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Leaf Conductance in Relation to Rate of CO_2 Assimilation

I. INFLUENCE OF NITROGEN NUTRITION, PHOSPHORUS NUTRITION, PHOTON FLUX DENSITY, AND AMBIENT PARTIAL PRESSURE OF CO_2 DURING ONTOGENY

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ABSTRACT

Plants of *Zea mays* were grown with different concentrations of nitrate (0.6, 4, 12, and 24 millimolar) and phosphate (0.04, 0.13, 0.53, and 1.33 millimolar) supplied to the roots, photon flux densities (0.12, 0.5, and 2 millimoles per square meter per second), and ambient partial pressures of CO_2 (305 and 610 microbars). Differences in mineral nutrition and irradiance led to a large variation in rate of CO_2 assimilation per unit leaf area (*A*, 11 to 58 micromoles per square meter per second) when measured under standard conditions. The variation was shown, with the plants that had received different amounts of nitrate, to be related to variations in the nitrogen and chlorophyll contents, and phosphoenolpyruvate and ribulose-1,5-bisphosphate carboxylase activities per unit leaf area. Irrespective of growth treatment, A and leaf conductance to CO_2 transfer (*g*), measured under standard conditions were in almost constant proportion, implying that intercellular partial pressure of CO_2 (p_i), was almost constant at 95 microbars. The same proportionality was maintained as *A* and *g* increased in an initially nitrogen-deficient plant that had been supplied with abundant nitrate. It was shown that p_i measured at a given ambient partial pressure was not affected by the ambient partial pressure at which the plants had been grown, although it was different when measured at different ambient partial pressures. This suggests that the close coupling between *A* and *g* in these experiments is not associated with sensitivity of stomata to change in p_i.

Similar, though less comprehensive, experiments were done with *Gossypium hirsutum*, and yielded similar conclusions, except that the proportionality between *A* and *g* at normal ambient partial pressure of CO_2 implied $P_i \approx 200$ microbars.

In a previous paper (8) we found that response of leaf conductance, *g*,[1] in leaves of *Eucalyptus pauciflora*, to change in photon flux density, I, was similar to response of rate of CO_2 assimilation, A, to I. Thus, intercellular partial pressure of CO_2, p_i, remained almost constant (257–243 μbar) when I changed 8-fold from 0.25 to 2 mmol m^{-2} s^{-1}. We also showed that the sensitivity of stomata to change in partial pressure of CO_2 was too small to maintain p_i so nearly constant; the variation in aperture was almost entirely due to a direct response of the variation of I. Following that study, we designed several experiments to further explore the relationship between leaf conductance and rate of assimilation. A brief summary of the results has been published (9). In these experiments, to be described in detail in this and subsequent papers (10, 11), the intrinsic capacity of leaf mesophyll tissue for photosynthesis was varied in a number of ways, involving different time scales. Although the means by which changes in conductance were attuned to changes in mesophyll capacity may have been quite different over the different time scales, the effect was that *A* and *g* were uniquely related in a given species, in such a way that p_i was almost constant.

In this paper we describe measurements of *A* and *g* in plants in which different photosynthetic capacities had been brought about by different nitrogen and phosphorus nutrition treatments, different photon flux densities during growth, and differing partial pressures of CO_2 during growth.

MATERIALS AND METHODS

Plant Material. Seeds of *Zea mays* L. and *Gossypium hirsutum* L. were sown in 5-L plastic pots containing sterilized garden soil. After emergence, seedlings were thinned from four to one per pot to obtain uniform plants. Plants were grown in a glasshouse under full sunlight, the photoperiod being 12 to 13 h, and the midday photon flux density (400–700 nm) being about 2 mmol m^{-2} s^{-1}. Air temperature in the glasshouse was 32 ± 2°C during the day and 18 ± 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 the late afternoon. During the daytime the plants were watered lightly every 3 h to compensate for loss by transpiration. Nutrient solutions were based on Hewitt's nitrate-type nutrient solution, consisting of 12 mM NO_3^-, 4 mM K^+, 4 mM Ca^{2+}, 1.5 mM Mg^{2+}, 1.33 mM PO_4^{3-} with balancing Na^+, SO_4^{2-}, Cl^- and micronutrients. Nitrogen nutrition experiments comprised four groups of 24 *Z. mays* plants and 10 *G. hirsutum* plants treated with 24, 12, 4, and 0.6 mM NO_3^- in nutrient solutions. Phosphorus nutrition experiments comprised four groups of 6 *Z. mays* plants treated with 1.33, 0.53, 0.133, and 0.040 mM PO_4^{3-} in nutrient solutions.

In experiments on the effect of ambient partial pressure of CO_2 during ontogeny, plants were grown in two glasshouses. One glasshouse was well ventilated, the partial pressure of CO_2 being about 320 ± 20 μbar (*i.e.* normal atmospheric partial pressure in Canberra). The partial pressure of CO_2 in the other glasshouse was maintained at 640 ± 15 μbar by injecting pure CO_2. The partial pressure of CO_2 was monitored and controlled with an URAS II (Hartman and Braun, Frankfurt, West Germany) IR gas analyzer. The plants in each glasshouse were divided into four groups, 8 *Z. mays* plants and 6 *G. hirsutum* plants in each group, which were given different nitrogen nutrient treatments as described previously.

Gas Exchange Methods. Measurements were made on *G.*

[1] Abbreviations: *g*, conductance to CO_2 transfer, mol m^{-2} s^{-1}; *A*, rate of CO_2 assimilation, μmol 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; PEP, phosphoenolpyruvate; RuP_2, ribulose-1,5-bisphosphate.

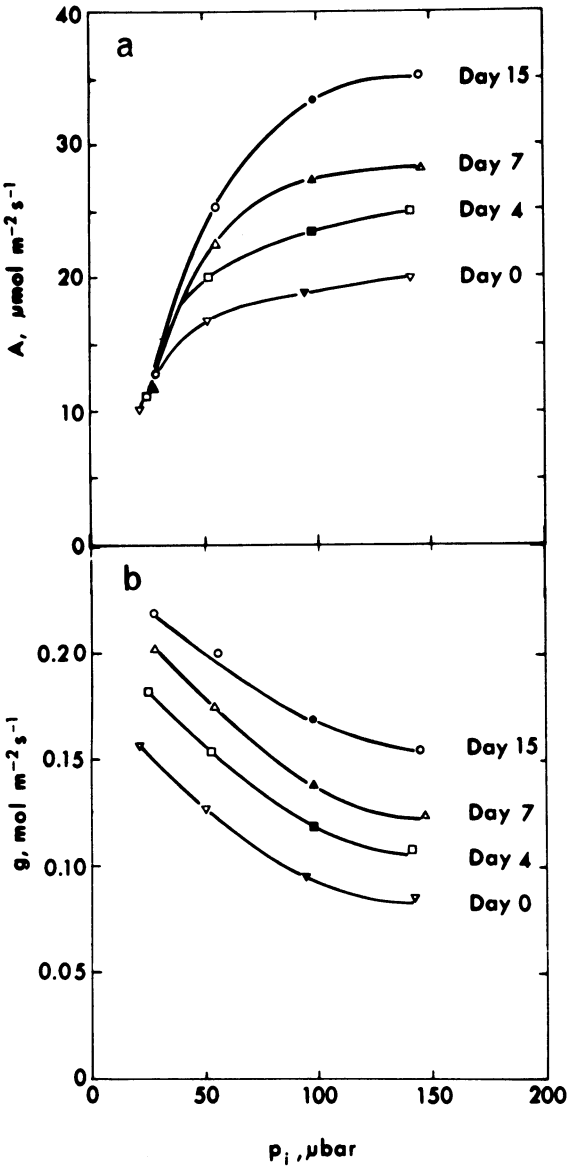

FIG. 1. Rate of CO_2 assimilation, A, and leaf conductance, g, as functions of intercellular partial pressure of CO_2, p_i, in *Z. mays* during recovery from nitrogen deficiency. Measurements were made with 2 mmol m^{-2} s^{-1} photon flux density at the illuminated leaf surface, p_a = 95, 190, 305, 380 μbar ambient partial pressure of CO_2, 30°C leaf temperature, and 20 mbar vapor pressure difference between leaf and air.

hirsutum 40 d after germination and were confined to the youngest fully expanded leaves. Measurements on *Z. mays* were made 30 d after germination and were done on the youngest fully expanded leaf, *i.e.* 7th leaf from the base of the plant. Two of the plants grown at normal ambient partial pressure of CO_2 and 0.6 mM NO_3^- were supplied with 24 mM NO_3^- thereafter, and measurements were repeated over a period of 15 d. Details of gas exchange methods have been described previously (7, 8). Briefly, rates of transpiration of water vapor and assimilation of CO_2 were measured independently for both sides of a leaf 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. Leaf temperature and vapor pressure difference between leaf and air were maintained at 30°C and 20 mbar, respectively. Except where otherwise stated, the photon flux density at the illuminated leaf surface was

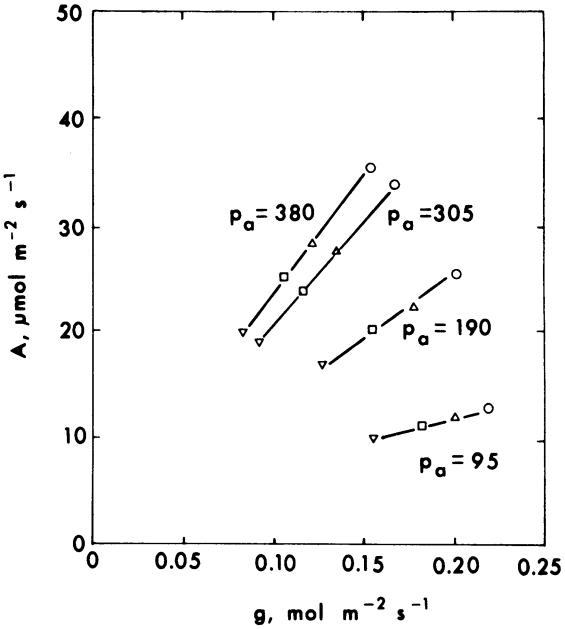

FIG. 2. Rate of CO_2 assimilation, A, as a function of leaf conductance, g, in *Z. mays* during recovery from nitrogen deficiency. Data from Figure 1. Symbols ▽, □, △, ○ indicate 0, 4, 7, and 15 d, respectively, from the time at which the plant roots were supplied with 24 mM NO_3^-.

2 mmol m^{-2} s^{-1}. Measurements were normally made with 320 μl/l ambient concentration of CO_2, equivalent to 305 μbar partial pressure at the altitude of Canberra. This was the concentration closest to normal ambient concentration that could conveniently be obtained using a Wosthoff pump gas mixing system.

The estimation of conductance, g, is described in the Appendix. It refers to transfer of CO_2 across the leaf epidermis and an external gas phase having an effective boundary layer conductance of 0.58 mol CO_2 m^{-2} s^{-1}. Intercellular partial pressure of CO_2, p_i, was found from the equation for rate of assimilation per unit area of leaf.

$$A = (g[p_a - p_i]/P) - (E[p_a + p_i]/[2P]) \quad (1)$$

where p_a is the effective ambient partial pressure of CO_2 and P is total gas pressure. The second expression in parentheses allows for the influence of vapor efflux on CO_2 transfer (1, 6). It was small in the conditions of our experiment, being approximately 8 and 3% of the first term with C_3 and C_4 plants, respectively. Equation 1 shows that the approximately proportional relationships between A and g that will be evident in the data to be presented imply approximate constancy in p_i.

Enzyme Activity Measurements. PEP carboxylase and RuP_2 carboxylase activities were determined in each leaf used in gas exchange measurement. The assay temperature was 30°C. The assay method, in which crude enzyme extract and $NaH^{14}CO_3$ as substrate are used has been described previously (7).

RESULTS

The relative increases in A and g with time in a *Z. mays* plant that had been grown with low availability of nitrogen and was then supplied with adequate nitrogen were similar at any particular magnitude of p_a (except possibly at the lowest partial pressure used, p_a = 95 μbar), with the corollary that p_i remained almost constant (Figs. 1 and 2). The proportionality between A and g measured at p_a = 305 μbar was similar to that found within groups of *Z. mays* plants that had been grown with various levels of nitrate, various levels of phosphate, and various photon flux

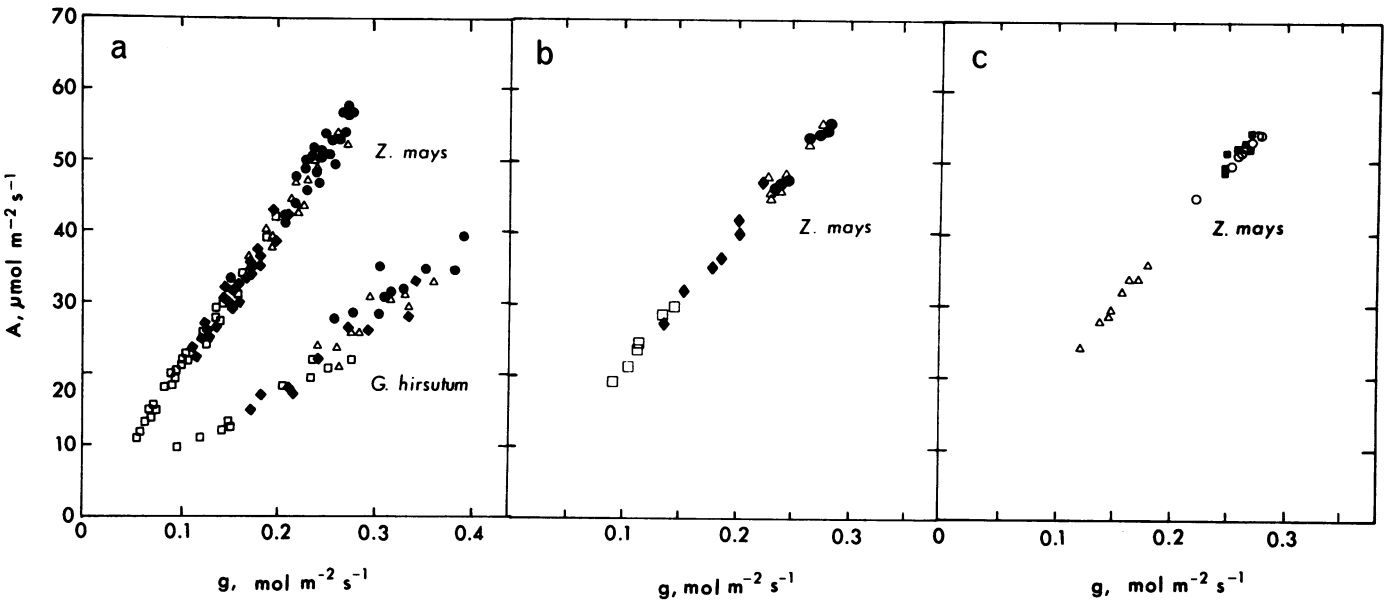

FIG. 3. Rate of CO_2 assimilation, A, as function of leaf conductance, g, in plants of *Z. mays* and *G. hirsutum* grown with (a) 0.6 (□), 4 (♦), 12 (△), 24 (●) mM NO_3^-; (b) 0.04 (□), 0.13 (♦), 0.53 (△), 1.33 (●) mM PO_4^{3-}; and (c) 2 (○), 0.5 (■), and 0.12 (△) mmol m^{-2} s^{-1} midday photon flux density. Except where otherwise specified, plants were grown with 12 mM NO_3^-, 1.33 mM PO_4^{3-}, and 2 mmol m^{-2} s^{-1} midday photon flux density. Each point represents a single plant. In (a) and (b) measurements were made with 2 mmol m^{-2} s^{-1} photon flux density. In (c) measurements were made at photon flux densities sufficient to saturate rate of assimilation, *i.e.* 2, 1.2, and 0.7 mmol m^{-2} s^{-1} with plants grown with 2, 0.5, and 0.12 mmol m^{-2} s^{-1} midday photon flux densities, respectively. All measurements were made with p_a = 305 μbar.

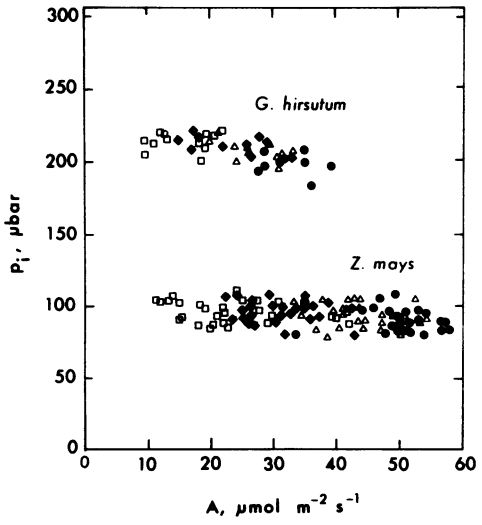

FIG. 4. Intercellular partial pressure of CO_2, p_i, against rate of CO_2 assimilation, A, in *Z. mays* and *G. hirsutum*. Data from Figure 3(a).

densities (Fig. 3). The different growth treatments led to large differences in A and g, and there were also differences due to natural variation among plants having the same treatment. But whatever the source of variation, the relationship between assimilation rate and conductance was closely approximated by the one relationship $A = 204g \times 10^{-6}$, implying that p_i was approximately constant at 95 μbar (Fig. 4). Figures 3a and 4 also contain data for *G. hirsutum* plants grown at four levels of nitrogen nutrition. These data are approximated by $A = 100g \times 10^{-6}$, and p_i = 200 μbar.

Because p_i was uniform among plants of each species, differences in rate of assimilation within each species reflected differences in the characteristic of photosynthetic carbon metabolism in the leaf mesophyll tissue. The biochemical bases of these differences in plants having different levels of nitrogen nutrition

Table I. *Rate of CO_2 Assimilation (μmol m^{-2} s^{-1}), A, Leaf Conductance to CO_2 Transfer (mol m^{-2} s^{-1}), g, and Intercellular Partial Pressure of CO_2 (μbar), p_i, Measured with 2 mmol m^{-2} s^{-1} Photon Flux Density at the Illuminated Surface, 305 μbar Ambient Partial Pressure of CO_2, 30°C Leaf Temperature, and 20 mbar Vapor Pressure Difference between Leaf and Air*

	A	g	p_i
C_3 species			
Atriplex hastata	29.8	0.35	225
Eucalyptus camaldulensis	35.4	0.32	201
Eucalyptus pauciflora	26.0	0.29	228
Gossypium hirsutum	33.0	0.35	216
Helianthus annuus	26.7	0.35	235
Phaseolus vulgaris	23.0	0.26	228
Rumex acetosa	14.0	0.12	194
Spinacia oleracia	22.2	0.20	196
C_4 species			
Amaranthus edulus	34.0	0.16	96
Imperata cylindrica	20.3	0.09	88
Pennisetum purpureum	55.7	0.26	98
Zea mays	53.0	0.25	94

are shown in Figure 5. The difference in p_i between *Z. mays* and *G. hirsutum* is typical of the differences between C_4 and C_3 species in general, although Table I indicates there is a slight variation in p_i among species having the same photosynthetic pathway.

To understand mechanisms it is useful to know whether p_i is similar in plants grown at different ambient partial pressures of CO_2. Two populations of *Z. mays* plants were treated similarly except that one was grown with p_a = 320 μbar, and the other with p_a = 640 μbar (Fig. 6). For both treatments $A = 204g \times 10^{-6}$ (as with the data shown in Fig. 3) when measurements were made at p_a = 305 μbar, and $A = 397g \times 10^{-6}$, implying p_i = 200 μbar, when measurements were made at p_a = 610 μbar. A similar

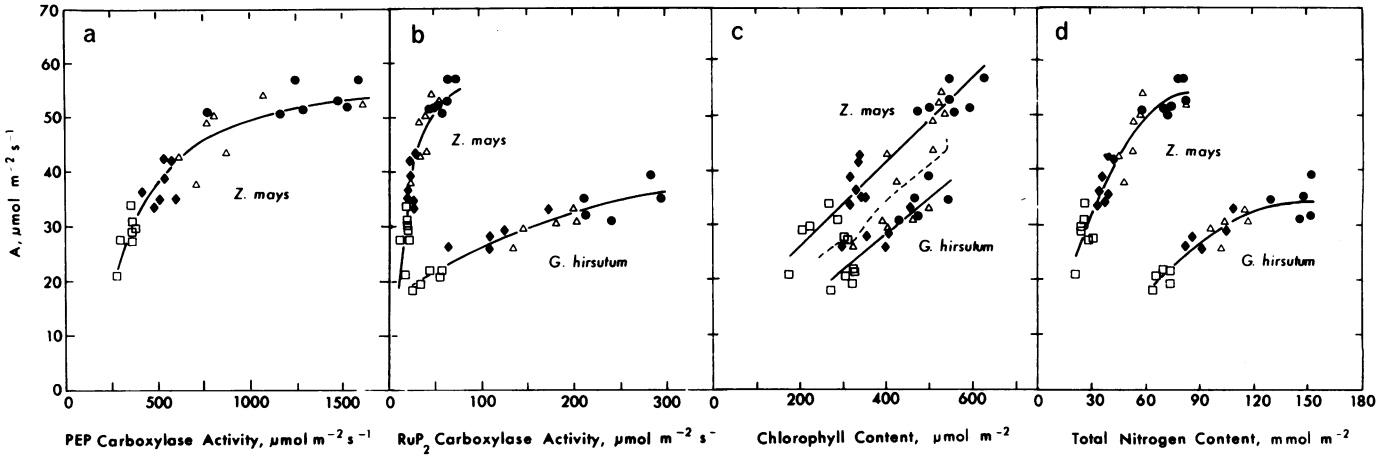

FIG. 5. Rate of CO_2 assimilation, A, as a function of (a) PEP carboxylase activity, (b) RuP_2 carboxylase activity, (c) Chl content, and (d) total nitrogen content in leaves of *Z. mays* and *G. hirsutum*. The measurements of A are those shown in Figure 3(a).

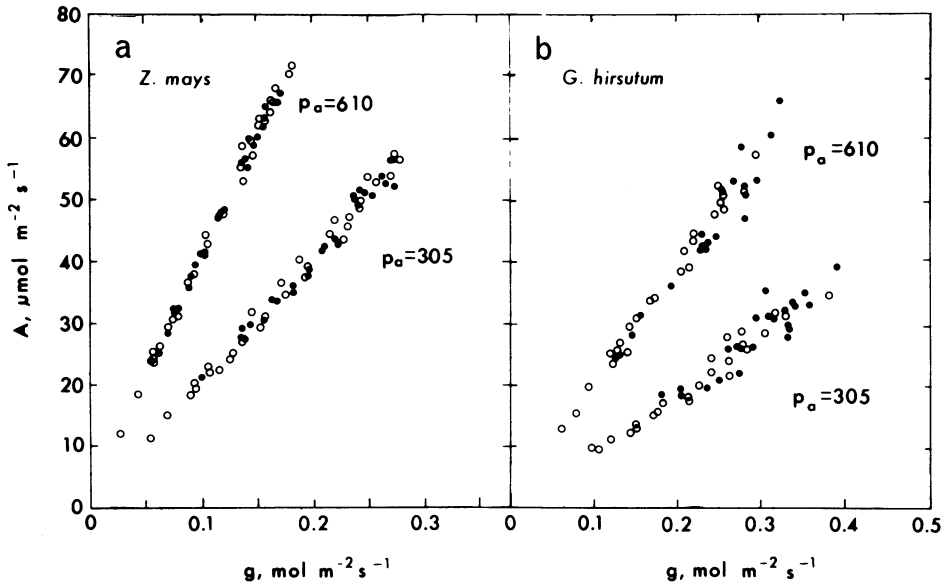

FIG. 6. Rate of CO_2 assimilation, A, and leaf conductance, g, measured at p_a = 305 and p_a = 610 μbar ambient partial pressure of CO_2 in plants of (a) *Z. mays*, (b) *G. hirsutum*, grown at 320 (●) and 640 (○) μbar ambient partial pressure of CO_2 and four levels of nitrate.

result was found with *G. hirsutum* (Fig. 6b) in which, irrespective of p_a during growth, $A = 100g \times 10^{-6}$ when p_a was 305 μbar and $A = 190g \times 10^{-6}$, implying $p_i = 410$ μbar, when p_a was 610 μbar.

DISCUSSION

Both *Z. mays* and *G. hirsutum* exhibit one-to-one relationships between A and g measured at normal ambient partial pressure of CO_2, p_a, irrespective of the source of variation in A and g (Figs. 2 and 3). The variations in A were undoubtedly due to variations in the characteristics of photosynthetic carbon metabolism in the leaf mesophyll, brought about primarily by differences in growth conditions. That is demonstrated in the case of plants having differing levels of nitrogen nutrition by the $A(p_i)$ relationships in Figure 1, and by the dependencies of A on leaf nitrogen content, Chl content, and enzyme activities shown in Figure 5.

That the relationships between A and g happen to be linear, with the corollary that p_i was nearly constant, is probably not of fundamental importance. In fact, there was a slight tendency, to be discerned in Figure 4, for p_i to decrease with increases in A and g. But, had the measurements been made with a larger boundary layer conductance, for example, then g would have been a positively curved function of A, and p_i would have increased with increase in A and g. The near constancy of p_i at a given p_a demonstrated in our results does have an implication, however. The differences in g associated with differences in A were not affected by sensitivity of the stomata to partial pressure of CO_2. The sensitivity of the stomata to change in p_i was, in any case, small (3, 4, 8). The characteristics in Figures 1 and 2, typical of many we have determined in *Z. mays*, shows that the response of g to change in p_i was inadequate to prevent substantial change in p_i with change in p_a. Figure 6 shows that the tendency for the stomata to adjust to maintain p_i constant was no greater in the long term than in the short.

Having ruled out p_i as a possible link, we are left with the question: what is responsible for the single relationship between A and g that is maintained when differences in A and g are due to differences in nitrogen nutrition, phosphorus nutrition, light during growth, and natural variation among plants having had the same growth treatment? Perhaps guard cell metabolism and leaf mesophyll metabolism had been influenced by growth independently, but in such a way that a single relationship between A and g was maintained. Or it may be that stomatal function was partially controlled by mesophyll function, the signal being transmitted via the epidermis rather than the intercellular air space (2, 5, 9). These possibilites require investigation.

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APPENDIX

The conductance to CO_2 transfer, g, used throughout this series of papers was found as the reciprocal of the resistance, $r = r_s + r_b + r_e$, where r_s and r_b are, respectively, the resistances to CO_2 transfer across the leaf epidermis and boundary layer, the r_e is an additional resistance allowing for the difference between the partial pressure on CO_2 in the air entering the chamber, which was usually maintained constant at 305 μbar, and that in the chamber itself. The stomatal and boundary layer resistances to CO_2 were taken to be 1.6 and 1.37 times the corresponding resistances to water vapor. The resistance r_b was 1.52 $m^2 \cdot s \cdot mol^{-1}$. As the partial pressure of CO_2 within the chamber was taken as the mean of those in the ingoing and outgoing air streams, then $r_e = 0.5a/u$, a being the area of leaf enclosed in the chamber and u the flux of air through the chamber. With $a = 2 \times 10^{-4}$ m^2 and $u = 0.51 \times 10^{-3}$ mol s^{-1}, $r_e = 0.19$ $m^2 \cdot s \cdot mol^{-1}$. This is an order of magnitude smaller than r_b and the smallest values of r_s; therefore, its influence on the estimate of g was slight. The sum $r_b + r_e$ represents an effective boundary layer resistance of 1.71 $m^2 \cdot s \cdot mol^{-1}$.