CO_2)$ is sometimes in or near the linear region. There has been a preoccupation with measurements of gas exchange under such conditions in order to take advantage of the simplicity of Equation 10. Ambient $p(CO_2)$ is varied and assimilation rate is plotted against the resulting intercellular $p(CO_2)$. The inverse of the slope of the linear portion is then given by $r_m + (Pk)^{-1}$. However, the intercellular $p(CO_2)$ is just as often outside the linear region, because of the inherent curvature in the kinetics of Rubisco, or because of limitations on RuP_2 regeneration. Fortunately, a simple procedure is available for determining the effect of stomatal conductance on assimilation rate, which is not restricted to the linear region.

The assimilation rate is given by the intersection of the A vs C_i relationship with that representing diffusion of CO_2 in the gaseous phase, Equation 4. The latter is represented by a line with slope $-g_c/P$ intersecting the abscissa at $C_i = C_a$ (see Figure 1). Raschke (84) referred to the A vs C_i relationship as the "demand function" and to Equation 4 as the "supply function." This graphical approach, first used by Jones (47), is useful for assessing stomatal limitations to photosynthesis, as we show later. At zero conductance, the "supply function" is a line intersecting the "demand function" at $C_i = \Gamma$, $A = 0$. As conductance increases, the assimilation rate and the $p(CO_2)$ at the point of intersection increase. The result is illustrated in Figure 2. Note that assimilation rate increases only marginally with conductance above the conductance at which RuP_2 becomes limiting (indicated by the arrow). At infinite conductance, the "supply function" in Figure 1 is a vertical line through $C_i = C_a$. In practice the boundary layer conductance r_a^{-1} is finite and the gas phase conductance cannot exceed r_a^{-1}. Thus the line with slope $-(r_aP)^{-1}$ would represent infinite stomatal conductance.

The "supply function" may be modified to include the water vapor/CO_2 interference effects described by Equation 6. The slope becomes $-(g_c + E/2)/P$, and at $C_i = C_a$, A takes the value $-Ec_a$. In principle, the effects of conductance on assimilation rate via changes in leaf temperature and water potential may be determined by plotting a series of "demand functions" corresponding to different transpiration rates. In practice, it is difficult to obtain so much data from a single leaf.

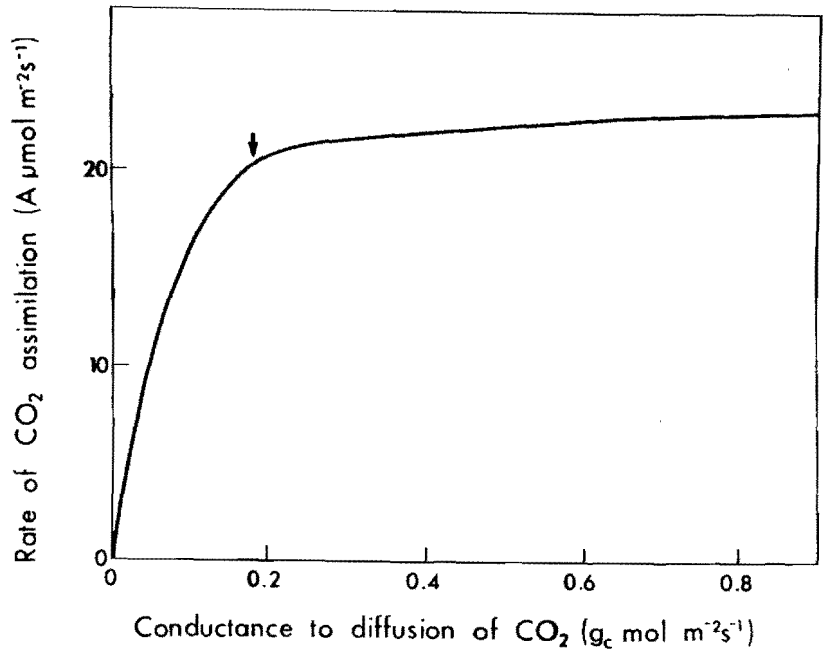

Figure 2 Rate of CO_2 assimilation versus the conductance to diffusion of CO_2 for the metabolic characteristics shown in Figure 1. The relationship is generated by varying the "supply function" in Figure 1. The arrow indicates the transition from RuP_2 saturation to RuP_2 limitation.

The effects of increased transpiration rate on assimilation via increased molecular interference and decreased water potential tend to make assimilation rate increase less with increased conductance than would be predicted from the simple graphical theory of Jones (49). However, at temperatures above the optimum for electron transport, the transpirational cooling has an opposite effect.

WHAT SHOULD WE EXPECT OF STOMATA?

We now ask a teleological question: what should we expect of stomata? or, what is an appropriate index of stomatal performance? Physiologists generally believe that stomata function to prevent desiccation while still allowing the passage of CO_2. It may be shown mathematically that in order to gain carbon most economically with respect to water loss, stomata should function such that the marginal water cost of assimilating more CO_2 ($\partial E/\partial A$) is constant all day.

The scene was set for the formalization of this idea in the conclusions to a theoretical study by Cowan & Troughton (17) of the generally nonlinear relationships between transpiration rate E and assimilation rate A as stomatal conductance varies. With the benefit of hindsight we can paraphrase one of their conclusions: the nature of the curvilinearity in the relationship between E and A affects the requirement for an optimum relationship between the two. When the ratio E/A increases with A (i.e. $\partial^2 E/\partial A^2 > 0$), an optimum relationship is achieved with an (at that time unspecified) uniform stomatal conductance.

An explicit expression for the optimal conductance was derived by Farquhar (21), who borrowed ideas from control theory and economics and suggested that stomata may tend to optimize some index of performance which depends on E and A. He assumed that there is a cost $a\ \mathrm{d}E$, associated with an increase $\mathrm{d}E$, in transpiration rate, and a benefit $b\ \mathrm{d}A$, associated with an increase $\mathrm{d}A$, in assimilation rate. The optimal conductance is then the one at which the marginal cost of change in conductance equals the marginal benefit

$$a\ \mathrm{d}E = b\ \mathrm{d}A \qquad 11.$$

That is, the optimal conductance is one at which

$$\partial E/\partial A = b/a \qquad 12.$$

where $\partial E/\partial A$ represents the slope of the relationship between E and A when conductance is the implicit source of variation. If the cost per unit water lost a and the benefit per unit carbon gained b remain in constant ratio, $\partial E/\partial A$ should also be constant in time and space. Farquhar derived an expression for conductance in terms of the same simple resistance analysis made by Cowan & Troughton (17) and pointed out that the increases of conductance and assimilation rate with irradiance are consistent with the optimization hypothesis. He noted that the solution is only optimal when the curvature ($\partial^2 E/\partial A^2$) is positive.

Cowan (13) defined an optimal time course of stomatal conductance as one which gives rise to the minimum loss of water over a certain period for a particular amount of carbon assimilated. He showed that this also requires constant $\partial E/\partial A$. Using a simple resistance analysis for the dependence of A on c_i, he showed that optimization sometimes demands direct responses of stomata to changes in humidity. Cowan & Farquhar (16) noted that for a maximum amount of carbon gained for a particular amount of water lost, conductance must still vary such that $\partial E/\partial A$ remains constant. They extended the treatment to include nonlinear dependence of A on c_i, irradiance and temperature. The notation for the constant value of $\partial E/\partial A$, written above as b/a was changed to λ, the usual notation for a Lagrange multiplier, since it was recognized that optimal conductance is the solution to a simple problem of the calculus of variations: minimize $\int E\ \mathrm{d}t$ over a period with the constraint that $\int A\ \mathrm{d}t$ is a constant over that period. The solution is $\partial E/\partial A = \lambda$, a constant. It was shown that in a hot climate, noonday stomatal closure should occur for low values of λ, but not for large values. From the previous simple linear analysis, noonday closure had been regarded as necessary for all values of $\partial E/\partial A$ (13). It was calculated that under certain conditions increased irradiance demanded increased c_i, a result which cannot be achieved solely by negative feedback control of c_i. Thus optimal behavior appears to demand feedforward responses to both light intensity and humidity. Farquhar (23) calculated that for $\partial E/\partial A$ to remain constant as photosynthetic capacity changes, stomatal conductance should change in a somewhat parallel fashion. This results in a close correlation between A and g as has been observed in such cases by Wong et al (104).

Cowan (14) developed a theory relating $\partial E/\partial A$ to the requirements for nonphotosynthesizing tissue to support the functioning and growth of photosynthesizing tissue and to provide water for transpiration.

The first experimental support for the optimization hypothesis was presented by Farquhar et al (27) who showed that $\partial E/\partial A$ remained substantially constant over a range of ambient humidities. Hall & Schulze (36) confirmed these findings in other species in the laboratory as did Field et al (31) in the field. Hall and Schulze also found that $\partial E/\partial A$ remained constant over a range of temperatures.

$\partial E/\partial A$ is often quite sensitive to small changes in conductance, especially at the transition between "RuP_2 saturation" and "RuP_2 limitation" (99). This is because the curvature, $\partial^2 E/\partial A^2$, is usually greatest in this region, as may be appreciated from Figure 2. Farquhar et al (27) showed that even when $\partial E/\partial A$ was not precisely constant, the gas exchange parameters (E, A, g, C_i) had values close to those which would occur if $\partial E/\partial A$ were constant. W. E. Williams (unpublished) has observed diurnal patterns of stomatal behavior in the field which conform to this relaxed definition of optimality. von Caemmerer & Farquhar (99) noted that because of the curvature at the transition between RuP_2 saturation and limitation, there is a large range of values of λ for which optimal conductance causes the photosynthetic system to be near this transition. They reported that the operational intercellular $p(CO_2)$ remained near the transition in the A vs C_i response under various growth conditions (irradiance and nutrition) when the measurements were made at temperatures and irradiances similar to those experienced during growth. von Caemmerer (98) also found that

C_i remained near the transition as water stress proceeded. Ball (2) observed similar results with imposition of increasing salinity around the roots of mangrove seedlings. In both these studies the stresses reduced assimilation rate and the $p(CO_2)$ at which the transition occurred.

von Caemmerer & Farquhar (99) suggested that at the transition, neither Rubisco nor RuP_2 regeneration capacity are in excess. Both components are expensive in terms of the nitrogen contained and so expensive photosynthetic machinery is not wasted.

Comins & Farquhar (12) showed that for an optimally behaving plant the marginal cost in terms of daily water use E_T, of a change in total daily assimilation A_T, is equal to λ (that is, $dE_T/dA_T = \lambda$). They termed λ the "marginal water cost of carbon assimilation." In the case of C_3 and C_4 plants it has been assumed that there is no effect of carbon gain at one time on carbon gain at some other time (and similarly for water loss). Consequently, conductance is optimal when $\partial E/\partial A = \lambda$. However, this is not the case with plants undergoing Crassulacean acid metabolism (CAM) since the extent of night-time fixation appears to be limited, thus introducing a dependence of A on the integrated carboxylation since the beginning of the night. Comins and Farquhar proposed that for optimal behavior in CAM plants, dE_T/dA_T should still equal λ, but that $\partial E/\partial A$ should not always equal λ, being less during early night-time CAM fixation. They discussed the optimal allocation of time between C_3 metabolism and CAM. Using a simple model of CAM, they predicted that at night stomatal conductance should be proportional to the square root of "carboxylation efficiency" and noted that the data of Nobel & Hartsock (68) were somewhat consistent with this. They contrasted this square root relationship with the linear one predicted for C_3 and C_4 metabolism (the latter relationship giving rise to a linear relationship between A and g).

Having examined how stomata may act to conserve water in acquiring carbon, we now ask a second question: how much carbon has the plant foregone in conserving water?

STOMATAL LIMITATION OF PHOTOSYNTHESIS

Calculation of Stomatal Limitation

The presence of a finite stomatal resistance to diffusion of CO_2, and of other resistances in the gaseous and liquid phases, cause the $p(CO_2)$ at the sites of carboxylation to be less than that in the atmosphere, thereby reducing the rate of CO_2 assimilation somewhat below its potential. In some desert annuals the stomatal resistance is so low that the boundary layer resistance is a very important component of the total resistance to diffusion (J. A. Berry, personal communication). Nevertheless, stomata usually impose the

largest resistance to diffusion. This is despite our uncertainty about the resistance to diffusion through the liquid phase. But we now intend to show that diffusion itself only marginally limits assimilation rate, a fact not widely appreciated.

The stomatal limitation of photosynthesis is often thought of as the contribution of stomatal resistance to some total "resistance" to CO_2 uptake. This is reasonable if the effects of transpiration rate on leaf temperature, water potential, and diffusion of CO_2 are ignored, and if the intercellular $p(CO_2)$, C_i, is in the region of linear response of A to C_i, *for all possible values of stomatal conductance.* In other words, if the "supply function" at infinite stomatal conductance intercepted the "demand function" in the linear region, Equation 10 could be used to estimate the stomatal limitation by comparing the actual rate of assimilation with that when $r_s = 0$. We call this the "linear resistance analysis" and in most cases it is invalid. The boundary layer conductance is usually sufficiently large to ensure that the intercellular $p(CO_2)$ at infinite stomatal conductance is beyond the linear region. The linear resistance analysis has led to several important misconceptions. It *appears* to indicate a large stomatal limitation of photosynthetic rate under most natural conditions (6, 58), and it *appears* to indicate large effects of stomatal closure on photosynthesis during water stress (62, 71, 95) and following source-sink manipulations (89). It has also been used to support the incorrect argument that stomata limit photosynthesis more in C_4 species than in C_3 species (52).

The problem is illustrated in Figure 3. The upper A vs C_i curve might represent a control leaf, and the lower curve a leaf after a treatment, for example water stress. We would describe these leaves as having the same levels of Rubisco, but differing in their respective capacities for regeneration of RuP_2. The "supply function," representing Equation 4 for the control leaf, is the dashed line labeled "*c*". Reduced stomatal conductance in the treated leaf is reflected in the less negative slope of the dashed line, denoted "*t*". The vertical dashed line, labeled "*o*," represents the "supply function" if resistance to diffusion were zero. The dotted extension of the initial linear portion of the A vs C_i curve is an implicit assumption in the linear resistance analysis of diffusional limitation.

Ignoring various effects of transpiration rate on assimilation rate, we see that resistance to diffusion reduces the rate of assimilation in the control leaf below its potential by the amount d. The incorrect linear resistance analysis suggests a reduction d', which is much larger. Similarly in the treated leaf, resistance to diffusion reduces the rate by e, whereas the incorrect analysis suggests a much larger reduction, e'. Note that the reduction e in assimilation rate of the stressed leaf by resistance to diffusion is less than that of the control leaf d. This is despite the decreased stomatal conductance and

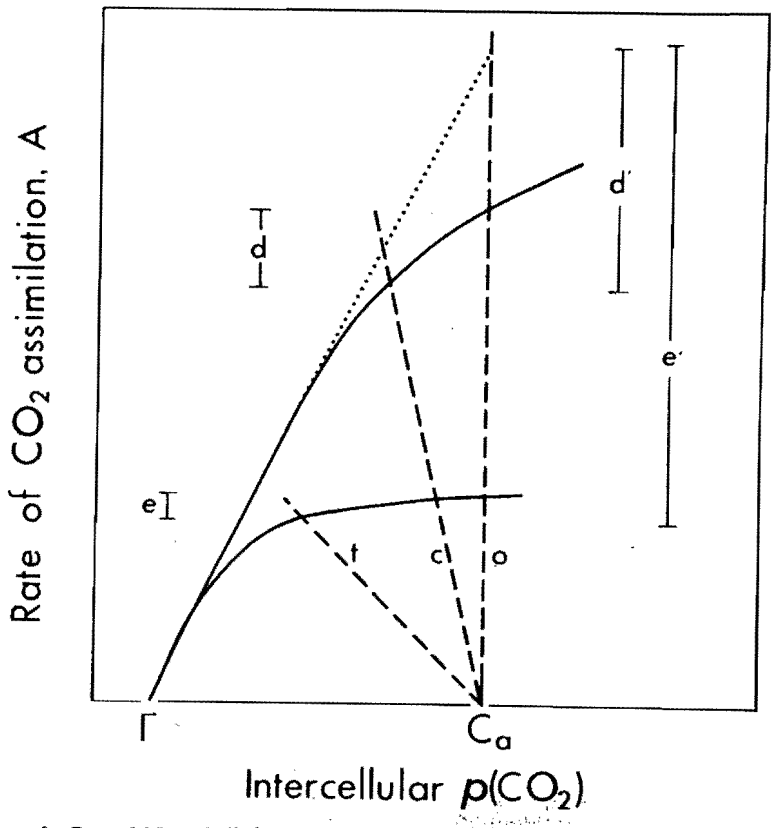

Figure 3 Rate of CO_2 assimilation versus the intercellular partial pressure of CO_2. The upper curve represents the "demand function" in a control leaf, and the lower curve that in the same leaf after some treatment. The respective "supply functions" are the dashed lines denoted *c* and *t*. The vertical dashed line, denoted *o*, represents the "supply function" if resistance to diffusion were zero. Because of the resistances that do occur, the control and treated leaves have assimilation rates less than their potential rates, by the amounts *d* and *e*, respectively. Incorrect extrapolation of the initial linear portions of the curves (the dotted line) would suggest that diffusional limitations have reduced the rates by *d'* and *e'*. The reduction of the assimilation rate by diffusion in the treated leaf *e* is less than that of the control *d*, despite the lower intercellular $p(CO_2)$ in the treated leaf. The incorrect linear resistance analysis suggests the opposite.

intercellular $p(CO_2)$ in the stressed leaf. In the linear resistance analysis the limitation due to gaseous diffusion is $(C_a\text{-}C_i)/(C_a\text{-}\Gamma)$, as a proportion to the "total limitation." In that sense, any treatment which causes a decrease in C_i would represent an "increased stomatal limitation" on photosynthesis. This need not be the case, as Figure 3 shows.

To reiterate, Equation 10 is often useful for predicting the effects of changes in stomatal conductance, providing the intercellular $p(CO_2)$ remains in the linear region. It is misleading, however, to extend the use of the equation into the nonlinear region, which is what is done implicitly in the linear resistance analysis of stomatal limitation of photosynthesis. Instead, the full A vs C_i relationship should be plotted together with the supply function, Equation 4, providing the effects of transpiration rate can be ignored.

The first criterion for establishing that stomatal responses are dominant in the response of assimilation rate to some perturbation is that C_i should change in the same direction as A. If the changes are opposite, the most important change must have been in the mesophyll cells. For example, Setter et al (89) recently described experiments in which they girdled the petiole of a soybean leaf. Assimilation rate and stomatal conductance declined, and the authors concluded that stomata imposed a large barrier to CO_2 diffusion which reduced the assimilation rate. From their data, however, we calculate that C_i was higher, after girdling, than in the control leaf. The dominant response was actually reduced mesophyll capacity for assimilation.

Change in C_i in the same direction as the change in A is a necessary, but not sufficient, condition to establish primacy of the stomatal response. The insufficiency was illustrated in Figure 3, where "stomatal limitation" decreased despite a decrease in C_i. In that case assimilation rate was less than it would have been had stomatal conductance remained the same. Nevertheless, to conclude that stomatal closure was the main cause of reduced assimilation rate, one should show that the stomatal limitation has increased.

In this context, T. D. Sharkey (unpublished) has suggested a simple method for assessing stomatal limitation. The assimilation rate that actually occurs, A, is subtracted from A_o, the rate which would occur if resistance to CO_2 diffusion were zero, and then divided by A_o to give a relative measure of stomatal limitation, l. Thus

$$l = \frac{A_o - A}{A_o} = 1 - \frac{A}{A_o} \qquad 13.$$

For the control leaf in Figure 3, $l = 17\%$, while the linear resistance analysis predicts a stomatal limitation of 37%.

The measure l actually includes the limitation imposed by boundary layer resistance, and tends to overestimate the stomatal limitation. In principle this could be taken into account, as could the modification of the supply function by transpirational interference, Equation 6. But given that other effects of transpiration rate on the "demand function" (the A vs C_i curve) are not included, there is little point in doing so. In using this definition of limitation it is assumed that the largest possible assimilation rate occurs at $C_i = C_a$.

In an analogous manner, the limitation imposed by RuP_2 carboxylase activity may be assessed by comparing the actual assimilation rate with the potential rate which would occur if the "RuP_2 saturated" region of the curve in Figure 1 were made very steep, but with an unchanged stomatal conductance and capacity for RuP_2 regeneration. In the situation represented in Figure 1, the "Rubisco limitation," so defined, would be zero, because RuP_2 regeneration is already co-limiting at that stomatal conductance. The Rubisco limitations are also small in the "control" and "treated" leaves in Figure 3.

Similarly, the limitation imposed by the capacity for RuP_2 regeneration may be assessed by estimating the assimilation rate that would occur if this capacity were much greater. Again the "regeneration limitation" would be zero in Figure 1 and small in Figure 3. In the cases exemplified, CO_2 assimilation rate is reduced if the capacity of any one component (stomatal, Rubisco, regeneration) is reduced. In contrast, the increase of CO_2 assimilation requires the simultaneous increase of each of the capacities.

The sum of the three limitations generally differs from unity. The stomatal limitation is frequently the largest of the three. Nevertheless, it has been overemphasized in many discussions of situations where both CO_2 assimilation rate and stomatal conductance are reduced for some reason.

Contributions of Changes in Stomatal Conductance to Observed Changes in Assimilation Rate

We now examine the contribution of stomata to limitations of photosynthesis, resulting from a number of short- and long-term treatments. We then briefly examine the situation in C_4 species.

LIGHT, NUTRITION, AND CHILLING At low irradiances assimilation rate is reduced. Although stomata close somewhat, they actually impose less limitation on photosynthesis, i.e. l is reduced (28, 99, 102, 103). Similar conclusions may be made from studies of plants grown at reduced light intensities (7, 99). Wong (102) found that photoinhibition reduced assimilation rate and conductance with little change in intercellular $p(CO_2)$. Calculations show that the stomatal limitation remained the same or was reduced.

With decreasing levels of nitrogen nutrition, it has been observed that assimilation rate and stomatal conductance decrease (99, 104), but calculations show that the stomatal limitation l actually decreases.

Drake & Raschke (19) reported that a pretreatment of *Xanthium strumarium* plants by chilling reduced stomatal conductance and assimilation rate, but increased intercellular $p(CO_2)$. Stomatal closure was therefore of secondary importance.

ABSCISIC ACID AND WATER STRESS One treatment that does increase the stomatal limitation is the addition of abscisic acid to the transpiration stream of detached leaves. From the measurements of Dubbe et al (20), we calculate that l increased from 12 to 29% as a result of feeding the hormone in a 10^{-5} M solution in the transpiration stream to a leaf of *Xanthium strumarium*. Since ABA did not affect the capacity for assimilation, the reduction in assimilation rate was entirely the result of stomatal closure.

It might be thought that water stress would have similar effects to those of ABA, but the evidence is not supportive. In cases where the intercellular $p(CO_2)$ is higher after water stress than in controls (27, 81), despite reduced stomatal conductance, stomatal closure is obviously of secondary importance in causing the reduction of assimilation rate. From the data of Nobel et al (69) we calculate that the intercellular $p(CO_2)$ was higher in *Encelia farinosa* plants growing in soil at a water potential of −1.8 MPa than in plants growing at −0.1 MPa.

Similar conclusions may be drawn when C_i is little changed despite reductions in conductance and assimilation rate. Wong et al (104) publicized this type of result which occurred in a number of treatments, including water stress in *Zea mays*.

In several experiments in this laboratory the initial effect of water stress appeared to be a reduction in the capacity for RuP_2 regeneration, while the "RuP_2 saturated" region of the A vs C_i curve was initially unaffected. In each case, intercellular $p(CO_2)$ was reduced but the stomatal limitation was actually less than in control plants because assimilation rate in the stressed plants tended to saturate at low intercellular $p(CO_2)$. [*Phaseolus vulgaris* (99); *Xanthium strumarium, Sorghum bicolor, Gossypium hirsutum* (T. D. Sharkey, M. R. Badger, and S. C. Wong, unpublished).]

Mooney et al (64) assessed the stomatal component of assimilation reduction after water stress by calculating the assimilation rate that would have occurred if the stomatal conductance had remained constant at the prestress level, and comparing that with the assimilation rate actually observed. Using this "constant conductance" analysis, they calculated that 20 to 25% of the reduction in assimilation rate was the result of stomatal closure. From their data we calculate that l increased from 28 to 40. From the data of Jones (48) on cotton, we calculate that l increased from 6 to 38. Reduced mesophyll capacity was a dominant feature in all cases of water stress-induced reduction of assimilation rate.

HUMIDITY One treatment in which reduced stomatal conductance is probably the prime cause of reduced assimilation rate is reduced ambient vapor pressure. It is important, however, that the relationship between assimilation and C_i be determined at both humidities since M. C. Ball, T. D. Sharkey, and I. R. Cowan (unpublished) have found that the capacity for RuP_2 regeneration was reduced by high transpiration rates in three species.

TRANSIENT BEHAVIOR Stomata take a finite time to respond to perturbations; during this time they may transiently limit assimilation more than in the steady state. M. C. Ball (unpublished) has observed that the delays in stomatal opening lengthen with increased salinity in the solutions surrounding roots of mangrove seedlings. Dramatic effects of nonsteady behavior are sometimes seen in the form of stomatal "overshoot" or oscillations (3). But before the above phenomena are discussed as imperfections or aberrations, it must be shown that the mesophyll chloroplasts are not undergoing a related time-dependent change in their characteristics.

C_4 METABOLISM We turn now to the stomatal limitation of photosynthesis in C_4 species, for which there are few data. Although the ratio of stomatal conductance to the initial slope of the A vs c_i response, Pk, is lower than in C_3 species, stomatal limitation appears to be no higher. The reason that the limitation is not high, despite the generally lower intercellular $p(CO_2)$ in C_4 species, is the earlier CO_2 saturation of assimilation rate.

SUMMARY From the foregoing, it appears that apart from some transients and from the effects of added abscisic acid and of reduced ambient humidity, reduced stomatal conductance is rarely the main cause of the reduced assimilation rates that occur in a number of situations. Others have been aware for some time (e.g. 7) of the small limitations generally placed on photosynthesis by stomata. In contrast, the stomatal limitation on transpiration rate is usually substantial (13). It appears then that stomata generally function to minimize water loss while only marginally limiting carbon assimilation. How is this achieved?

TO WHAT SIGNALS DO STOMATA RESPOND?

Introduction

For efficient functioning of the plant, stomatal conductance must be tuned to the environment and photosynthetic metabolism of the leaf and to the hydraulic characteristics of the soil and plant. To some extent all of these factors vary stochastically, and this requires that stomata be able to sense the changes that occur. In this section we discuss how stomata may obtain the necessary information, first about factors affecting carbon assimilation and then, briefly, about those affecting transpiration.

Light Intensity and Quality

Scarth (88) suggested that the responses of stomata to light are mediated by changes in intercellular $p(CO_2)$ and this may be so in limited cases (86). In general, however, the gain of the CO_2 feedback loop (13, 24) is too low to account for observed responses to light (65, 90, 104).

Blue light has almost always been found to be more effective than red light in causing stomatal opening or preventing stomatal closure while extremely high quantum fluxes of green light are required for stomatal opening. This pattern of response is observed with branches (65), intact leaves (60, 65, 91, 100), epidermal strips (41, 54, 70), and isolated guard cell protoplasts (107). The blue light response may be the result of light absorption by a flavin (70) while the red light response and part of the blue light response is the result of light absorption by chlorophyll (91).

Zeiger et al (105) and Outlaw et al (73) have demonstrated that guard cell protoplasts contain both photosystem II and I reaction centers. It appears that whole chain electron transport can occur in guard cells, even though net carbon reduction cannot (72, 85). Electron transport of mesophyll chloroplasts has a Michaelis-Menten type response to irradiance (29), and the responses of stomatal conductance are similar (44). Treatments that reduce electron transport capacity of the mesophyll (for example photoinhibition) may also effect that of the guard cells.

The presence of a second (blue) light absorbing pigment could serve as a means of perceiving the spectral quality of the light environment (91). Zeiger et al (106) have observed a blue light-dependent, early phase of stomatal opening and suggested that it may constitute an adaption of plants to the light quality prevailing at dawn.

Carbon Dioxide

Stomatal response to increased $p(CO_2)$ is variable. To the extent that it occurs it is usually one of decreased opening. This is so in light and in darkness, in leaves, epidermal strips, and isolated individual guard cells (84). The sensing mechanism is unknown, but interesting observations have been made recently by E. Zeiger and A. Melis (unpublished) of quenching of fluorescence in guard cell chloroplasts by CO_2.

At high light intensity in C_3 species, stomatal conductance is often insensitive to low partial pressures of CO_2 (20, 65, 81, 102, 103), becoming sensitive at the transition from "RuP_2 saturation" to "RuP_2 limitation," and again less sensitive at still higher $p(CO_2)$. Similar results are seen in C_4 species, where the transition occurs at lower intercellular $p(CO_2)$ (20, 67, 102).

These patterns are consistent with our general interpretation of stomatal functioning. In the case of a C_4 plant, in order not to restrict assimilation, stomata should be insensitive to C_i when C_i is lower than the level at which saturation occurs. But when C_i is above this level, stomata should be very sensitive to C_i so that they close and reduce water loss (without affecting assimilation). In C_3 plants the same is true except that any reduction in evaporation rate is generally at the cost of some reduction in assimilation rate.

Stomata become more sensitive to CO_2 in the presence of abscisic acid (ABA) (20, 83), which may explain why stomata in water stressed and chilled plants are also more sensitive.

Leaf Water Status

Assimilation rate often begins to decline when the leaf turgor is reduced to zero (8), and ABA production begins when turgor is near zero (80). Most of the ABA in nonstressed leaves is in the chloroplast (59), and since the chloroplast envelope becomes leaky when water stressed (50), ABA may be partially responsible for the rapid responses of stomatal conductance to reduced water status and for the longer term correlation between declining conductance and assimilation rate.

Mesophyll Metabolites

To the extent that stomata respond to intercellular $p(CO_2)$, they are responding to a parameter affected by mesophyll metabolism. However, abscisic acid is the best example of a mesophyll metabolite which may affect stomatal conductance. Synthesized in the mesophyll (59) in response to water stress (101), ABA may diffuse to the guard cells thereby reducing stomatal aperture (83).

I. R. Cowan, J.A. Raven, W. Hartung, and G. D. Farquhar (unpublished) have hypothesized a more pervasive role of ABA in modulating stomatal conductance in a manner which is related to the capacity of the mesophyll for photosynthesis. They noted that because ABA is a weak acid, it will tend to accumulate in regions of high pH, such as the mesophyll chloroplast stroma in the light. They suggested that irradiance of mesophyll tissue should reduce the amount of ABA available via the free space, to the guard cells, and enhance stomatal opening. Conversely, any factor which causes a reduction of stromal pH should tend to increase the amount of ABA available and promote stomatal closure.

A number of other compounds, synthesized in the mesophyll, are known to enter the epidermis rapidly (18, 94). Another possible linkage between the mesophyll and guard cells is via phosphate levels. Phosphate added to the transpiration stream is known to promote stomatal closure, while mannose, which binds phosphate, promotes opening (G. Harris and D. A. Walker, unpublished). There could perhaps be electrical connections between the mesophyll and epidermis (97), as was first suggested by Heath and Russell (38).

The remarkable correlation between stomatal conductance and assimilation rate observed by Wong et al (104) led them to suggest that their results might be explained by the movement of some metabolite. This correlation led to a constancy of the intercellular $p(CO_2)$ under the conditions examined. Of course C_i varies much more widely under natural conditions, as may be inferred from the range of naturally occurring carbon isotope compositions (25).

In the long term, the capacity of the mesophyll to assimilate carbon will affect stomatal conductance, since the guard cell lacks Rubisco and depends on the mesophyll for its reduced carbon (72, 85). If rapid changes in assimilation capacity are via changes in RuP_2 regeneration capacity (e.g. after irradiance changes or water stress), the absence of Rubisco will not be a handicap to guard cell sensing of the photosynthetic capacity of the leaf.

The question for stomatal physiologists is not whether metabolites from the mesophyll affect stomatal conductance, but to what extent and on what time scale does it occur.

Metabolites from Roots

A supply of cytokinins from the roots is necessary to prevent chlorosis and protein degradation (51). It has often been shown that detached leaves placed in a solution of kinetin (or some other cytokinin) will transpire more water than detached leaves placed in water only (see 57 for a review). For a long time, no distinction was made between cytokinins increasing stomatal conductance and the ability of cytokinins to retard senescence. If the detached leaf in water senesces more rapidly than the leaf in the cytokinin solution, we might expect the "control" leaf to lose less water because of stomatal closure. Many of the reports of cytokinin-induced stomatal opening can be interpreted in this manner (4). While exogenous cytokinins have no effect on stomata in epidermal strips of some species (39), Jewer & Incoll (46) report that in *Anthephora pubescens* cytokinins applied to epidermal strips can stimulate stomatal opening. An increase in the cytokinin supply from the roots (perhaps signaling increased root growth) could affect photosynthesis by stimulating chlorophyll formation and protein synthesis and at the same time promote stomatal opening so that the stomata would not limit the increased capacity for photosynthesis. Bengtson et al (4) found that placing detached leaves in a cytokinin solution resulted in a lower ABA content. Much more research is needed on the role of cytokinins in regulating photosynthesis and stomatal conductance.

Recently Bradford & Yang (9) have shown that the ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC) may serve as a messenger to the leaf of the distress of water-logged roots. Water-logging often leads to stomatal closure, but Bradford and Yang do not believe that this necessarily results from the temporary wilting that can occur. It will be interesting to see if there is a substantial stomatal limitation of photosynthesis after flooding.

Humidity

In addition to responding to changes in parameters that affect the rate of assimilation of CO_2, stomata also repond to factors that influence the rate of transpiration. One such example is the leaf to air vapor pressure difference (VPD) (37). To some extent this may be due to changes in leaf water potential, and this would be a form of negative feedback. Bunce (10) made the interesting observation that sensitivity of stomatal conductance to change in VPD was negatively correlated with the length of the root system per unit of plant area.

However, in some cases stomata close sufficiently in response to increased VPD that the resulting transpiration rate is lowered. This demands feedforward (13, 22) and is presumably caused by peristomatal transpiration, i.e. evaporation from beyond the throat of the stoma, which is unaffected by the stomatal closure.

Careful studies are needed of the effects of changes in boundary layer conductance on stomatal conductance, as in many ways these should be similar to changes in VPD.

CONCLUSION

In general, the responses we have examined are qualitatively consistent with a role for stomata minimizing plant water loss while only marginally limiting carbon gain. It appears that the stomatal limitation of photosynthesis is *usually* slight, whether the plants have C_3 or C_4 metabolism and whether or not they are stressed.

This does not in any way reduce the importance of stomata, for only by being well tuned to the metabolism and environment of the plant can they achieve this role. A current challenge in guard cell physiology is to determine the nature of this fine tuning.

Optimal stomatal behavior ($\partial E/\partial A = \lambda$, a constant) leads to only a small benefit in terms of water saved when compared to the situation which would occur if the stomata were uniformly open all day, with the same carbon gain (16). Nevertheless, such savings may represent a significantly increased rate of growth or reduced probability of mortality (15).

More measurements are needed of the marginal water cost of carbon assimilation $\partial E/\partial A$ and of the factors affecting it. These will tax the physiologists so inclined, since they require accurate measurements not only of rates of transpiration and CO_2 assimilation, but of what these would be if stomatal conductance were slightly different from that which is actually found. The above formulation of optimal stomatal behavior does not take into account possibly damaging effects of stomatal closure in high light by photoinhibition or thermal damage, or of damage to the photosynthetic apparatus by local water deficits when transpiration rate is high. Indices of stomatal performance that take into account these and other aspects that might affect plant survival need to be formalized.

ACKNOWLEDGMENTS

We wish to thank our colleagues in stomatal physiology for their cooperation and, in particular, Drs. James Morison and Ian Cowan. We thank Brian Weir for assistance in preparation of the manuscript.

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