

Gain of the Feedback Loop Involving Carbon Dioxide and Stomata

THEORY AND MEASUREMENT¹

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ABSTRACT

The physiological and physical components of the feedback loop involving intercellular CO₂ concentration (*c_i*) and stomata are identified. The loop gain (*G*) is a measure of the degree of homeostasis in a negative feedback loop [the expression 1/(1-*G*) represents the fraction to which feedback reduces a perturbation]. Estimates are given for the effects of *G* on responses of stomata and *c_i* to changes in ambient CO₂ concentration, light intensity, and perturbations in the water relations of a leaf. At normal ambient CO₂ concentration, the gain of the loop involving stomatal conductance and *c_i* was found to be -2.2 in field-grown *Zea mays*, -3.6 if plants of this species were grown in a growth chamber, and zero in well watered *Xanthium strumarium* in the vegetative state.

In land plants, both CO₂ and water vapor exchange are affected by the movement of the same turgor-operated valves, the stomata. Assimilation of CO₂ from the atmosphere requires maximal gas exchange; the prevention of excessive water loss demands that gas exchange be kept minimal. In order to balance these opposing priorities, plants make use of at least two major feedback loops affecting stomatal conductance, one responding to the need for CO₂, the other one limiting water loss (16). We wished to describe the action of these two major feedback loops in quantitative terms. We shall describe a possible approach to this goal and exemplify it by measurements conducted on leaves of two species that differ in stomatal behavior with respect to CO₂.

In order to maximize their efficiency of water use, plants must synchronize stomatal opening with the CO₂ requirement of the assimilatory tissue. One means of achieving this is to have the stomata sense the CO₂ depletion of the intercellular spaces (16). When this is the case a reduction of the [CO₂] inside the leaf, *c_i*, causes an increase in stomatal conductance, *g* (measured in mol m⁻² sec⁻¹; units used in this paper are discussed in the Appendix), and more CO₂ diffuses into the leaf which tends to increase *c_i*. This is a feedback loop because a causal chain exists that may be summarized by *c_i* → *g* → *c_i* (where → is read as "affects"). In technological systems the feedback is a conscious addition by man and is readily distinguishable from the process it regulates (10). Causal loops occur also in many biological processes; but some of these loops are rather trivial and hardly warrant the term feedback. In an attempt to recognize feedback involved in the regulation

within a biological system we first have to identify the important processes requiring regulation and then determine whether and how this regulation is accomplished. In order to assess the importance of various interacting regulatory loops and the biological consequences of this regulation, it is essential to measure the degree of regulation that occurs. In classical feedback theory the gain of the feedback loop, which is the amplification that a signal receives moving around a feedback loop, is the appropriate measure (3, 5). Determining the gain of a stomatal feedback loop is particularly important since dynamic and steady-state stomatal behavior depend on the functioning of various feedback loops. Results of most experiments on stomata are ambiguous because they do not allow one to distinguish feedback loops. Recognizing these loops and measuring their properties will aid in understanding stomatal behavior and interpreting experimental results. In this paper, we examine the loop gains of the individual components of these loops. We develop appropriate theory and techniques for measuring the gains and then present results obtained with *Zea mays* (a C₄ plant whose stomata are always sensitive to CO₂) and *Xanthium strumarium* (a C₃ plant whose stomata can be insensitive to CO₂). Applications of the concept of loop gain are discussed.

THEORY

We first consider the steady-state relationships between the intercellular CO₂ concentration, *c_i*, and the conductance of stomata to diffusion of water vapor, *g*.

We know that *c_i* depends on the ambient CO₂ concentration, *c*, the stomatal conductance, *g*, and the net rate of assimilation, *A*. The following partial differential equation describes changes in *c_i*:

$$dc_i = (\partial c_i / \partial c)_{A,g} dc + (\partial c_i / \partial g)_{A,c} dg + (\partial c_i / \partial A)_{g,c} dA \quad (1)$$

We also know that stomatal conductance and net assimilation depend on metabolic and environmental factors. Conductance is a function of *c_i*, light quality and perhaps intensity, *I*, concentration of ABA (16), and other not necessarily unimportant factors which we will call *n_g*. Assimilation rate is a function of *c_i*, *I*, temperature, *T*, and other factors which we will call *n_A*. Both *g* and *A* can now be expressed in terms of the following two partial differential equations:

$$dg = (\partial g / \partial c_i)_{I,[ABA],n_g} dc_i + (\partial g / \partial I)_{c_i,[ABA],n_g} dI + (\partial g / \partial [ABA])_{c_i,I,n_g} d[ABA] + (\partial g / \partial n_g)_{c_i,I,[ABA]} dn_g \quad (2)$$

$$dA = (\partial A / \partial c_i)_{I,T,n_A} dc_i + (\partial A / \partial I)_{c_i,T,n_A} dI + (\partial A / \partial T)_{c_i,I,n_A} dT + (\partial A / \partial n_A)_{c_i,I,T} dn_A \quad (3)$$

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Initially, for the sake of simplicity, we assume that all factors except intercellular CO₂ concentration, stomatal conductance, assimilation rate, and ambient CO₂ concentration are held constant. The partial differentials $\partial g/\partial c_i$ and $\partial A/\partial c_i$ become total differentials and equations 2 and 3 simplify to:

$$dg = (dg/dc_i)dc_i \tag{4}$$

$$dA = (dA/dc_i)dc_i \tag{5}$$

The two feedback loops of interest to us here are indicated in Figure 1, which is a diagrammatic representation of equations 1, 4, and 5. One loop involves conductance and the other assimilation rate and both affect the intercellular [CO₂]. We define dg/dc_i and dA/dc_i as the physiological gains of the conductance loop and the assimilation loop, respectively. These gains are so named because they are dependent on metabolic processes in the guard cells and assimilatory tissue. The partial differentials $(\partial c_i/\partial g)_{A,c}$ and $(\partial c_i/\partial A)_{g,c}$ will be called the physical gains of the conductance loop and the assimilation loop, respectively. The magnitudes of these gains depend on physical characteristics of the stomatal pore as expressed in the following Ohm's Law type of relationship:

$$c_i = c - 1.6A/g \tag{6}$$

The factor 1.6 is the ratio of the diffusivities of water vapor and CO₂ in air.

The open loop gains of the feedback loops involving stomatal conductance and assimilation are now defined as the products of their respective physiological and physical gains. In both loops this product is usually negative in the steady-state. The open loop gain is so called because if a change, dc_i , were imposed causing a change, $(dg/dc_i)dc_i = dg$ in conductance, and if it were possible to open the loop artificially and examine the change in c_i , $(\partial c_i/\partial g)_{c,A}dg$, resulting from the change in conductance, under conditions not affecting the original perturbation of c_i , that change would be $(\partial c_i/\partial g)_{c,A}(dg/dc_i)dc_i$. Thus, the amplification that the small perturbation has undergone after one transit around the feedback loop is $(\partial c_i/\partial g)_{c,A}(dg/dc_i) = G_g$ (10). An analogous example can be worked through for the assimilation loop, and here the amplification would be $(\partial c_i/\partial A)_{c,g}(dA/dc_i) = G_A$. It is important to note that it does not matter which factor is initially perturbed within the loop. The amplification after one transit around the loop will always be equal to the loop gain.

Apart from being independent of the way the loop is broken up the measure is also independent of the choice of units of the components. For example, if instead of conductance we chose stomatal resistance as our measure of stomatal opening, the new measures of the physiological and physical gains would have different magnitudes and opposite signs, but the product of the two gains would have the same magnitude and sign as that obtained using conductance.

We now examine how the open loop gain comes into play in the normal closed loop situation. Returning to equation 1, we see that dg and dA can be replaced by using equations 4 and 5. This yields the following equation:

$$dc_i = \left(\frac{\partial c_i}{\partial c}\right)_{g,A} dc + \left(\frac{\partial c_i}{\partial g}\right)_{c,A} \left(\frac{dg}{dc_i}\right) dc_i + \left(\frac{\partial c_i}{\partial A}\right)_{c,g} \left(\frac{dA}{dc_i}\right) dc_i \tag{7}$$

Equation 7 can then be rearranged:

$$dc_i = \frac{(\partial c_i/\partial c)_{g,A} dc}{1 - (\partial c_i/\partial g)_{c,A}(dg/dc_i) - (\partial c_i/\partial A)_{c,g}(dA/dc_i)} = \frac{(\partial c_i/\partial c)_{g,A} dc}{1 - G_g - G_A} \tag{8}$$

We see that the open loop gains of both the conductance loop and the assimilation loop appear in the denominator of this equation. Thus, in effect, a change in intercellular CO₂ concentration that one might expect to see if one perturbed the ambient CO₂ without

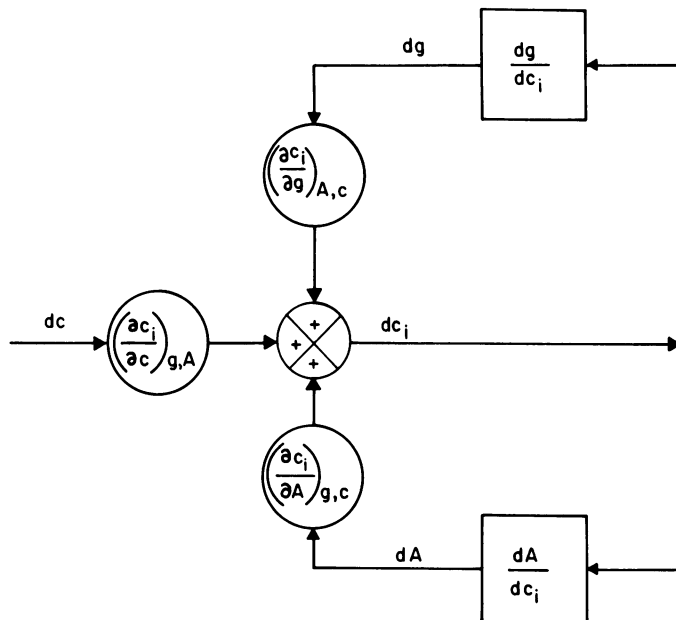


FIG. 1. Diagrammatic representation of equations 1, 4, and 5, describing the feedback loops involving stomata, net assimilation rate, and CO₂. All factors affecting stomata and photosynthesis but not mentioned are held constant. Changes in ambient CO₂ concentration c , stomatal conductance, g , and rate of CO₂ assimilation, A , affect the intercellular CO₂ concentration, c_i , which in turn affects stomatal conductance and rate of CO₂ assimilation. The differentials dg/dc_i and dA/dc_i are the physiological gains of the conductance loop and the assimilation loop, respectively; the partial differentials $(\partial c_i/\partial g)_{A,c}$ and $(\partial c_i/\partial A)_{g,c}$ are the physical gains of the conductance loop and the assimilation loop, respectively. The product of the physiological and the physical gain of each loop is the open loop gain.

modifying conductance or assimilation will be modified by $1/(1 - G_g - G_A)$. Since both loop gains are normally negative in the steady-state, the denominator becomes greater than 1, thus reducing the expected change in c_i . Therefore, the greater the absolute value of the loop gain becomes, the more "resistant" c_i is to change.

We can also look at the effect of the open loop gain on the response of conductance. If we take equation 4 and substitute for dc_i using equation 8 we obtain the following:

$$dg = \frac{(dg/dc_i)(\partial c_i/\partial c)_{g,A} dc}{1 - G_g - G_A} \tag{9}$$

A change in g that one would expect from a change in ambient CO₂ concentration is also modified by $1/(1 - G_g - G_A)$. From equations 8 and 9 one can see that both conductance and intercellular CO₂ concentration are under the influence of feedback. The degree to which this occurs depends on the magnitudes of the loop gains.

Thus far, we have considered only the influence of a change in ambient CO₂ concentration on conductance or intercellular CO₂ concentration. Let us now examine the effect of a perturbation of conductance. Consider for example the effect of a change in water potential, $d\psi$, that would have an effect $(\partial g/\partial \psi)_{c_i} d\psi$ on conductance if $[CO_2]_i$ did not change. We denote this as $(dg)_{c_i}$ i.e.:

$$(dg)_{c_i} = (\partial g/\partial \psi)_{c_i} d\psi \tag{10}$$

What happens when this effect is modified by the feedback loops involving CO₂? With all variables except conductance and intercellular CO₂ concentration kept constant and ψ substituting for n_g , equation 2 becomes:

$$dg = (dg)_{c_i} + (\partial g/\partial c_i)_{\psi} dc_i \tag{11}$$

and from equations 1 and 5, the change in $[CO_2]$ is:

$$dc_i = (\partial c_i / \partial g)_{c,A} dg + G_A dc_i$$

which on rearrangement yields:

$$dc_i = \frac{(\partial c_i / \partial g)_{c,A} dg}{1 - G_A} \quad (12)$$

Combining equation 12 with equation 11 and defining

$$G_g^\psi = \left(\frac{\partial c_i}{\partial g} \right)_{c,A} \left(\frac{\partial g}{\partial c_i} \right)_\psi$$

we find that the change in intercellular $[CO_2]$ is:

$$dc_i = \frac{(\partial c_i / \partial g)_{c,A} (dg)_{c_i}}{1 - G_g^\psi - G_A} \quad (13)$$

Again both loops tend to minimize changes in c_i since the gains are usually negative in the steady-state. Substituting equation 13 back into equation 11 we find that the change in conductance is:

$$dg = \frac{(dg)_{c_i}}{1 - G_g^\psi / (1 - G_A)} \quad (14)$$

As before, the greater the absolute value of the conductance loop gain, G_g^ψ , the more resistant g is to change; but this time the assimilation loop acts in a different way. As far as "conductance control" is concerned, the assimilation loop is a subloop with negative feedback that tends to reduce the effective physical gain. Obviously one could continue to identify more subloops in the carbon metabolism of the leaf (8). Alternatively we could have subsumed the assimilation loop into the effective physical gain of the conductance loop. In fact:

$$(\partial c_i / \partial g)_c = (\partial c_i / \partial g)_{c,A} / (1 - G_A) \quad (15)$$

and if the loop gain of the conductance loop were defined as:

$$G_g' = (\partial c_i / \partial g)_c (dg / dc_i) \quad (16)$$

then the response of conductance to a perturbation not directly affecting the assimilation loop would be:

$$dg = \frac{(dg)_{c_i}}{1 - G_g'}$$

which is apparently simpler than equation 14. In any examination of feedback loops in biology one must arbitrarily choose the degree of fineness desired in the analysis of the structure to be examined.

Finally we examine the effects of a change, dI , in light quality or intensity, I , that may involve direct effects on both conductance and assimilation. We rewrite equations 4 and 5 as:

$$dg = (\partial g / \partial c_i)_i dc_i + (\partial g / \partial I)_{c_i} dI \quad (17)$$

and

$$dA = (\partial A / \partial c_i)_i dc_i + (\partial A / \partial I)_{c_i} dI \quad (18)$$

These two expressions for dg and dA can then be substituted in equation 1. If the atmospheric $[CO_2]$ is constant ($dc = 0$) the change in c_i is:

$$dc_i = \frac{(\partial c_i / \partial g)_A (\partial g / \partial I)_{c_i} dI + (\partial c_i / \partial A)_A (\partial A / \partial I)_{c_i} dI}{1 - \hat{G}_g - \hat{G}_A} \quad (19)$$

where the gains are defined by $\hat{G}_g = (\partial c_i / \partial g)_A (\partial g / \partial c_i)_i$ and $\hat{G}_A = (\partial c_i / \partial A)_A (\partial A / \partial c_i)_i$. It can be seen that light indirectly affects c_i by changing the conductance and assimilation rate. This effect is modified by the loop gains of both the conductance and assimilation loops. For example, if $\hat{G}_g = -1.5$ and $\hat{G}_A = -0.5$ the denominator becomes 3 and the effect of a change of light intensity on c_i would be 33% of what one would expect if there were no feedback.

Similarly, we can look at the effect of light on conductance. Substituting equation 19 into equation 17, one can derive the following:

$$dg = dI \left[\frac{(\partial g / \partial I)_{c_i} - (\partial g / \partial I)_{c_i} (\partial A / \partial c_i)_i (\partial c_i / \partial A)_A + (\partial g / \partial c_i)_i (\partial c_i / \partial A)_A (\partial A / \partial I)_{c_i}}{1 - \hat{G}_g - \hat{G}_A} \right] \quad (20)$$

From the equation, one can see that light affects conductance in three ways. There is the direct effect and the two indirect effects which operate via the CO_2 feedback loops. The loop gains can have a large influence on all three effects.

It is apparent that the feedback loops involving c_i , g , and A have a significant influence on the response of stomata to environmental variables and the control of intercellular CO_2 concentration. By measuring or calculating the various gains we can now gain a better understanding of how stomata respond to environmental perturbations, how these responses vary under different conditions, and how the responses affect both the assimilation and water status of the plant (since water vapor exchange is also affected by stomatal movement, dg also affects water status and the treatment can be expanded to describe this effect).

MATERIALS AND METHODS

Plants. Plants of *Xanthium strumarium* L. were grown in a soil mixture in the greenhouse of the MSU-ERDA Plant Research Laboratory. The strain used is a strict short day plant. The natural daily light period was extended to 20 hr day⁻¹ by supplementary illumination with 0.3 w m⁻² from Sylvania Gro-lux fluorescent lamps so that the plants were kept in the vegetative state. Temperature maxima in the greenhouse were between 23 and 29 C; the RH was between 70 and 80%. The plants were pruned to carry five or six leaves. Fully expanded leaves from 2-month-old plants were used. The fifth leaf from the apex was detached under water after submerging the top of the plant for about 2 min (to produce temporary hydropassive stomatal closure); the cut end of the petiole was kept under water throughout the experiment.

Plants of *Zea mays* L. were grown either in the field or in growth chambers. Leaves 4 and 5 (from the top) of field-grown plants (cv. Michigan 572) were cut in August, when the plants were 3 months old; the plants had tassels and were watered 24 hr before the leaves were taken. For the measurement of gas exchange, the leaf lamina was trimmed to an area measuring 4 to 5 cm × 15 to 18 cm.

Plants of the cultivar Michigan 500 were cultivated in a sand-Vermiculite mixture in a high light intensity growth chamber. They were illuminated for 20 hr day⁻¹ (peak irradiance was 240 w m⁻²) from a combination of General Electric lamps H 400DX33-1 (mercury vapor) LU 400 (high temperature discharge sodium vapor). The day temperature was 31 C and the night temperature was 20 C; the RH was 70%. The sixth leaf from the base of 1-month old plants was used.

Measurement of Gas Exchange. Water-jacketed chambers were attached to the upper and lower surface of each detached leaf, and water of 22.5 C was circulated through the jackets. Four pairs of chambers were available for simultaneous measurements on four leaves. Air of known water vapor (dew point = 18.5 C) and CO_2 contents was passed at 50 liters hr⁻¹ through these chambers. The exposed leaf surface was 2.44 cm² in each chamber. Formation of endogenous ABA in the leaf tissue in response to low water potentials was kept low by trimming all parts of the leaf not covered by the gas exchange chambers, thereby reducing the transpiring area (flag-shaped leaves; 15).

Changes in gas composition were measured with differential IR gas analyzers (Uras 2; Hartmann und Braun, Frankfurt, a.M., Germany). The absolute CO_2 content of the air was monitored with an additional Uras. Thermocouples were used to measure leaf temperature and the temperature of the condenser setting the

dew point of the air supplied to the chambers.

The leaves were illuminated with 340 w m^{-2} of photosynthetically usable light (corresponding to $1.58 \text{ mE m}^{-2} \text{ sec}^{-1}$). The light source was a water-cooled 6,000-w xenon arc lamp (Osram XBF 6000 W/1) behind IR absorbing glass (Corning 4600) and neutral density filters (Plexiglas No. 800; Röhm und Haas, Darmstadt, Germany). Voltages from the thermocouples and the gas analyzers were amplified and fed into a Hewlett-Packard 2100S minicomputer which computed the time courses of the exchange of CO_2 and water vapor through the upper and lower epidermes, epidermal conductances to water vapor exchange, and intercellular CO_2 concentrations (= CO_2 concentration at the sites of evaporation). The intercellular CO_2 concentrations presented in this paper were calculated using equation 6. Values for assimilation and conductance are combined values from the upper and lower epidermis. Results are from a 20-min time span after the stomata had reached a steady-state. Readings for each leaf were taken once every 5 min.

Measurements of Various Gains. The basic experiment consisted of varying the ambient CO_2 concentration, c , from 0 to about $600 \mu\text{l l}^{-1}$ over a time span of 5 hr and measuring the steady-state values of the conductance, g , net assimilation rate, A , and intercellular CO_2 concentration, c_i , at each value of c . From these values, plots of conductance and net assimilation rate versus c_i were made. Using these plots and a rearranged equation 6, plots showing the relationship between c_i and c were drawn. An operating point was chosen at some particular c , and from the above mentioned graphs, values were obtained for g , A , and c_i at this point. It is necessary to choose an operating point because the physical gains $(\partial c_i / \partial g)_{c,A}$ and $(\partial c_i / \partial A)_{c,g}$ are defined at some particular constant c . As will be seen, choosing different operating points results in different values for the various gains. From the plots of g and A versus c_i , the physiological gains of the conductance (dg/dc_i) and assimilation (dA/dc_i) loops were determined by measuring the slope of these curves at the operating point.

The physical gains at the operating point were obtained from the following equations which were derived from equation 6:

$$(\partial c_i / \partial g)_{A,c} = 1.6 A/g^2 \quad (21)$$

$$(\partial c_i / \partial A)_{c,g} = -1.6/g \quad (22)$$

$$(\partial c_i / \partial c)_{A,g} = 1 \quad (23)$$

The open loop gain of each loop is then the product of the physical and physiological gains of that loop.

Example: calculation of the physiological, physical, and loop gains for the feedback loops involving conductance, assimilation, and c_i . We will use *Zea mays* at an operating point of $c = 300 \mu\text{l l}^{-1}$. Using Figure 2 we determine that:

$$\begin{aligned} c_i &= 137 \mu\text{l l}^{-1} \\ A &= 27.5 \mu\text{mol m}^{-2} \text{sec}^{-1} \\ g &= 0.254 \text{ mol m}^{-2} \text{sec}^{-1} \end{aligned}$$

The calculations of the physiological gains are shown in Figure 2:

$$\begin{aligned} (dA/dc_i) &= 38.7 \times 10^{-3} \text{ mol m}^{-2} \text{sec}^{-1} \\ (dg/dc_i) &= -1700 \text{ mol m}^{-2} \text{sec}^{-1} \end{aligned}$$

The physical gains would be:

$$\begin{aligned} (\partial c_i / \partial A)_{c,g} &= -1.6 / (0.254 \text{ mol m}^{-2} \text{sec}^{-1}) \\ &= -6.30 \text{ m}^2 \text{sec mol}^{-1} \end{aligned}$$

$$\begin{aligned} (\partial c_i / \partial g)_{A,c} &= (1.6)(27.5 \mu\text{mol m}^{-2} \text{sec}^{-1}) / (0.254 \text{ mol m}^{-2} \text{sec}^{-1})^2 \\ &= 0.682 \cdot 10^{-3} \text{ m}^2 \text{sec mol}^{-1} \end{aligned}$$

The loop gains would be:

$$\begin{aligned} G_n &= (dA/dc_i)(\partial c_i / \partial A)_{c,g} = -0.243 \\ G_g &= (dg/dc_i)(\partial c_i / \partial g)_{A,c} = -1.16 \end{aligned}$$

RESULTS AND DISCUSSION

Figure 2 shows how the intercellular $[\text{CO}_2]$, c_i , changed in leaves

of *Z. mays* and *X. strumarium* when the $[\text{CO}_2]$ in the ambient air was varied. Response curves of the photosynthetic uptake of CO_2 and of epidermal conductance are juxtaposed. If conductance is mainly determined by the degrees of stomatal opening, it can be seen that the stomata of *Z. mays* are sensitive to changes in c_i , whereas those from *X. strumarium* are virtually insensitive.

For evaluation, we have chosen three operating points (Table I). Operating point II is representative of conditions in a natural environment; operating points I and III are normally not encountered.

Using diagrams like the one presented in Figure 2 and Table I, we have calculated the physiological, physical, and loop gains at each operating point. The values for these gains are given in Table II. They include a comparison of values determined on leaves of plants of *Z. mays* grown in the field and those cultivated in growth chambers. Growth chamber plants were characterized by rates of photosynthesis less than half of those of field-grown plants and by a higher stomatal sensitivity to CO_2 .

We now examine in more detail the loop gains at operating point II and the significance that they have for the plant. The absolute values of the loop gains of both the conductance and assimilation loops for *Z. mays* are greater than those for *X. strumarium*. Going back to equation 8, we see that an imposed change in ambient c causes an equal change in c_i if no feedback exists (*i.e.* if the loop gains are zero). If we substitute the values of the loop gains for *X. strumarium* into this equation, we see that in a natural atmosphere (operating point II) a change in c would result in a change of c_i of $1/(1 + 0.08) = 0.92$ of that which would be expected without any feedback. For *Z. mays*, the equivalent result varies between 0.21 and 0.41, depending on the conditions under which the leaves grew. These gains are the slopes of the relationship between c_i and c in the topmost diagrams of Figure 2. A slope of 1, *i.e.* a rise of the curve at an angle of 45° at the point of interest, would indicate the absence of feedback; a horizontal line would occur if the negative gain was infinite. Clearly, there is greater regulation of c_i in *Z. mays* than there is in *X. strumarium*. We predict that the loop gains should also influence the response of stomata to other perturbations. In this case, the large G_g of *Z. mays* is not significantly offset by G_A and in growth chamber-grown plants the change in conductance resulting from a change in, say, water potential would be only 0.25 of that which would occur if there were no feedback operating; in the two leaves sampled from the field the fractions are 0.37 and 0.52 (equations 14 and 16; G'_g from Table II).

We now speculate on what significance these differences in gains might have to each plant. For *Z. mays*, the value of c_i at the normal c corresponds approximately to that found at the "break-point" (3) of the photosynthetic curve of C_4 plants; this is the point at which CO_2 assimilation saturates rather abruptly with respect to CO_2 (Fig. 2). Further increases in c_i caused by increased conductance would not increase net assimilation appreciably and would only lead to greater water loss. In *Z. mays*, a high gain of the conductance loop helps to husband water. In *X. strumarium*, as in other C_3 plants, increases in c_i always result in increases of net assimilation (within the range investigated). If a plant of this species is not suffering from water stress, it will benefit from keeping stomata as widely open as possible in order to obtain maximal photosynthesis. A low gain of the conductance loop is of advantage to leaves of *X. strumarium* under the condition of excellent water supply to the leaf tissue.

In feedback systems with delays (corresponding here to the response times of stomata to changes in $[\text{CO}_2]$) instability can arise when the loop gain is negative with a magnitude greater than unity (5, 10). Oscillations of stomatal conductance have been observed in leaves of *Z. mays* and ascribed to feedback involving CO_2 (1, 14). This explanation is consistent with the loop gains we determined: subsuming the gain of the conductance loop with that of the assimilation loop (equations 14 and 16) we obtain a gain, $G'_g = G_g / (1 - G_A)$, which in the leaves of *Z. mays* we tested

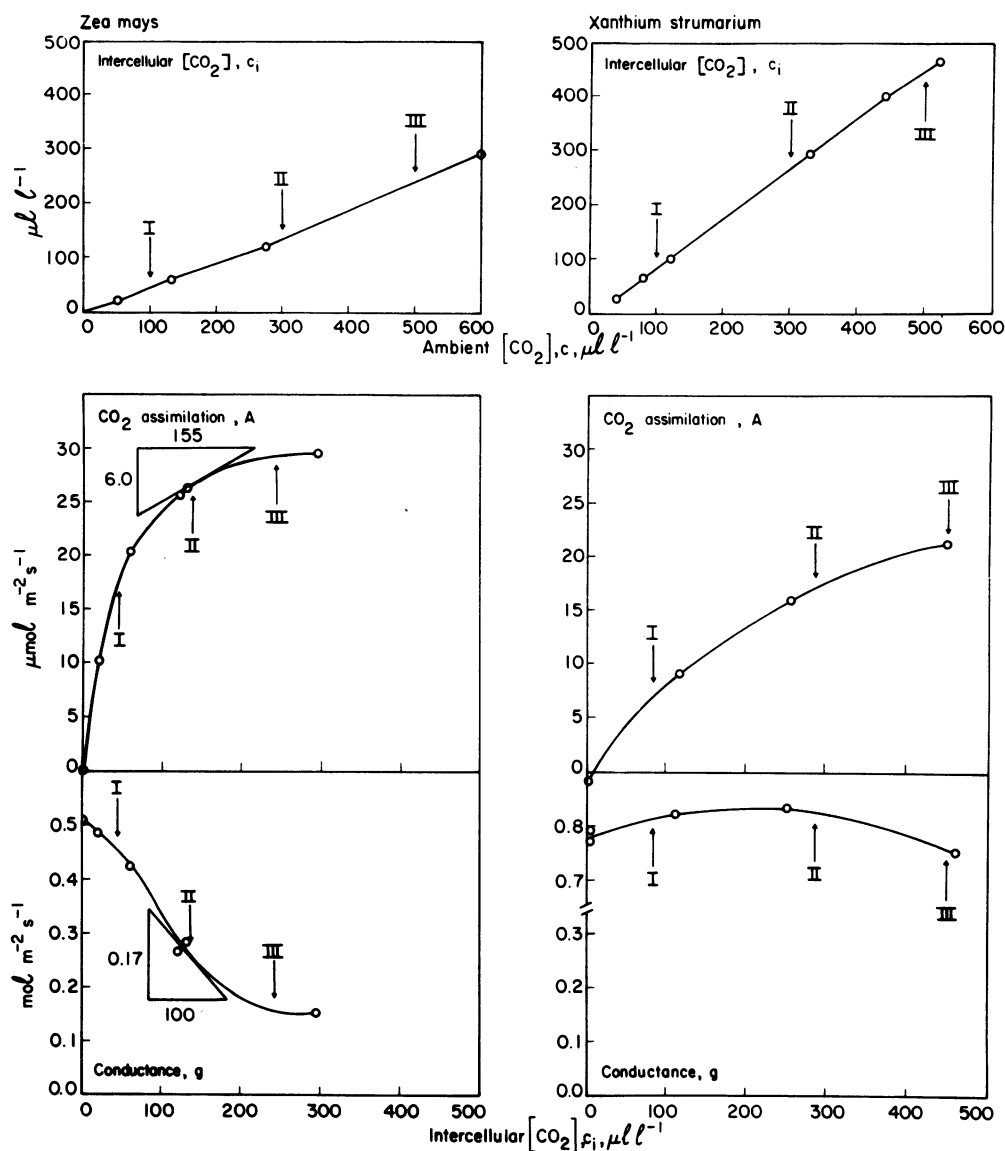


FIG. 2. Intercellular $[\text{CO}_2]_i$'s, c_i , occurring in leaves of *Z. mays* and *X. strumarium* at various ambient $[\text{CO}_2]_c$'s, c , and the relationship between c_i and the rate of CO_2 assimilation, A , and epidermal conductance for water vapor, g , in these leaves. Arrows I, II, and III indicate the three operating points at which loop gains were determined (Tables I and II). The assimilation and conductance plots for *Z. mays* contain examples for the determination of the physiological gains dA/dc_i ($6.0 \mu\text{mol m}^{-2} \text{sec}^{-1} + 155 \mu\text{l l}^{-1} = 38.7 \text{mmol m}^{-2} \text{sec}^{-1}$) and dg/dc_i ($0.17 \text{mol m}^{-2} \text{sec}^{-1} + 100 \mu\text{l l}^{-1} = 1,700 \text{mol m}^{-2} \text{sec}^{-1}$) at the operating point II. Data for *Z. mays* are from one leaf of a plant grown in the field (leaf 1 of Tables I and II); those for *X. strumarium* are means from four leaves from plants grown in a greenhouse. Conditions during measurement: quantum flux, $1.58 \text{mE m}^{-2} \text{sec}^{-1}$; air temperature, 22.5°C ; dew point, 18.5°C .

exceeded unity in magnitude in several cases, particularly when the leaves were exposed to normal $[\text{CO}_2]$ in the air (Table II).

The open loop gains we determined are in the range of those previously reported for other biological systems. The gain (or homeostatic index, as it is called in human and animal physiology) for the pupillary reflex arc of the eye has been measured as -0.16 . For the semicircular canal control of balance it is -0.1 ; for eye tracking, -1.1 ; for eye movement control, -4 (17). Larger homeostatic indices of -9 and -16 have been measured for body temperature control and for the respiratory CO_2 chemostats, respectively (17).

Stomatal loop gains, like gains of many other physiological systems are low; stomata moderate rather than eliminate disturbances. One can think of at least two reasons for a selective advantage of low gains. One is a reduced likelihood of the occurrence of instabilities in the operation of the control system; the other is the requirement to reconcile opposing priorities. For example, precise control of assimilation would mean imprecise

control of water use, and vice versa. A high gain in one loop makes additional regulation by another feedback loop ineffective if the second loop has a lower gain.

The simultaneous operation of several feedback loops has another aspect; it introduces uncertainty into the measurement of individual gains. Biological control systems are characteristically "rich," a description that the systems engineer uses when there are many interacting feedback loops. If these loops have negative loop gains, measurements of the individual gains are underestimates and estimates of the moderation of effects of perturbations are inaccurate. For example, if stomata are involved in feedback loops involving other unknown parameters, x , y , z , \dots , than our measured CO_2 loop gain needs to be multiplied by:

$$\left(1 - \frac{dx}{dg} \left(\frac{dg}{dx} \right)_{c, x, \dots} - \frac{dy}{dg} \left(\frac{dg}{dy} \right)_{c, x, \dots} - \frac{dz}{dg} \left(\frac{dg}{dz} \right)_{c, x, y, \dots} - \dots \right)$$

This necessity for correction was evident when we examined the

Table I. Values of intercellular $[CO_2]$, rate of CO_2 assimilation, and epidermal conductance for water vapor for three different ambient CO_2 concentrations in *Zea mays* and *Xanthium strumarium*

Plant Material	Operating Point	c Ambient $[CO_2]$ $\mu\ell\ell^{-1}$	c_i Intercellular $[CO_2]$ $\mu\ell\ell^{-1}$	A Assimilation $\mu\text{mol m}^{-2}\text{s}^{-1}$	g Conductance $\text{mol m}^{-2}\text{s}^{-1}$	
<i>Zea mays</i> from field, leaf 1 ¹	I	100	45	18.0	0.46	
	II	300	137	27.5	0.25	
	III	500	243	28.6	0.16	
	leaf 2	I	100	55	27.5	0.32
		II	300	90	30.2	0.21
		III	500	167	31.3	0.14
from growth chambers mean of 4 leaves	I	100	50	8.8	0.26	
	II	300	157	13.3	0.14	
	III	500	176	14.0	0.07	
<i>Xanthium strumarium</i> from greenhouse mean of 4 leaves ¹	I	100	83	6.5	0.72	
	II	300	287	16.0	0.72	
	III	500	450	21.0	0.65	

¹Data plotted in Fig. 2

Table II. Values of the loop gains and their components, the physiological and physical gains, at three different ambient CO_2 concentrations in *Zea mays* and *Xanthium strumarium*

Plant Material	Operating point	Physiological Gains		Physical Gains		Loop Gains		$\frac{G_g}{1-G_A}$	
		$\frac{dg}{dc_i}$	$\frac{dA}{dc_i}$	$\left(\frac{\partial c_i}{\partial g}\right)_{A,c}$	$\left(\frac{\partial c_i}{\partial A}\right)_{g,c}$	$\left(\frac{dg}{dc_i}\right)\left(\frac{\partial c_i}{\partial g}\right)$	$\left(\frac{dA}{dc_i}\right)\left(\frac{\partial c_i}{\partial A}\right)$		
		$\frac{\text{mol}}{\text{m}^2\text{s}}$	$\frac{10^{-3}\text{mol}}{\text{m}^2\text{s}}$	$\frac{10^{-6}\text{m}^2\text{s}}{\text{mol}}$	$\frac{\text{m}^2\text{s}}{\text{mol}}$	G_g	G_A	G_g'	
<i>Zea mays</i> from field, leaf 1	I	-1350	243	136	-3.47	-1.84	-0.84	-1.00	
	II	-1700	38.7	682	-6.30	-1.16	-0.24	-0.94	
	III	-244	6.2	1788	-10.00	-0.44	-0.06	-0.41	
	leaf 2	I	-3480	123	416	-4.92	-1.44	-0.60	-0.90
		II	-2070	37.6	1065	-7.51	-2.20	-0.28	-1.72
		III	-48	8.3	2765	-11.85	-1.33	-0.10	-1.21
from growth chamber, mean of 4 leaves	I	+200	119	198	-4.82	+0.04	-0.57	+0.02	
	II	-3330	18.4	1079	-11.40	-3.59	-0.21	-2.96	
	III	-2700	7.5	4814	-23.40	-13.00	-0.18	-11.0	
<i>Xanthium strumarium</i> from greenhouse mean of 4 leaves	I	+88	75.1	19.9	-2.21	0.00	-0.17	0.00	
	II	0	37.5	49.1	-2.21	0.00	-0.08	0.00	
	III	-750	13.3	79.0	-2.45	-0.06	-0.03	-0.06	

effect of subsuming the assimilation loop (equation 15). Similar considerations apply when stomatal moderation of water loss is to be included in the treatment. When, for instance in the surrounding of leaves of *Z. mays*, c is increased stomata close and the rate of evaporation, E , declines. The water status of the leaf improves, reducing through feedback the degree of stomatal closure.

The gain of this loop has two components: the environmental gain, dE/dg ; and the physiological gain $(\partial g/\partial E)_c$, (6). In our present study we neglected effects of this loop because we tried to keep the environmental gain small by using only a moderate humidity gradient and to minimize the physiological gain by using detached leaves and thus removing the drop in water potential through the rest of the plant.

Stomatal action provides an example of the reconciliation of opposing priorities through the simultaneous operation of several feedback loops. Optimal control theory (11) should be applicable to the analysis of stomatal functioning. If stomata indeed optimize the gas exchange of plants one should expect variations in the relative importance of the individual feedback loops in response to changes in the environment or in the physiological or devel-

opmental state of the plant. Measurements of loop gains provide a means to bring out such responses of the plant; in a subsequent paper we shall report an example (4).

APPENDIX ON UNITS

The diffusion of a gas in air is given in reference 2:

$$J = CD\nabla w \quad (A1)$$

where J ($\text{mol m}^{-2}\text{sec}^{-1}$) is the molar flux density, C (mol m^{-3}) is the molar density of the air plus gas, D ($\text{m}^2\text{sec}^{-1}$) the diffusivity of the gas in air, and w ($\text{mol/mol} = l/l$) is the mole fraction of the gas in air (2). Penman and Schofield (12) examined the volume fluxes per unit area J/C (m sec^{-1}) of CO_2 and water vapor into and out of leaves. They assumed diffusion in one dimension and introduced "equivalent lengths" L_s and L_a of the stomatal array and the external atmosphere. Forgetting the complexity of actual geometries, L_s can be thought of as the depth of a stomatal pore divided by the proportion of the area of leaf occupied by pores. Thus the rate of evaporation per unit area of leaf is:

$$J/C = D(w_i - w_a)/L_s + L_a \quad (\text{A2})$$

where w_i is the mol fraction of water in the air inside the leaf and w_a is that outside in the ambient air. If $D\Delta w$ is thought of as a potential difference and J/C as a flux then $L_s + L_a$ could be regarded as resistances, using an analogy with electrical resistors.

Raschke (13) examined the fluxes of latent and sensible heat, $\text{cal cm}^{-2} \text{min}^{-1}$, resulting from gradients of enthalpy, cal cm^{-3} , and resistances with the dimension min cm^{-1} emerged.

Gaastra (7) considered the volume flux per unit area (cm sec^{-1}) of CO_2 into a leaf as resulting from an effective gradient of mol fraction of CO_2 in air and resistances with dimensions sec cm^{-1} again emerged. These resistances were effective lengths divided by a diffusivity.

We see then that mass flux densities of water, E , could be described by:

$$E = M_w J = M_w C(e_i - e_a)/Pr' \quad (\text{A3})$$

where M_w is the molecular weight of water, e_i and e_a are partial pressures of vapor inside the leaf and in the ambient air, respectively, and r' is the sum of the resistances. This equation is derived assuming that the total pressure, P , is uniform throughout. Equation A3 may be rewritten as:

$$E = M_w(e_i - e_a)/RT r' \quad (\text{A4})$$

where R is the gas constant and T the temperature.

The error involved in using leaf or air temperature here in what is not actually an isothermal system is small.

Equation A4 may be rewritten in terms of gradient in absolute humidity:

$$E = (X_i - X_a)/r' \quad (\text{A5})$$

where X_i and X_a (g cm^{-3}) are the absolute humidities inside and outside the leaf. This time the isothermal assumption is obscured, but the potential errors are further confounded (3).

A drawback of the above formulation of resistance (equations A3, A4, and A5) is that it introduces dependences on pressure and temperature which have nothing to do with changes in the stomatal geometry. This is because diffusivity is given by:

$$D = D_0(T/273)^{1.75}(760/P)$$

where D_0 is the diffusivity at 273 K and a pressure P of 760 mm Hg (9). Recognizing this, Cowan (3) introduced a new measure of resistance, r , equal to L/CD . This removes the intrinsic pressure dependence and reduces the temperature dependence of resistance. The use of molar fluxes and mole fractions then gives the equation defining resistance a particularly simple form:

$$J = (w_i - w_a)/r$$

Similar considerations apply to the fluxes of CO_2 and sensible heat.

We have chosen to adopt this new formulation for resistance, r , and conductance, g ($= 1/r$). The relationship between this new measure of conductance, g ($\text{mol m}^{-2} \text{sec}^{-1}$), and the previous measure, g' (m sec^{-1}) ($= 1/r'$) is given in reference 3:

$$g = Cg'$$

where C is the molar density of air (mol m^{-3} ; ref. 3). At a temperature of 25 C and atmospheric pressure a conductance of $0.4 \text{ mol m}^{-2} \text{sec}^{-1}$ corresponds to 1 cm sec^{-1} . The new conductance unit has the dimension of a permeability (flux per unit potential difference).

Mol fractions of water vapor and CO_2 in air are usefully scaled by using the units ml l^{-1} and $\mu\text{l l}^{-1}$, respectively, giving rates of evaporation and assimilation of convenient magnitude measured as $\text{mmol m}^{-2} \text{sec}^{-1}$ and $\mu\text{mol m}^{-2} \text{sec}^{-1}$, respectively.

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LITERATURE CITED

1. APEL IP 1967 Über rhythmisch verlaufende Änderungen in der CO_2 -Aufnahme von Blättern. *Ber Deutsch Bot Ges* 80: 3-9
2. BIRD RB, WE STEWART, EN LIGHTFOOT 1963 Transport Phenomena. John Wiley & Sons, New York
3. COWAN IR 1977 Stomatal behaviour and environment. *Adv Bot Res* 4: 117-227
4. DUBBE DR, GD FARQUHAR, K. RASCHKE 1978 Effect of abscisic acid on the gain of the feedback loop involving carbon dioxide and stomata. *Plant Physiol* 62: 413-417
5. FARQUHAR GD 1973 A study of the responses of stomata to perturbations of environment. PhD thesis, Australian National University, Canberra
6. FARQUHAR GD, IR COWAN 1974 Oscillations in stomatal conductance. Influence of environmental gain. *Plant Physiol* 54: 769-772
7. GAASTRA P 1959 Photosynthesis of crop plants as influenced by light, carbon dioxide, temperature and stomatal diffusion resistance. *Meded Landb Hogesch Wageningen* 59(13): 1-68
8. HALL AE, O BJÖRKMÄN 1975 Model of leaf photosynthesis and respiration. In DM Gates, RB Schmerl, eds, *Perspectives of Biophysical Ecology*. Ecological Studies, Vol 12. Springer-Verlag, New York, pp 55-72
9. International Critical Tables in Numerical Data, Physics, Chemistry and Technology 1929 Volume 5. McGraw-Hill, New York, p. 62
10. JONES RW 1973 Principles of Biological Regulation: An Introduction to Feedback Systems. Academic Press, New York
11. KIRK DE 1970 Optimal Control Theory. An Introduction. Prentice-Hall, Englewood Cliffs NJ
12. PENMAN HL, RK SCHOFIELD 1951 Some physical aspects of assimilation and transpiration. *Symp Soc Exp Biol* 5: 115-129
13. RASCHKE K 1958 Über den Einfluß der Diffusionswiderstände auf die Transpiration und die Temperatur eines Blattes. *Flora* 146: 546-578
14. RASCHKE K 1965 Die Stomata als Glieder eines schwingungsfähigen CO_2 -Regelsystems. Experimenteller Nachweis an *Zea mays* L. *Z Naturforsch* 20b: 1261-1270
15. RASCHKE K 1974 Abscisic acid sensitizes stomata to CO_2 in leaves of *Xanthium strumarium* L. In *Plant Growth Substances* 1973. Proc VIII Intern Conf Plant Growth Substances. Hirokawa Publ Co, Tokyo, pp 1151-1158
16. RASCHKE K 1975 Stomatal action. *Annu Rev Plant Physiol* 26: 309-340
17. RIGGS DS 1970 Control Theory and Physiological Feedback Mechanisms. Williams & Wilkins, Baltimore