

Stomatal Behavior and CO₂ Exchange Characteristics in Amphistomatous Leaves¹

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ABSTRACT

The possibility that differences in stomatal conductance between upper and lower surfaces of amphistomatous leaves are adaptations to differences in CO₂ exchange characteristics for the two surfaces was investigated. The ratio of upper to lower stomatal conductance was found to change little in response to light and humidity for well-watered sunflower (*Helianthus annuus* L.) plants. Stressing the plants ($\psi = -17$ bars) and rewatering 1 day before gas exchange measurements reduced upper conductance more severely than lower in both indoor- and outdoor-grown plants, and caused small changes in conductance ratio with light and humidity. A similar pattern was found using outdoor grown sunflower and cocklebur (*Xanthium strumarium* L.) plants. Calculated intercellular CO₂ concentrations for upper and lower surfaces were always close to identical for a particular set of environmental conditions for both sunflower and cocklebur, indicating that no differences in CO₂ exchange characteristics exist between the two surfaces. By artificially creating a CO₂ gradient across the leaf, the resistance to CO₂ diffusion through the mesophyll was estimated and found to be so low that despite possible nonhomogeneity of the mesophyll, differences in CO₂ exchange characteristics for the two surfaces are unlikely. It is concluded that differences in conductance between upper and lower stomata are not adaptations to differences in CO₂ exchange characteristics.

thermal conductivity for leaves (4) make significant temperature gradients across the leaf improbable in most leaves, and although small differences in ambient humidity may exist between adaxial and abaxial surfaces, under reasonably well-stirred conditions these differences are likely to be small. However, Jones and Slatyer (5) reported a higher mesophyll resistance for CO₂ entering through the upper stomata than for the lower, and the data of Vaclavik (12) appear to support this conclusion. These data, plus consideration of the anisolateral nature of the mesophyll in many C₃ dicotyledonous species, raise the possibility that different CO₂ exchange characteristics may exist for CO₂ entering through one surface or the other, caused by either differences in resistance to CO₂ diffusion through the intercellular spaces or differences in photosynthetic characteristics between palisade and spongy mesophyll cells. Although differences in carbon metabolism between these two types of cells have been shown not to exist (7), differences in electron transport reactions are indicated by differences in fluorescence characteristics between upper and lower surfaces of leaves (1).

The goals of this study were to clarify responses for upper and lower stomata to environmental factors, to determine if differences in CO₂ uptake characteristics exist for the two surfaces, and hence decide if the observed differences in conductance are adaptive.

MATERIALS AND METHODS

A significant proportion of terrestrial vascular plants have leaves with stomata on both surfaces (termed amphistomatous), including most open field herbs and grasses and virtually all annual crop plants. Amphistomaty has been discussed by Parkhurst (8) and more recently by Mott *et al.* (6), but these discussions have centered on the adaptive significance of amphistomatous leaves as opposed to hypostomatous leaves, and very little has been written concerning the adaptive significance of reported differences between upper and lower stomata. Upper stomata are usually distinct from lower in density, conductance, and behavior. Typically, density is higher on the lower surface, but due to differences in size and response to environmental factors, conductance ratios are poorly related to density ratios for the two surfaces (10). Although often reported, few studies have carefully quantified these differences and discussed their relevance to overall gas exchange.

For differences in behavior between upper and lower stomata to be important in the regulation of gas exchange, there must be differences in either H₂O or CO₂ diffusion characteristics for the two surfaces. Differences in transpiration rate per conductance are unlikely, since the process is purely physical and gradients and basic pathways are likely to be similar. High values of

Sunflower (*Helianthus annuus* L.) and cocklebur (*Xanthium strumarium* L.) were grown indoors under fluorescent light banks with light intensity at the top of the plant maintained at approximately 350 $\mu\text{E m}^{-2} \text{s}^{-1}$. Day-night cycle was 18 and 6 h at 32 and 27°C, respectively. Plants were watered once a day with one-quarter strength modified Hoagland solution, and as necessary with tap water. Plants were also grown outdoors in large pots for diurnal measurements of stomatal conductance. Water stress was created by withholding water, and stressed plants were always rewatered around noon on the day prior to gas exchange measurements. Water potential was measured with a Wescor 33T Dewpoint Hygrometer using a C-51 chamber. Sunflower was used for detailed study of the response of upper and lower stomatal conductances to environmental factors, and both cocklebur and sunflower were used to verify the response to water stress for outdoor-grown plants, and for CO₂ uptake experiments.

Photosynthesis and transpiration were determined using a gas exchange system which allowed measurements of upper and lower surfaces independently. A clamp-on type chamber with the leaf forming the barrier between the two chambers was used, and pressure was equalized in the two chambers to prevent gas flow through the leaf. Light was provided by a 300-w cool-beam floodlight, or for later experiments, by a 400-w metal halide lamp. Light intensity on the adaxial leaf surface was determined with a Li-Cor model Li-170 light meter, using the quantum sensor, and was attenuated as necessary with ordinary cheese-

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cloth. All leaves were illuminated only from above.

To determine stomatal responses to light and humidity, air was pumped from outside the building, dried, and rehumidified to the desired level by bubbling part of the air stream through distilled H₂O and remixing. Air streams for upper and lower chambers were humidified separately to achieve the same chamber humidity for both surfaces despite differing stomatal conductances. For photosynthesis *versus* internal CO₂ concentration experiments, different CO₂ concentrations were achieved by mixing CO₂-free air with 2% CO₂ in air. In all cases, initial CO₂ concentration was measured using a Beckman model 215B CO₂ gas analyzer set in the absolute mode. CO₂ depletion was measured using an ADC Series 225 Mk. II CO₂ analyzer set in the differential mode. Both analyzers were calibrated against each other and against a standard gas. Water vapor loss was measured using an EG & G model 880 Dewpoint Hygrometer, and leaf temperature was measured using a fine wire thermocouple pressed to the underside of the leaf. Photosynthesis and transpiration rates and internal CO₂ concentrations were calculated according to the equations given by von Caemmerer and Farquhar (13).

Conductance measurements for outdoor-grown plants were made using a Li-Cor model Li-1600 steady-state porometer, and meteorological data such as air temperature, humidity, and light intensity were recorded to insure that experimental days were similar in these regards.

RESULTS

Effects of Environmental Factors on Upper and Lower Stomatal Conductances of Sunflower. Conductances for the two surfaces and the ratio of upper conductance to lower conductance, termed conductance ratio, were quite variable among plants and even among adjacent fully matured leaves on the same plant. For well-watered sunflower plants, upper conductance generally exceeded lower conductance slightly, leading to conductance ratios ranging from 1.0 to 2.0. Both upper and lower conductances declined curvilinearly with decreasing light intensity (Fig. 1), and the two conductances behaved in parallel, leading to constant conductance ratios across a wide range of light intensities (Fig. 2a). The variation present in conductance ratio in Figure 2a is due to variation among leaves; the conductance ratio for one leaf was remarkably constant for the light intensities used.

The after-effect of water stress ($\psi = -17$ bars) relieved 24 h prior to gas exchange measurements was to reduce both upper and lower conductances from the well-watered condition. Upper conductance was more severely reduced than lower (Fig. 1), indicating a differential response of the two surfaces to stress, and leading to conductance ratios less than 1.0 (Fig. 2b). If plants were allowed to recover from stress for 4 d instead of 1 d, conductance ratios were found to be similar to nonstressed plants (Fig. 2c). A slight decline in conductance ratio is seen with decreasing light intensity for plants allowed to recover for 1 or 4 d (Fig. 2, b and c), indicating that the stomatal conductances of the two surfaces were not responding in parallel to light intensity under these conditions. The deviation from parallel is not pronounced, however, and is difficult to discern from plots of stomatal conductance alone.

A milder stress ($\psi = -12$ bars) relieved 24 h prior to gas exchange measurements produced extremely variable results, including some plants with conductances and conductance ratios much higher and some much lower than for well-watered plants (Fig. 2d). Perhaps fortuitously, average conductances and conductance ratios were essentially unchanged from those of well-watered plants.

Figure 3 shows the response of stomatal conductance to vapor pressure difference across the stomatal pore for well-watered and

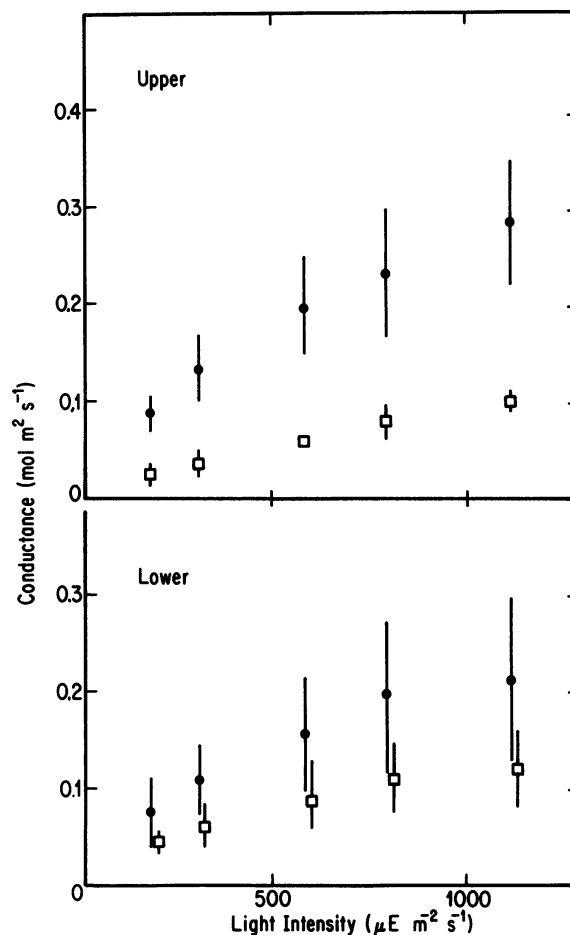


FIG. 1. Upper and lower stomatal conductance responses to light intensity for nonstressed (●, seven plants) and stressed (□, five plants) sunflower plants. Lines indicate on either side of the mean ($0.4 \text{ mol m}^{-2} \text{ s}^{-1} = 1 \text{ cm}^{-1}$).

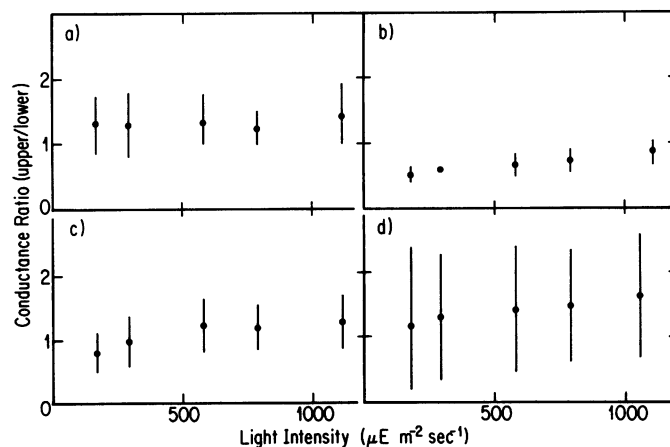


FIG. 2. Conductance ratio (upper/lower) of sunflower plants as affected by light intensity for (a) nonstressed plants (seven); (b) stressed ($\psi = -17$ bars) and rewatered 1 d before measurements (five); (c) stressed ($\psi = -17$ bars) and rewatered 4 d before measurements (five); (d) stressed ($\psi = -12$ bars) and rewatered 1 d before measurements (four).

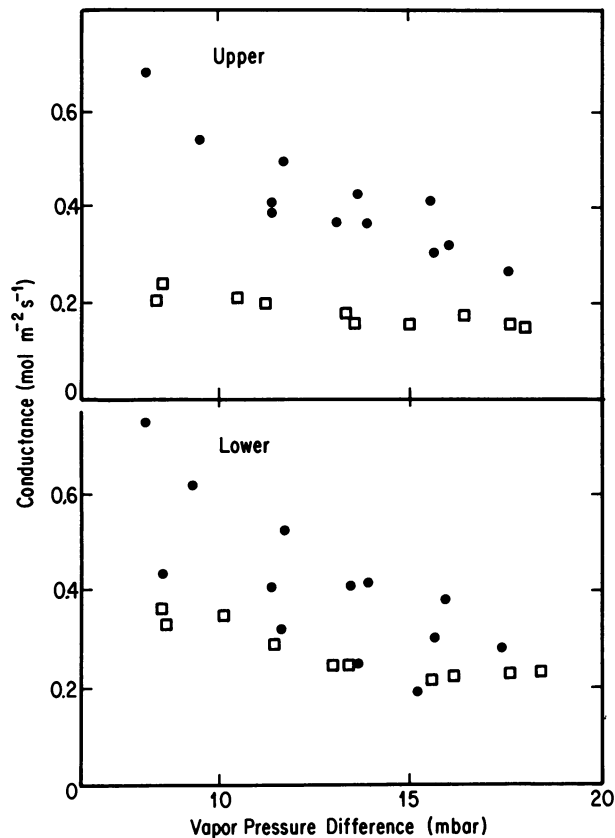


FIG. 3. Upper and lower stomatal conductance responses to vapor pressure difference across the stomatal pore for nonstressed (●, three plants) and stressed (□, three plants) sunflower plants ($0.4 \text{ mol m}^{-2} \text{ s}^{-1} = 1.0 \text{ cm s}^{-1}$).

stressed sunflower plants. Both upper and lower conductances increased as vapor pressure differences were decreased, and, as with the light response, the two conductances behaved in parallel leading to a constant conductance ratio over the range of vapor pressure differences examined. Again, average conductance ratios were slightly greater than 1.0 for well-watered plants, but stressing the plant reduced upper conductance more than lower conductance causing conductance ratios to be less than 1.0.

Stomatal Conductances of Outdoor-Grown Plants. To determine if this preferential reduction of the upper conductance by stress was restricted to plants grown in the growth room, diurnal conductances of sunflower and cocklebur plants growing outdoors were measured, and conductance ratios calculated. Meteorological conditions were extremely similar for days on which conductance measurements were taken. Data for well-watered plants show that conductance ratios were 0.8 to 1.0 over most of the day for both species (Figs. 4 and 5), rather than 1.0 to 2.0 as for growth room sunflower plants. However, stressing the plants did cause low conductance ratios for the day following rewatering in both species.

CO₂ Uptake Characteristics for Sunflower and Cocklebur. Photosynthetic rate was determined as a function of C_i^2 for both sunflower and cocklebur plants grown under the fluorescent light banks. Photosynthesis and transpiration were measured separately for the two surfaces, and a C_i value for each surface was calculated. For both cocklebur and sunflower, these two values

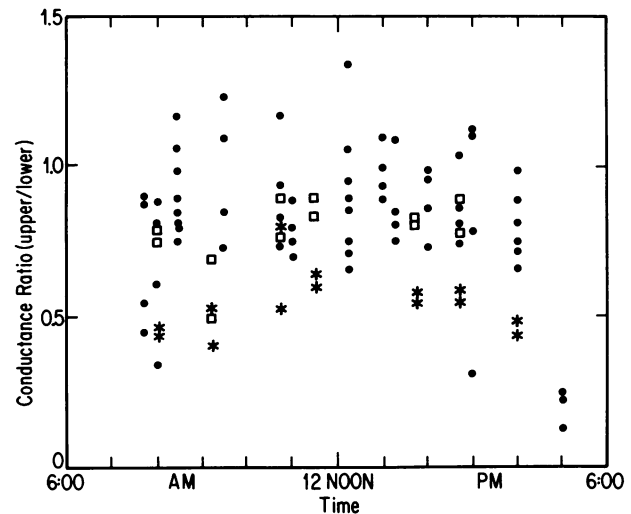


FIG. 4. Diurnal conductance ratios (upper/lower) for four sunflower plants grown outdoors. (●), Conductance ratios for each of the four plants before stressing; (*), for plants stressed and rewatered 1 d prior to measurements; (□), for control plants on the same day.

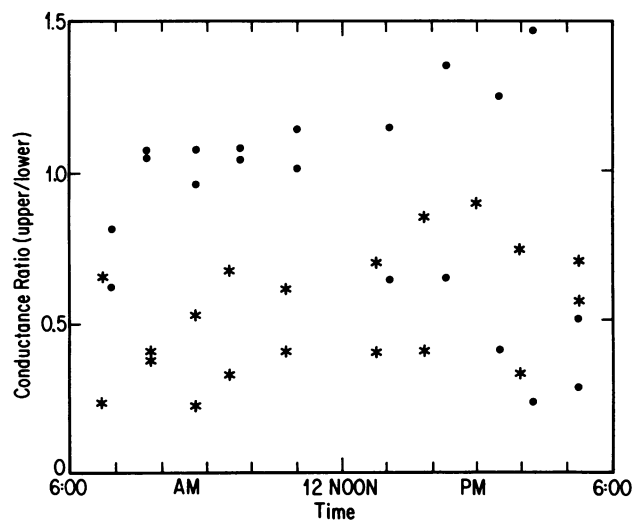


FIG. 5. Diurnal conductance ratios for two cocklebur plants (●) before stress, and (*) 1 d after recovery from stress.

were always in close agreement for a particular set of conditions (Fig. 6), and this was true over a wide range of external CO₂ concentrations despite widely differing stomatal conductances between the surfaces in some cases.

By lowering C_a in one chamber (either upper or lower) until no net CO₂ exchange was occurring across that surface, a situation was created in which both sets of stomata were open, yet all net CO₂ exchange was occurring across only one surface. Under these conditions, $C_a = C_i$ for one surface and C_i can be calculated for the other. For sunflower, these two C_i values were always close to identical, and the photosynthetic rate for the leaf at that C_i was the same as the photosynthetic rate for two surfaces at the same C_i value (Fig. 7). These data indicate an extremely low

² Abbreviations: C_i , internal CO₂ concentration; C_a , ambient or external CO₂ concentration; r_i , resistance to CO₂ diffusion through the mesophyll; A , photosynthetic rate; A_1 and A_2 , photosynthetic rate of upper and lower surface, respectively.

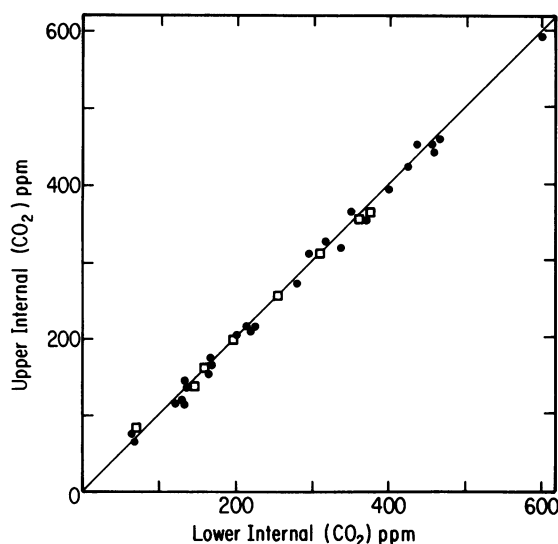


FIG. 6. Internal CO_2 concentration calculated for the upper surface versus that calculated for the lower surface under a particular set of environmental conditions for sunflower (●) and cocklebur (□).

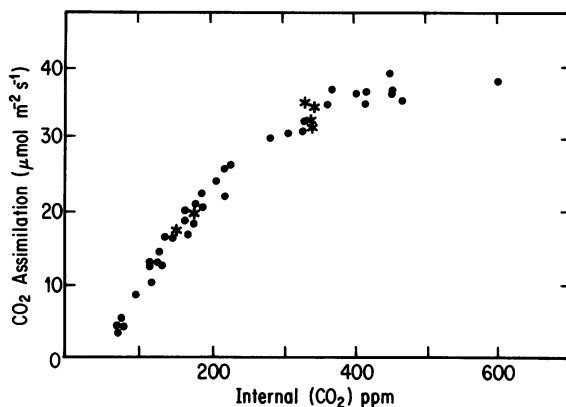


FIG. 7. Photosynthetic carbon assimilation versus calculated internal CO_2 concentration for sunflower with CO_2 exchange occurring across both surfaces (●), and with CO_2 exchange occurring across only one surface (*; see text).

resistance to CO_2 diffusion through the mesophyll. For cocklebur, differences in calculated C_i for the two surfaces were found under the conditions described above, with C_i for the surface with no CO_2 exchange across it always lower than the C_i value for the other surface. Using higher C_a values accentuated these differences in calculated C_i . Since there is a unidirectional gradient of CO_2 across the leaf under these conditions, these C_i values, along with the photosynthetic rate, can be used to get an approximation of the resistance to CO_2 diffusion through the leaf. If the gradient through the leaf is approximated as linear, the resistance to CO_2 diffusion through the leaf is given by $2(C_i)/A$ (3). Using data from cocklebur, we obtain values for this resistance of approximately $3.0 \text{ m}^2 \text{ s mol}^{-1}$ (1.2 s cm^{-1}). If the maximum difference in CO_2 concentration gradient within an amphistomatous leaf is given by $((A_1 A_2)/(A_1 + A_2))/(r_i/2)$ (see Ref. 3), then at C_i and photosynthetic rate values under ambient atmospheric conditions and high light intensities the maximum CO_2 concentration difference within the leaf is only 5 to 10 ppm.

DISCUSSION

It is evident from the data presented here and from various literature sources (see 10) that upper and lower stomata often

respond differently to environmental factors. However, for well-watered sunflower plants the responses to light and humidity appear parallel, as indicated by the constant conductance ratios found. In stressed plants, both 1 and 4 d following rewatering the responses are not perfectly parallel, but the differences are not large.

Although differences in behavior between upper and lower stomata probably do exist, many of those reported are differences only in absolute conductance changes for the two surfaces, not in conductance ratio. The surface with the higher stomatal conductance must have a larger absolute change to achieve the same proportional change as the other surface and hence maintain conductance ratio constant. The problem is accentuated by the practice of reporting diffusive resistances rather than conductances. For many plants, stomatal resistances are low for most physiologically important environmental conditions, and low resistance values mean small changes in absolute value for large percentage changes and hence large changes in diffusion rates. The use of aspirated diffusion porometers compounds the problem because these devices are inaccurate at low resistance values. Finally, although no time-course data were taken, we observed that the upper stomata generally responded more slowly to changes in environmental factors than did the lower, especially in cocklebur. If measurements are taken before steady state is reached, differences in stomatal conductance between the two surfaces will be miscalculated.

A large difference in stomatal response between upper and lower surfaces was observed for stressed plants. Although both conductances were reduced by the treatment, the reduction in the upper conductance was much more pronounced than for the lower. This effect was not an artifact of the growth conditions used because it was found in outdoor-grown plants, despite differences in conductance ratios in well-watered plants for the two growth conditions. The effect was reversible within 4 d, and it is possible that it is the result of differing sensitivities of the two sets of stomata to ABA. Pemadasa (9) has shown that, in isolated epidermes of *Commelina communis*, upper stomata show a greater response to a given concentration of ABA than do lower, and ABA levels are known to rise with water stress, cause stomata to close, and remain high for periods of time following resumption of high water potentials.

Any differences in CO_2 uptake characteristics between the two surfaces, either due to differences in diffusional resistance to CO_2 through the intercellular spaces or due to differences in photosynthetic characteristics between palisade and spongy mesophyll cells, should be reflected in differences in calculated C_i for the two surfaces. The similarity of these two values over wide ranges of C_a and for large differences in stomatal conductance between the two surfaces is strong evidence that no differences in CO_2 uptake characteristics exist. This argument is supported by the low resistances to CO_2 diffusion through the mesophyll which our data suggest. The technique of lowering C_a for one surface until $C_a = C_i$ for that surface produces a gradient in CO_2 concentration across the leaf which is measurable by calculating C_i for each surface. In sunflower, the resistance to CO_2 diffusion across the leaf was apparently so low that we were unable to obtain consistently lower C_i values for the surface across which no CO_2 exchange was occurring. For cocklebur, consistently differing C_i values were obtained, but the calculated resistance for CO_2 diffusion across the mesophyll was only $3.0 \text{ m}^2 \text{ s mol}^{-1}$. This value compares favorably with the value of $3.2 \text{ m}^2 \text{ s mol}^{-1}$ calculated by Farquhar and Raschke (2). However, using a technique similar to ours, Sharkey *et al.* (11) estimate r_i in *Xanthium* to be closer to $1.0 \text{ m}^2 \text{ s mol}^{-1}$, and state that they believe the estimate of $3.2 \text{ m}^2 \text{ s mol}^{-1}$ to be high. Based on these data and our measurements on sunflower and cocklebur, it seems that estimated r_i values are quite variable, possibly due to differ-

ences in technique, plant material, and because of the difficulty in accurately measuring the low resistances which apparently exist. Regardless of actual values, it appears that resistance across the mesophyll in many species is low, hence CO₂ concentration differences within the leaf under ambient CO₂ concentrations are small. Therefore, differences in resistances in the intercellular spaces or differences in photosynthetic characteristics between palisade and spongy mesophyll cells will not show up as differences in CO₂ uptake characteristics between the two surfaces.

In summary, differences in upper and lower stomatal response to light intensity and vapor pressure difference were found to be negligible for well-watered sunflower plants, and small for stressed ones. Stressing the plants reduced both conductances, but the effect was greater on the upper, causing a large change in conductance ratio. However, the extremely low resistances to CO₂ diffusion through the mesophyll in sunflower and cocklebur preclude any differences in CO₂ uptake characteristics, despite possible nonhomogeneity of the mesophyll. We conclude that differences in behavior between upper and lower stomata are not adaptations to differences in CO₂ uptake characteristics for these plants. The low values for resistance to CO₂ diffusion through the mesophyll found for *Gossypium hirsutum* and *Zea mays* by Farquhar and Raschke (2) indicate that this conclusion may be widely applicable, but exceptions are possible for sclerophyllous or succulent leaves.

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