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Leaf Conductance in Relation to Rate of CO_2 Assimilation

III. INFLUENCES OF WATER STRESS AND PHOTOINHIBITION

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ABSTRACT

Rates of CO_2 assimilation and leaf conductances to CO_2 transfer were measured in plants of *Zea mays* during a period of 14 days in which the plants were not rewatered, and leaf water potential decreased from −0.5 to −8.0 bar. At any given ambient partial pressure of CO_2, water stress reduced rate of assimilation and leaf conductance similarly, so that intercellular partial pressure of CO_2 remained almost constant. At normal ambient partial pressure of CO_2, the intercellular partial pressure of CO_2 was estimated to be 95 microbars. This is the same as had been estimated in plants of *Zea mays* grown with various levels of nitrogen supply, phosphate supply and irradiance, and in plants of *Zea mays* examined at different irradiances.

After leaves of *Phaseolus vulgaris* L. and *Eucalyptus pauciflora* Sieb. ex Spreng had been exposed to high irradiance in an atmosphere of CO_2-free N_2 with 10 millibars O_2, rates of assimilation and leaf conductances measured in standard conditions had decreased in similar proportions, so that intercellular partial pressure of CO_2 remained almost unchanged. As the conductance of each epidermis that had not been directly irradiated had declined as much as that in the opposite, irradiated surface it was hypothesized that conductance may have been influenced by photoinhibition within the mesophyll tissue.

In Part I (14) of this series we showed that rate of assimilation, A,[1] and leaf conductance to CO_2 transfer, g, in plants of *Zea mays* were uniquely related, irrespective of whether the variations in A and g were due to differences in nitrogen supply, phosphate supply, or irradiance during growth, or were associated with natural variation among plants grown in similar conditions. Under the standard conditions in which plants were examined, A was proportional to g and therefore intercellular partial pressure of CO_2, p_i, was constant. Assimilation rate was also proportional to g in plants of *Gossypium hirsutum* grown with different levels of nitrogen supply. In Part II (15) we showed that the same relationship between A and g in *Z. mays* was again obtained when single plants were examined at various irradiances, the other conditions being the same as those used in Part I. Relationships between A and g for *Phaseolus vulgaris* with varying photon flux density were the same in plants that had been grown under low, as in plants that had been grown under high, light intensity, although the maximum magnitudes of A and g attainable were much greater in the latter.

These findings caused us to ask whether guard cells are autonomous in controlling g, or are influenced, along with A, by the photosynthetic metabolism of the mesophyll tissue. It was found that variations in irradiance of leaves of *Eucalyptus pauciflora* influenced conductance in the opposite epidermis more than could be explained on the basis of light transmission through the leaf.

In this paper, we examine the effect of water stress on A and g in *Z. mays*, and photoinhibitory stress on A and g in *E. pauciflora* and *P. vulgaris*. We pay particular attention to the way in which photoinhibition, brought about by high irradiance with low ambient CO_2 and O_2 partial pressures, influences conductances in the two epidermes of the leaves.

MATERIALS AND METHODS

Plant Materials. Seeds of *Phaseolus vulgaris* L. were sown in sterilized garden soil in 5-L plastic pots. Uniform seedlings were obtained by thinning from four to one per pot after germination. Seeds of *Eucalyptus pauciflora* Sieb. ex Spreng were stratified at 4°C on moist filter paper for 30 d before germination. Seedlings were later grown in 5-L pots. Plants were grown in a glasshouse under full sunlight, the midday photon flux density being about 2 mmol m^{-2} s^{-1}. Air temperature was 32 ± 2°C during the day and 20 ± 2°C at night. RH varied between 50 and 70%. The soil in each pot was flushed daily with 1 L of nutrient solution in late afternoon (14). During the daytime the plants were watered lightly every 3 h to compensate for water loss due to transpiration. The youngest, fully expanded, attached leaves from 30-d-old plants of *P. vulgaris*, and 4-month-old plants of *E. pauciflora* were used in experiments.

For the experiments with water stress, seeds of *Zea mays* L. were sown in 45-L plastic bins containing sterilized garden soil. Soil in the bin was flushed daily with 10 L of nutrient solution in late afternoon. Water was withheld 30 d after germination. Gas exchange measurements were made on the 8th leaf from the base, *i.e.* the youngest fully collared leaf.

Gas Exchange Methods. Transpiration rates and assimilation of CO_2 were measured for both sides of the leaf, independently, using a small, double-sided glass and aluminum leaf chamber clamped to the leaf. The whole-leaf data presented in this paper were obtained by summing the fluxes at each side. Rate of CO_2 assimilation, A, and g were measured at a photon flux density of 2 mmol m^{-2} s^{-1} and at ambient partial pressures of CO_2, p_a, of 95, 190, 305, and 380 μbar. Leaf temperature and leaf to air vapor pressure difference were maintained at 30°C and 20 mbar, respectively, Details of gas exchange methods have been described previously (12, 13). The measurement of conductance to CO_2 transfer, g, was described previously (14). Leaf water potentials were measured with a pressure chamber.

To promote photoinhibition, leaves were maintained for 2, 3, or 7 h in CO_2-free N_2 containing 10 mbar O_2, and were irradiated

[1] Abbreviations: A, rate of CO_2 assimilation, μmol m^{-2} s^{-1}; g, leaf conductance to CO_2 transfer, mol m^{-2} s^{-1}; I, photon flux density (400–700 nm), mmol m^{-2} s^{-1}; p_a, ambient partial pressure of CO_2, μbar; p_i, intercellular partial pressure of CO_2, μbar.

with 2 mmol m^{-2} s^{-1} photon flux density. Responses of A and g to changes in p_i were measured immediately before and after the photoinhibitory treatment.

RESULTS

Influence of Water Stress. During the 14-d period when water was withheld from *Z. mays*, the predawn leaf water potential declined from −0.5 to −8.0 bar. Rate of assimilation, A, and leaf conductance, g, also declined (Fig. 1). At any given ambient partial pressure of CO_2, p_a, the relative reductions in A and g were in almost the same proportion (Fig. 2), so that p_i remained almost constant (Table I). The line drawn in Figure 2 is $A = 204\ g \times 10^{-6}$, the same as that closely describing A and g in plants of

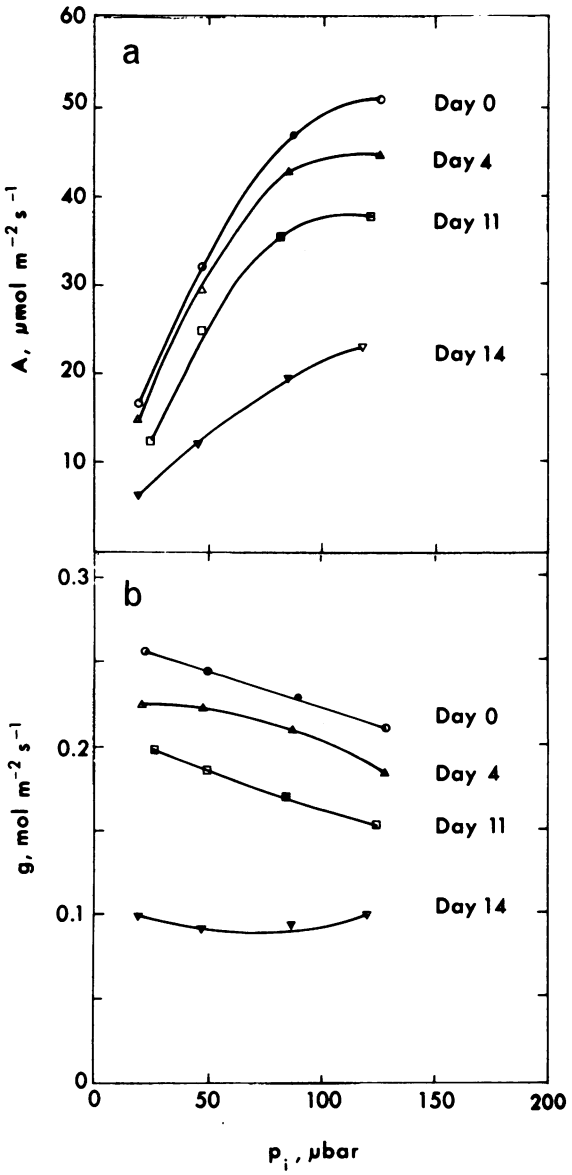

FIG. 1. Rates of assimilation, A, and leaf conductances, g, as functions of intercellular partial pressure of CO_2, p_i, in *Z. mays* on various days after withholding watering. Measurements made with 95, 190, 305, and 380 μbar ambient partial pressure of CO_2, 2 mmol m^{-2} s^{-1} photon flux density, 30°C leaf temperature, and 20 mbar vapor pressure difference between leaf and air. Closed symbols represent measurements with 305 μbar ambient partial pressure of CO_2. Leaf water potentials were −0.5, −2, −5, and −8 bars on days 0, 4, 11, and 14, respectively.

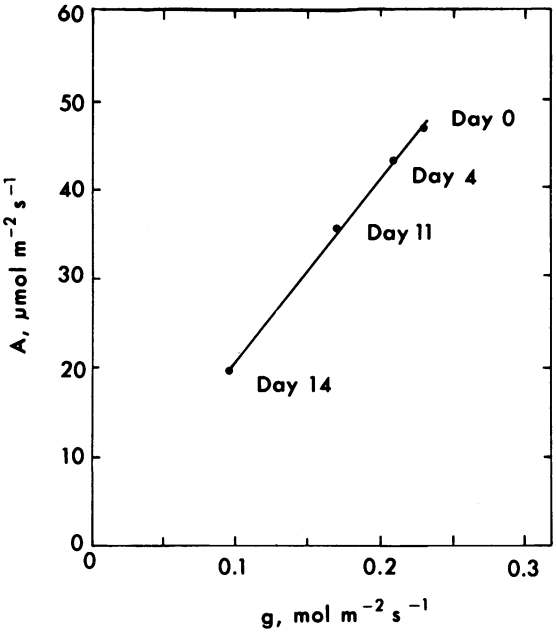

FIG. 2. Rate of assimilation, A, and leaf conductance, g, in *Z. mays* on various days after withholding watering. Data are those shown in Figure 1 with 305 μbar ambient partial pressure of CO_2.

Table I. *Rate of Assimilation, A (μmol m^{-2} s^{-1}), Leaf Conductance, g (mol m^{-2} s^{-1}), and Intercellular Partial Pressure of CO_2 p_i (μbar), in Two Mature Leaves from Two Zea mays Plants during the Imposition of Water Stress*

Predawn water potential, ψ, is expressed in bar. Measurements were made with 2 mmol m^{-2} s^{-1} photon flux density, 305 μbar ambient partial pressure of CO_2, 30°C leaf temperature, and 20 mbar vapor pressure difference between leaf and air.

Day	A	Reduction	g	Reduction	p_i	ψ
		%		%		
0	46.8		0.23		98	−0.5
4	43.0	8	0.21	9	95	−2
11	35.5	24	0.17	26	92	−5
14	19.5	58	0.09	59	95	−8
0	46.0		0.23		99	−0.5
4	43.3	6	0.20	13	95	−2
11	35.2	23	0.17	26	98	−5
14	21.2	54	0.10	57	92	−7

Z. mays grown with various levels of nitrogen supply, phosphate supply, and irradiance (14), and in plants of *Z. mays* examined at various irradiances (15). It implies p_i is approximately 95 μbar.

Influence of Photoinhibition. The effects of 2 h of photoinhibitory treatment on A and g in *P. vulgaris* are shown in Figure 3. At any given p_a, A and g were reduced by almost the same relative amounts, so that p_i was changed very little. At 305 μbar ambient partial pressure of CO_2, A and g had been reduced by 34 and 31%, respectively, after photoinhibition. The reduction of g was as great in the abaxial surface, which had not been directly irradiated during the photoinhibitory treatment, as in the adaxial surface (Table II).

Rather similar results were obtained with *E. pauciflora*. At any given magnitude of p_a, the relative reductions in A and g after 3 h of photoinhibitory treatment, and after a further 7 h of photoinhibitory treatment on the following day, were almost the same, and p_i remained almost constant (Fig. 4). At normal ambient partial pressure of CO_2, A and g were reduced by 30%

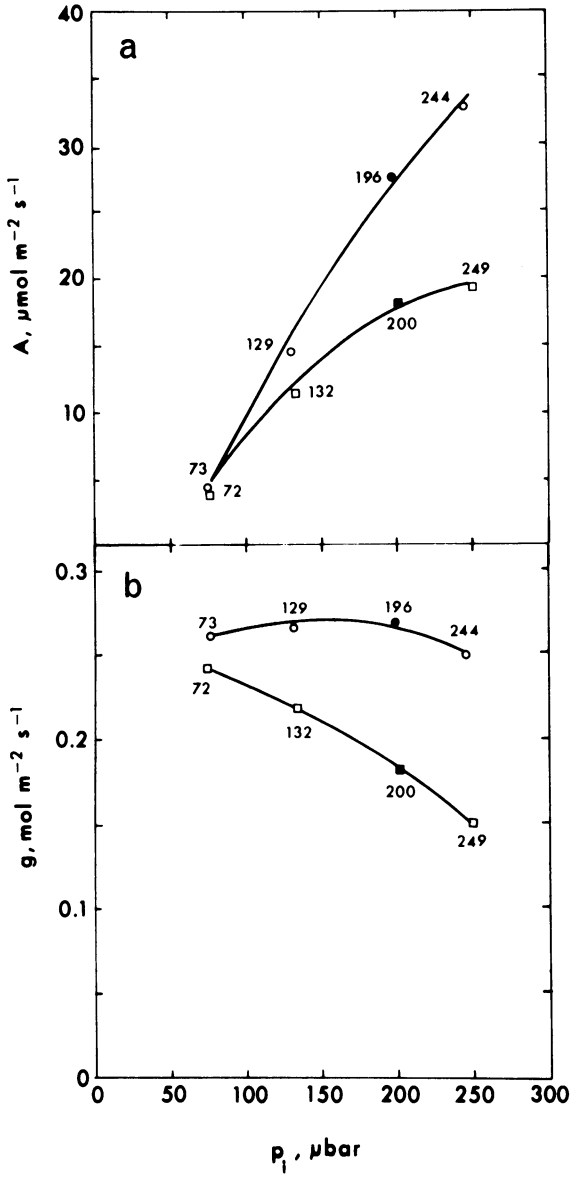

FIG. 3. Assimilation rates, A, and leaf conductances, g, as functions of intercellular partial pressure of CO_2, p_i, in *P. vulgaris* before (O) and after (□) 2-h exposure to 2 mmol m^{-2} s^{-1} photon flux density in an atmosphere of CO_2-free N_2 containing 10 mbar O_2. Measurements were made with conditions described for *Z. mays* in Figure 1. Closed symbols represent measurements made at p_a = 306 μbar. Numbers indicate values of p_i (μbar).

after the first period, and 46% after the subsequent period. In this experiment the reduction of g was even greater in the abaxial surface, which had not been directly irradiated, than in the adaxial surface (Table III).

DISCUSSION

Both water stress and photoinhibitory treatment caused a decline in the photosynthetic metabolism of leaf mesophyll tissue. As with other treatments engendering differences and changes in photosynthetic metabolism (14, 15), g was changed by approximately the same relative magnitudes as the changes in A. Changes in g were very little influenced by sensitivity of the stomata to intercellular partial pressure of CO_2. At any given magnitude of p_a, the change in p_i due to water stress or photoinhibition was no more than a few microbars. It is evident from Figures 1, 3, and 4 that the responses of g to changes in p_i of this magnitude were negligible.

Table II. *Rate of CO_2 Assimilation, A (μmol m^{-2} s^{-1}), Leaf Conductance, g (mol m^{-2} s^{-1}), and Intercellular Partial Pressure of CO_2, p_i (μbar), Measured in Adaxial, Abaxial, and Both Leaf Surfaces Taken Together in Two Leaves from Two Phaseolus vulgaris Plants before and after the 2-h Photoinhibitory Treatment*

Measurements were made with conditions as for *Z. mays* described in Table I.

Surface	Treatment	A	Reduction	g	Reduction	p_i
			%		%	
Both	Before	27.4		0.27		204
	After	18.2	34	0.18	31	208
Adaxial	Before	9.3		0.10		211
	After	5.6	40	0.07	30	227
Abaxial	Before	18.1		0.17		200
	After	12.6	30	0.11	33	197
Both	Before	25.3		0.29		225
	After	17.7	30	0.19	34	218
Adaxial	Before	8.5		0.10		221
	After	5.5	35	0.06	40	225
Abaxial	Before	16.8		0.20		227
	After	12.2	27	0.13	35	214

The mechanisms by which water stress affects photosynthetic capacity are unclear. Potter and Boyer (7) reported a reduction in electron transport in PSII of chloroplasts isolated from desiccated sunflower (*Helianthus annuus*) leaves. There was also a reduction in the quantum yield of CO_2 fixation in these leaves (5). Powles and Björkman (8) showed that water stress caused an inactivation of the primary photochemistry of the PSII reaction center complex. On the other hand, Sharkey and Badger (11) found no reduction in the uncoupled electron transport capacity of intact spinach (*Spinacia oleracea*) chloroplasts and *Xanthium strumarium* cells subjected to reduced water potentials in the suspension medium, but concluded that ATP formation was affected. Caemmerer and Farquhar (1) observed that electron transport was unchanged in thylakoids of water-stressed *P. vulgaris* leaves. They concluded that ribulose bisphosphate regeneration capacity was affected in some way. Regardless of the way mesophyll energy transduction is affected, it is possible that similar effects are occurring in guard cells.

It is possible, also, that electron transport in guard cells (6, 16) can be photoinhibited in much the same way as electron transport in mesophyll chloroplasts. Powles and Critchley (9) have reported a 50% reduction in Hill reaction activity in chloroplasts isolated from leaves from *P. vulgaris* grown under full sunlight and exposed to 3 h of photoinhibitory treatment (at photon flux density of 2 mmol m^{-2} s^{-1} and in CO_2-free N_2 containing 10 mbar O_2).

However, the results of photoinhibition shown in Tables II and III seem inconsistent with the supposition that stomatal closure is entirely due to photoinhibition of guard cell metabolism, and suggest that the stomata may have been influenced by changes that had taken place in the leaf mesophyll tissue. Leaf conductance declined as much in the epidermis that had not been directly irradiated during the photoinhibitory treatment as in the epidermis that had been directly irradiated.

Typically, leaves of *P. vulgaris* were observed to transmit 5% of incident light through the mesophyll. Therefore, the abaxial surface of the leaf transmits 100 μmol photon m^{-2} s^{-1} when the incident photon flux density on the adaxial surface is 2 mmol m^{-2} s^{-1}. Although the photon flux impinging on the abaxial surface will be somewhat greater than 100 μmol m^{-2} s^{-1} because

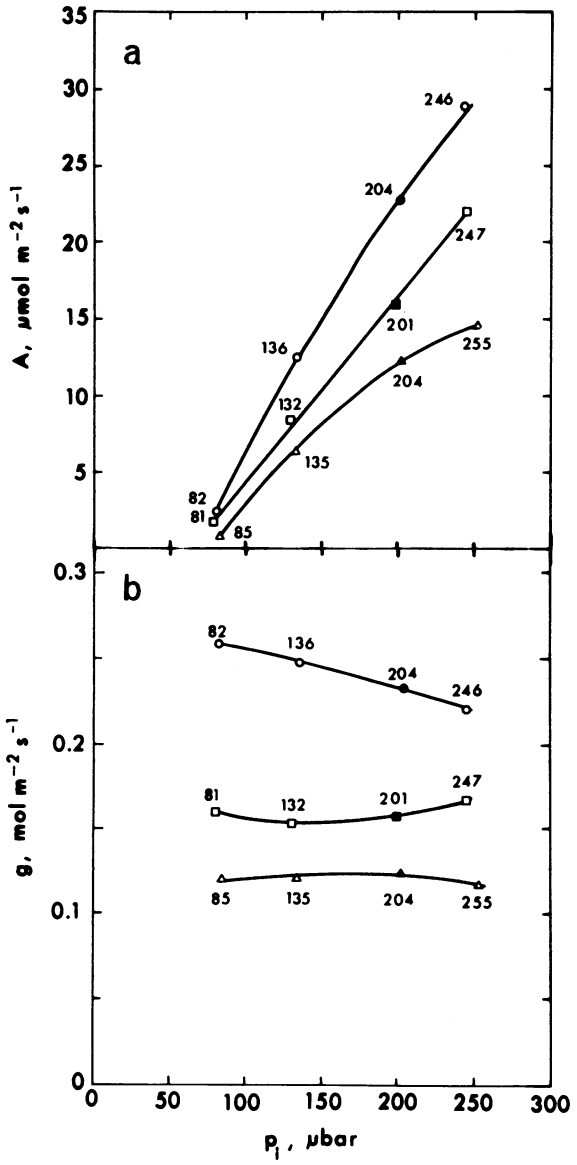

FIG. 4. Assimilation rates, A, and leaf conductances, g, as functions of intercellular partial pressure of CO_2, p_i, in *E. pauciflora* before (O) and after (□) 3-h exposure to 2 mmol m^{-2} s^{-1} photon flux density in an atmosphere of CO_2-free N_2 containing 10 mbar O_2. A second 7-h photoinhibitory treatment was given on the second day of the experiment (△). Conditions for measurements were those described in Figure 1. Closed symbols represent measurements made at p_a = 305 μbar. Numbers indicate values of p_i (μbar).

of internal reflection, it seems unlikely that it would be great enough to cause photoinhibition in the guard cells, let alone an inhibition as great in its effect on stomatal aperture as that in the adaxial surface. As reported by Powles and Osmond (10), photoinhibition is a function of the photon flux density during the photoinhibitory treatment; no photoinhibition was detected in leaves of *P. vulgaris* at a photon flux density of 100 μmol m^{-2} s^{-1}.

The paradox, that stomata in the nonirradiated surface are as much affected by photoinhibitory treatment as those in the irradiated surface, is even more surprising in *E. pauciflora*, the leaves of which transmitted only 1% of the incident light. While there is undoubtedly a great deal of reflection and refraction of light within leaves, the presence of Chl in the mesophyll ensures that the photon flux density at the abaxial surface must be much

Table III. *Rate of CO_2 Assimilation, A (μmol m^{-2} s^{-1}), Leaf Conductance, g (mol m^{-2} s^{-1}), and Intercellular Partial Pressure of CO_2, p_i (μbar), Measured from Adaxial, Abaxial, and Both Leaf Surfaces Taken Together in E. pauciflora before and after 3-h and 7-h Photoinhibitory Treatment*

Measurements were made with conditions as for *Z. mays* described in Table I.

Surface	Treatment	A	Control	g	Control	p_i
			%		%	
Both	Before	22.7		0.24		210
	After 1st-	16.0	71	0.16	66	208
	After 2nd-	12.3	54	0.13	53	210
Adaxial	Before	13.9		0.14		204
	After 1st-	10.3	74	0.10	68	196
	After 2nd-	9.1	66	0.09	63	204
Abaxial	Before	8.8		0.10		219
	After 1st-	5.7	65	0.06	63	223
	After 2nd-	3.2	36	0.04	38	224

less than that at the adaxial surface when the latter is illuminated. Yet g in the abaxial surface was reduced to an even greater extent than that in the adaxial surface (Table III). The observation is consistent with the results described earlier (15), in which variation of the irradiance of the adaxial or abaxial epidermis in *E. pauciflora* (ambient partial pressures of CO_2 and O_2 being normal) had an anomalously large effect on conductance to CO_2 transfer across the opposite epidermis.

We have previously asked the question (14, 15), are the differences and variation in leaf conductance which are so closely correlated with differences and variations in mesophyll photosynthetic metabolism independent of the latter, or are there control mechanisms, not involving intercellular partial pressure of CO_2, which maintain conductance in tune with the capacity of the mesophyll tissue for photosynthesis? The answer may depend on the time scale being considered; it is perhaps unlikely that one control mechanism is responsible for the correlation between g and A in a group of plants that had developed under different nutritional regimes, and in a single plant examined at various irradiances or sustaining progressively increased photoinhibition. It is only in these latter two examples that we have firm evidence that the variations in g are not entirely autonomous—that they can be induced, or at least modulated, by events occurring in the mesophyll tissue.

We have no concrete suggestion as to how this modulation might take place. Farquhar and Wong (4) have shown that variation of g with changes in irradiance, intercellular partial pressure of CO_2, and temperature is an analog of estimated variation in the ATP content of the mesophyll chloroplasts, but do not propose a mechanistic link. Cowan *et al.* (3) have shown theoretically how irradiance may influence the partitioning of ABA between the chloroplast and other compartments in leaf tissue, and suggested this may influence the amount of ABA available to the guard cells via the free space.

Whatever the mechanism, it is apparent that a remarkable concordance between leaf conductance and photosynthetic metabolism is maintained in plants subjected to water stress and photoinhibition, and that this concordance is similar to that found among plants of the same species subjected to a variety of growth conditions and in single plants of the same species examined at different irradiances. In ecological terms, this concordance will assist to minimize rate of transpiration and the probability of plant desiccation, given the requirement in plants to assimilate carbon in order to grow and reproduce (2).

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