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How Stomata Resolve the Dilemma of Opposing Priorities

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How stomata resolve the dilemma of opposing priorities

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Satisfaction of a leaf's need for CO_2 requires an intensive gas exchange between mesophyll and atmosphere; prevention of excessive water loss demands that gas exchange be kept low. Stomata open when a low CO_2 concentration in the guard cells triggers (*a*) uptake of K^+ in exchange of H^+, (*b*) production of organic acids, and (*c*) import of Cl^-. 'Hydropassive' stomatal closure (i.e. turgor loss without reduction of the solute content of the guard cell) appears insufficient to protect the plant from desiccation. An additional 'hydroactive' solute loss is necessary; it is brought about by (+)-abscisic acid (ABA) acting as feedback messenger between mesophyll and epidermis.

Stomatal closure not only curbs water loss but improves water-use efficiency because transpiration is proportional to stomatal conductance (at constant temperature). In contrast, assimilation, following saturation kinetics with respect to intercellular CO_2, is relatively insensitive to changes in stomatal conductance (as long as stomata are wide open).

In *Xanthium strumarium*, the amplitude of stomatal responses to ABA depends on the concentration of CO_2 in the guard cells; the opposite statement is also true. These interactions cause stomata to behave like 'adjustable control systems' capable of giving priority either to CO_2 assimilation or to water husbandry.

The plant's dilemma

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. Even if a leaf had a carboxylating mechanism with an infinitely high affinity for CO_2 and if CO_2 uptake was limited by the boundary layer of the air around the leaf only, at least 20 molecules of water would be lost for each molecule of CO_2 taken up (at 20 °C and 70% relative humidity). This ratio increases further with increasing temperature and decreasing humidity. At 50 °C and 10% relative humidity about 430 molecules of water would escape from the leaf for each assimilated molecule of CO_2. Under actual conditions water losses are at least twice, or even ten times higher than these estimates because the affinity of the carboxylating enzymes for CO_2 is limited, and some CO_2 is continuously liberated to the air during the processes of respiration and photorespiration.

The plant's dilemma could be solved if a substance existed whose permeability for CO_2 was several times greater than that for water vapour. 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. Instead, plants have evolved an epidermis which in turn is covered by a cuticle of low permeability for gases, including water vapour and CO_2. Gas exchange with the atmosphere is made possible through the evolution in the epidermis of mechanical devices of

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microscopic dimensions, the stomata. They are able to regulate the exchange of gases between the interior of leaves and the atmosphere. To them the plant has delegated the task of providing food while preventing thirst.

The stomatal mechanism

The present state of knowledge on stomatal action has just been reviewed (Raschke 1975*a*). I do not need to quote, therefore, original papers when I briefly describe the mechanism by which the plant controls gas exchange.

In most plant species, stomata consist of pairs of kidney-shaped guard cells. They swell when the solute content of their vacuoles increases and water moves into the guard cells by osmosis from the surrounding epidermal tissue. Owing to the radial micellation of the cellulose microfibrils in the cell walls, guard cells become longer and bend during inflation. As the ends of the cells expand, the two guard cells push each other apart. A pore forms between the middle portions of the guard cells and the width of the aperture is roughly proportional to the solute content of the guard cells. The pore is usually a few micrometres wide but may in exceptional cases measure as much as 20 μm. The volume of a pair of guard cells is of the order of 10 pl; it may double during an opening movement.

Potassium salts serve as osmotica in guard cells of open stomata; this has been shown for at least 50 species. When stomata open, guard cells take up K^+ from their environment in exchange for H^+. It has been demonstrated that the amounts of K^+ accumulated in guard cells, in association with various anions, are sufficient to account for the observed changes in osmotic pressure. Potassium ions are also transported fast enough into guard cells and out of them to explain the observed rapid stomatal movements. Electroneutrality is maintained in guard cells mainly by the production of organic anions and to a smaller extent also by import and export of Cl^-.

The organic acid in guard cells of *Vicia faba* was found to be mainly malic acid. Most probably it is made by the carboxylation of phosphoenolpyruvate (PEP; Willmer & Dittrich 1974) derived from starch (figure 1). Epidermal samples possess PEP carboxylase activity and this activity was found to be proportional to the number of stomata in the epidermal samples (Willmer, Pallas & Black 1973). When stomata close, K^+ and Cl^- are released from guard cells and H^+ is taken up. The fate of the organic anions is not known; probably they are partly burnt up and partly used to resynthesize starch. However, gluconeogenesis has not yet been demonstrated in guard cells.

The stomatal apparatus of grasses and sedges appears to be more highly developed than that of plants with kidney-shaped guard cells. In the latter, the hydraulically inactive middle portion of the cells constitutes one-half of the total cell volume or even more. About one-half of the volume work done during inflation is therefore expended in the middle portion. In grasses like maize, the volume of the middle portion is reduced to such an extent that almost 90% of the work is expended in opening the pore. Stomata of grasses are more highly differentiated than ordinary stomata in two other respects. Stomata of maize, and possibly also of other grasses, utilize more Cl^- as counter ion to K^+ than, for instance, *Vicia faba*. Chloride ion is univalent and therefore provides more osmotically active anions per K^+ ion than divalent or trivalent anions. Finally, stomata of grasses possess subsidiary cells which function as ion stores. During stomatal movement, the total K^+ and Cl^- content of a stomatal apparatus of *Zea mays* does not change. Only the distribution of these two ions changes. During stomatal

opening, K^+ and Cl^- migrate rapidly from the subsidiary cells to the guard cells, during closing they return rapidly to the subsidiary cells. This may be one of the reasons why for instance stomata of *Zea mays* respond more quickly to perturbations than stomata with kidney-shaped guard cells.

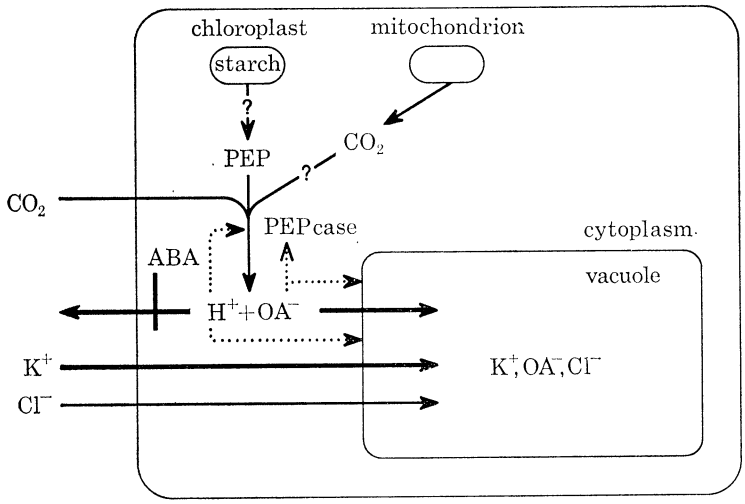

FIGURE 1. Transport and hypothetical metabolism of ions involved in the production of turgor in guard cells. OA^-: organic anion; PEP: phosphoenolpyruvate; PEPcase: phosphoenolpyruvate carboxylase. The inhibition of proton expulsion by abscisic acid (ABA) is hypothetical (Raschke 1975*a*, *b*).

I shall now try to outline a view of how the stomatal mechanism fulfils its dual task of admitting CO_2 from the atmosphere to the mesophyll when need arises while watching over the water economy of the plant.

ADMISSION OF CARBON DIOXIDE TO THE MESOPHYLL

Stomata open in response to the depletion of CO_2 in the intercellular spaces by the assimilatory activity of the mesophyll. Much evidence supporting this view has accumulated since the discoveries of stomatal sensitivity to CO_2 by Linsbauer (1916), Freudenberger (1940) and Heath (1948), (see also Meidner & Mansfield 1968). The ability of the guard cells to recognize a demand for CO_2 closes a feedback loop which allows stomatal conductance to be adjusted in proportion to the assimilatory activity of the mesophyll (Raschke 1965). Feedback through the partial pressure of CO_2 in the leaf has thus an advantage over the synchronization of stomatal opening with the assimilatory activity through a response to light or a circadian endogenous rhythm. Photosynthetic activity of the chloroplasts in the guard cells is not essential for stomatal opening, although it may modify stomatal behaviour as explained below. Plants equipped with the crassulacean acid metabolism assimilate CO_2 during the night and their stomata open in darkness in response to CO_2 depletion of the intercellular spaces (Kluge & Fischer 1967). Night opening can also be induced in plants which can assimilate CO_2 in the light only if one can reduce the partial pressure of CO_2 in the intercellular spaces by some other means. Plants of *Xanthium strumarium*, well supplied with water, have stomata which are not completely closed during the night. If these leaves are brought into CO_2-free air, enough CO_2 diffuses out of the leaves, the CO_2 level within the leaves drops, and the stomata respond immediately and open very rapidly (Raschke & Pierce 1974).

That light is not essential for stomatal opening was shown by an experiment of Hanebuth & Raschke (1973) with leaves of *Zea mays*. Leaf samples were illumintaed with white, red or blue light. Of the monochromatic light applied, 99.5% was absorbed by the leaf. The epidermis facing the light source thus received 200 times more light than the epidermis away from the light. Nevertheless, stomatal conductances were the same whether or not the epidermis, upper or lower, was illuminated directly, as long as the quantum flux received by the mesophyll and, consequently, the rate of photosynthesis in the mesophyll remained the same. In further experiments, stomatal conductances were determined while the CO_2 content of the air was varied in the light and in darkness. Regardless of the method of altering the intercellular $[CO_2]$, whether by light, darkness or by the $[CO_2]$ in the air surrounding the leaf, one common curve could be fitted to the data points when plotted against the intercellular $[CO_2]$, indicating that stomatal aperture in *Zea mays* was controlled by the $[CO_2]$ in the substomatal cavity. At equal intercellular values of $[CO_2]$, however, illuminated stomata were a little wider open than those in darkness. A direct stomatal response to light was, therefore, superimposed on the curve representing the relationship between stomatal aperture and $[CO_2]$. This direct response can be attributed to the photosynthetic reduction of CO_2 by the chloroplasts in the guard cells. In darkness the intracellular $[CO_2]$ is probably above the intercellular $[CO_2]$; in the light it is below. The photosynthetic removal of intracellular CO_2 may be important for sharpening the stomatal response to CO_2. Stomatal closure follows a saturation hyperbola with respect to CO_2 (Raschke 1972, 1975*b*). Half saturation occurs roughly at 200 cm^3/m^3, implying that stomata would not be closed in darkness at a CO_2 concentration near the ambient were it not for the CO_2 evolved by the numerous mitochondria in the guard cells. On the other hand, depletion of intercellular spaces of CO_2 may not be enough to produce maximal opening. Maximal opening will occur if the photosynthetic removal of endogenous CO_2 lowers the $[CO_2]$ in the guard cells to a level optimal for opening. It should be clear that the CO_2-scrubbing activity of the guard cell chloroplasts need not be very effective to exert an effect on intracellular $[CO_2]$. It is to be expected that small differences in the strength of sources and sinks for CO_2 in the guard cells determine whether a stoma opens or closes. Species differ in numbers and sizes of chloroplasts in guard cells. Whether these differences manifest themselves in different modifications of stomatal behaviour by light has not yet been investigated.

There remain the questions how CO_2 controls the transport of ions into guard cells and out of them and how CO_2 affects the metabolism of organic acids in guard cells. We do not have the answers yet. But there is the observation that in several species, including *Xanthium strumarium* (Drake & Raschke 1974), *Zea mays* and *Gossypium hirsutum* (unpublished), maximal stomatal opening does not always occur in CO_2-free air but near 100 cm^3/m^3. It is very likely that some CO_2 is needed for stomatal opening (namely for the production of malate from phosphoenol pyruvate) and that only the excess of CO_2 leads to stomatal closure because the removal of malic acid from the cytoplasm of the guard cells cannot keep pace with its production. As a result, the acid level in the cytoplasm rises and this in turn stops or reverses stomatal opening (Raschke 1975*a*). We shall return to this hypothesis later after considering further observations on the effect of CO_2 on guard cells.

Stomata insensitive to CO_2

Stomata of some species lose their sensitivity to CO_2 when they open in the morning if the water content of the tissue remains high. For instance, stomata of greenhouse-grown and well-

watered plants of *Xanthium strumarium*, once open, do not respond to CO_2. These stomata can, however, be sensitized to CO_2 by raising the level of abscisic acid in the leaf (Raschke 1974, and see below).

Prevention of excessive water loss

When a plant loses water to the atmosphere, the chemical potential of water drops at the sites of evaporation. A gradient in water potential is formed which drives water from the soil through the plant. Stomata do not usually respond to the reductions in water potential necessary to maintain the transpiration stream unless the water potential in the leaf sinks below a threshold. Then stomata begin to close rapidly and more or less completely. This threshold can be at water potentials anywhere between -0.7 and -1.8 MPa (-7 and -18 bar) (Hsiao 1973). We observed in unpublished experiments with *Vicia faba* that due to this stomatal reaction leaf water potential did not drop below -0.7 MPa. The leaves aged and died rather than lose more water.

Most textbooks convey the impression that stomata close in response to a lowered water potential because guard cells passively lose water to their neighbouring cells. Turgor declines; the guard cells shrink; the pore closes. The switch-like action of stomata at the threshold may be inherent in their mechanics. However, a test of this hypothesis on epidermal strips of *Vicia faba* did not confirm this notion: the decline in stomatal aperture with sinking water potential turned out to be gradual and the stomata remained open at water potentials as low as -6 MPa (Raschke, Dickerson & Pierce 1973). A threshold appeared if more than a few minutes was allowed for stomatal adjustment; it was particularly pronounced if the mesophyll was left attached to the epidermal sample. This result reminds one of Stålfelt's reports of 1929. He found that water loss led to stomatal closure only after a delay of about 13 min. This closure could not be reversed by supplying the leaves with water again. Stålfelt called this response the 'hydroactive' closure. We now know, through the work of Hiron & Wright (1973) and others that production and action of the plant hormone abscisic acid (ABA) are responsible for the hydroactive closure. ABA is formed within minutes in leaves suffering water stress. Guard cells lose K^+ and close if ABA is present in the transpiration stream. This closure may lead to a recovery of leaf turgor and water potential. The hydroactive system is highly sensitive to small changes in water potential. Changes in water supply to the plant may result in relatively small changes in water potential in the leaf. If the water potential in the leaf happens to be near the threshold mentioned above, the synthesis of ABA is triggered and stomata close in response to it. Perhaps plants under water stress produce additional substances acting in a similar way. Wellburn, Ogunkanmi, Fenton & Mansfield (1974) report that all-*trans*-farnesol may possibly be one of them.

The stomatal response to ABA increases with the concentration of ABA in the transpiration stream. The velocity of the transpiration stream intensifies the effect of ABA. This observation indicates that the accumulation of ABA at the terminal point of the transpiration stream is important. Besides, this velocity dependence constitutes an additional negative feedback of transpiration on stomatal aperture (Raschke, unpublished).

It is very likely that stomata respond to the (+)-enantiomer of ABA only (Cummins & Sondheimer 1973; Kriedemann, Loveys, Fuller & Leopold 1972). As required for a response to a feed-back signal, stomata are not permanently affected by ABA. When the supply of ABA stops, stomata reopen again (Cummins, Kende & Raschke 1971).

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Open-loop control by atmospheric humidity

Stomata of some species have an additional means of preventing rapid water loss; the epidermis loses water to the atmosphere directly and may not gain an equal amount of water back from the leaf tissue. As a result, stomatal apertures are narrower in dry air than in moist air, and leaf water content in moist air may be lower than in dry air. Schulze *et al.* (1972) found this phenomenon to occur in plants growing in a desert. Control of stomatal aperture by cuticular transpiration does not always occur. This uncertainty makes prediction of stomatal behaviour difficult.

Interaction between responses to carbon dioxide and abscisic acid

What do guard cells do when a low [CO_2] in the leaf calls for stomatal opening and the presence of ABA signals water stress? For an answer to this question I wish to return to stomata which are insensitive to CO_2. Zelitch (1969) reported that stomata did not respond to CO_2 in leaf sections floating on water; I observed that stomata of leaves of *Xanthium strumarium* stay open when the [CO_2] is raised. Similar observations could be made on other species (like *Gossypium hirsutum*). Such stomata can be sensitized to CO_2 if the level of ABA in the leaves is raised (Raschke 1974). Exogenous ABA administered through the transpiration stream is as effective as endogenous ABA produced in response to rapid transpiration (Raschke 1974) or to exposure to cold (as found in plants of *Xanthium strumarium*, Drake & Raschke 1974, in combination with unpublished work). Further work showed that in leaves of *Xanthium strumarium* the converse was also true: stomata did not respond to ABA in the absence of CO_2 (Raschke 1975*b*). Abscisic acid and CO_2 were simultaneously required for the modulation of stomatal aperture. These two messengers of the stomatal CO_2 and water feedback loops appear to act on sites in the metabolic network of guard cells which are close to each other and very likely in series. A study of the interactions between response to CO_2 and to ABA showed that the sensitization of stomata to ABA by CO_2 was not an on-off response but followed saturation kinetics with respect to CO_2. Half saturation occurred when [CO_2] in the vicinity of guard cells was about 200 cm^3/m^3. The enhancement by ABA of stomatal responses to CO_2 did not follow saturation kinetics exactly; apparent half-saturation occurred between 10^{-6} and 10^{-5} M (±)-ABA in the transpiration stream. Stomatal aperture is thus effectively modulated by the concentrations of the two feedback messengers of the two major control loops of the stomata. A quantitative study of the interaction has been conducted on only one species so far, namely *Xanthium strumarium* (Raschke 1975*b*). As expected, high [ABA] in the transpiration stream ($\geqslant 10^{-4}$ M (±)-ABA) overrides opening commands given to the guard cells in the absence of CO_2.

The simultaneous requirement of CO_2 and ABA for stomatal closure in *Xanthium strumarium* is the basis for speculating on the mechanism of CO_2 and ABA in guard cells. The observations could be explained if CO_2 is used by guard cells to form organic acids, as mentioned above. If the organic anions formed are not removed fast enough into the vacuole and if the H^+ ions are not exchanged fast enough for K^+ ions the cytoplasm of the guard cells will acidify. By analogy with processes occurring in roots it is postulated that this acidification causes a solute loss from the vacuoles and stops the production of further acids. Abscisic acid is suspected of blocking the active expulsion of H^+ from guard cells and would therefore, enhance the acidification

of the cytoplasm in the presence of CO_2. In the absence of CO_2 this acidification cannot occur and then ABA is ineffective. Conversely, in species possessing a strong H^+ pump in guard cells, CO_2 can initiate stomatal closure only if ABA inhibits the expulsion of H^+ from guard cells. Export of H^+ is necessary for an import of K^+. It is known that the fungal toxin fusicoccin stimulates the expulsion of H^+ from plant cells (Marrè, Lado, Ferroni & Ballarin Denti 1974), and Squire & Mansfield (1972) have shown that fusicoccin overcomes the inhibitory action of ABA on stomata. This finding could be looked upon as evidence in favour of the hypothesis presented above.

Effects of stomatal closure on carbon dioxide uptake and water loss

Stomatal movement affects the uptake of CO_2 and transpiration to different degrees. Transpiration is nearly linearly related to stomatal conductance. (The deviation from linearity results from the cooling effect of transpiration). The relation between stomatal conductance and the uptake of CO_2 is more complicated because the assimilation of CO_2 follows approximately saturation kinetics with respect to the intercellular $[CO_2]$. Since the $[CO_2]$ at which CO_2 assimilation is half saturated is at the level which normally occurs in illuminated leaves (200–300 cm^3/m^3) stomatal movements affect CO_2 uptake relatively less than transpiration. With larger apertures, the effect may be of the order of a few percent only. But modulation of CO_2 uptake by stomatal movements increases rapidly as stomata become narrower. Quantitatively, the sensitivity of CO_2 uptake to stomatal movements is inversely proportional to the square of stomatal conductance.

If respiration as well as the drop in $[CO_2]$ from the walls of the mesophyll cells to the chloroplasts are neglected, CO_2 assimilation follows saturation kinetics with respect to the intercellular $[CO_2]$:

$$\frac{P}{P_{max}} = \Pi = \frac{1}{1+K_p/C_w} \tag{1}$$

with P, rate of CO_2 assimilation; P_{max}, maximal rate of CO_2 assimilation; Π, relative rate of assimilation; K_p, $[CO_2]$ at half saturation of CO_2 assimilation; C_w, $[CO_2]$ at the cells walls of the mesophyll.

The $[CO_2]$ in the intercellular spaces depends on the $[CO_2]$ in the atmosphere and on the concentration drop across the boundary layer around the leaf and the stomata.

$$C_w = C_a - Pk_s^{-1}, \tag{2}$$

with C_a, $[CO_2]$ in the atmosphere; k_s effective conductance of the stomata (and the boundary layer; under most conditions the drop across the boundary layer is a small fraction of the total drop).

The sensitivity of the $[CO_2]$ at the cell walls to changes in conductance is then

$$\frac{\partial C_w}{\partial k_s} = \frac{P}{k_s^2}, \tag{3}$$

and the sensitivity of CO_2 assimilation to changes in the $[CO_2]$ at the cell walls is

$$\frac{dP}{dC_w} = \frac{K_p}{(K_p+C_w)^2}. \tag{4}$$

From equations (1) and (2), particular examples can be computed for the effects of stomatal movements on CO_2 assimilation in comparison with transpiration. For the example given in figure 2, coefficients were used derived from measurements on leaves of *Xanthium strumarium*. At $k_s > 0.5$ cm s^{-1} changes in stomatal aperture affect transpiration but hardly change the assimilation of CO_2. In other words, stomatal narrowing reduces the amount of water spent per amount of CO_2 taken up virtually without reducing the absolute amount of the latter. The situation is different at $k_s < 0.2$ cm s^{-1}; CO_2 uptake is greatly reduced by any further reduction in stomatal aperture. The transpiration ratio diminishes from close to 300 molecules of water lost per molecule of CO_2 absorbed at a conductance of 1.4 cm s^{-1} to about 50 at 0.1 cm s^{-1}. Saturation kinetics of CO_2 assimilation must be considered, in addition to the consideration of the energy exchange of the leaf (Cowan & Troughton 1971), when assessing the relative roles of stomatal movement in transpiration and assimilation.

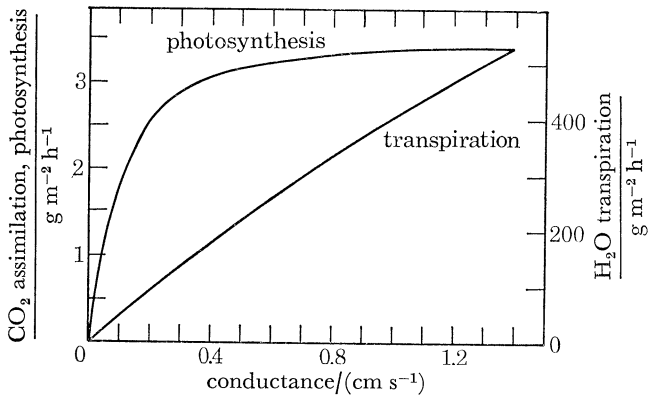

FIGURE 2. Computed dependence of CO_2 assimilation and transpiration on stomatal conductance (k_s) using coefficients obtained from measurements on leaves of *Xanthium strumarium*. K_p: $[CO_2]$ in the intercellular spaces at half-saturation of CO_2 assimilation (= 200 cm^3/m^3); P_{max}: maximal rate of CO_2 assimilation (= 5.6 g m^{-2} h^{-1}). Transpiration was computed according to Raschke (1958). Conditions for transpiration: saturation deficit of the air 1 kPa, air temperature 20 °C, radiation balance of the leaf 420 W m^{-2}, total conductance of the boundary layers 10 cm s^{-1}. $[CO_2]_{air}$ = 350 cm^3/m^3.

TABLE 1. TRANSPIRATION RATIOS OF DETACHED LEAVES OF *XANTHIUM STRUMARIUM* BEFORE AND AFTER ADDITION OF (±) – ABA TO THE IRRIGATION WATER (FROM RASCHKE 1974)

[ABA]	transpiration ratio g H_2O/g CO_2		relative transpiration	relative net photosynthesis
M	before	after	(%)	(%)
10^{-7}	62	63	102	100
10^{-6}	59	60	102	97
10^{-5}	68	38	49	86
10^{-4}	68	31	25	54

The improvement in water-use efficiency by stomatal closure in response to ABA can be demonstrated experimentally (see table 1). The reduction in the transpiration ratio by ABA also appears in long term experiments. Jones & Mansfield (1972) report that the transpiration ratio of young barley plants was reduced by 30% in the ABA treatments while the dry weight was only 5% less than that of the controls after 9 days. The most striking example was given by Mizrahi, Scherings, Malis Arad & Richmond (1974) who grew wheat seedlings in a dry

environment without irrigation and found after 32 days of cultivation that the plants sprayed with 3.8×10^{-4} M ABA every three days were heavier than the control plants by as much as 47 %.

Shifts in priority in the stomatal regulation of gas exchange

Stomata resolve their dilemma (*a*) by going through several stages of sensitivity to CO_2 depletion and water stress and (*b*) by affecting water-vapour loss more than CO_2 uptake, particularly at large stomatal apertures. The latter phenomenon is fortuitous; it is caused by the saturation kinetics of CO_2 uptake with respect to the $[CO_2]$ in the intercellular spaces. The advantage of changing sensitivities is best explained by the example of *Xanthium strumarium* whose stomata lose their sensitivity to CO_2 as they open in the morning. Theoretically, the rate of photosynthesis is never maximal as long as stomata are sensitive to CO_2. A measurable drop in CO_2 concentration across the stomatal pore is necessary to enable the guard cells to measure the current of CO_2 molecules flowing into the leaf in order to adjust stomatal permeability for CO_2. It could, therefore, be beneficial to a plant to open its stomata to maximal aperture as long as water supply was ample. This wide opening might also be beneficial in a humid environment in order to maintain a transpiration stream necessary for the transport of nutrients from root to shoot. If transpiration increases during the course of the day, the principle of optimization may shift from maximal photosynthesis to an improvement of the water-use efficiency. The compromise between the optimization of photosynthesis and water-use efficiency is made on a sliding scale, depending on the amount of ABA formed and its interaction with CO_2. Each leaf can respond individually. If these regulatory actions are insufficient to maintain the water balance of the plant, more ABA will be formed which leads to a further solute loss from the guard cells. Stomata will close despite a low partial pressure of CO_2 in the leaf. This is an idealized interpretation of the experiments conducted with *Xanthium strumarium*. Other species may not have the full scale of stomatal responses at their disposal. For instance, I have never seen stomata of *Zea mays* which were insensitive to CO_2. On the other hand, observations of Stålfelt (1959) of a promotion of hydroactive closing by CO_2 in *Vicia faba* and *Rumex sativa* indicate that the interaction between effects of CO_2 and ABA is not restricted to *Xanthium strumarium*. Much work remains to be done on whole plants and on partial mechanisms to understand how the stomatal feedback system compromises between the requirements of CO_2 assimilation and maintenance of water balance like an industrial 'adjustable control system' which is able to choose between several principles of optimization.

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