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# Light Adaptation/Acclimation of Photosynthesis and the Regulation of Ribulose-1,5-Bisphosphate Carboxylase Activity in Sun and Shade Plants[1]

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## ABSTRACT

**The consequences of light adaptation and acclimation of photosynthesis on photosynthetic nitrogen use efficiency (NUE), particularly as it relates to the efficiency of ribulose-1,5-bisphosphate carboxylase (Rubisco) use in photosynthetic $CO_2$ assimilation, was studied in the sun species *Glycine max* and the shade species *Alocasia macrorrhiza*. Both *G. max* and *A. macrorrhiza* were found to possess the capacity for light acclimation of $CO_2$ assimilation, but over distinctly different ranges of photon flux density (PFD). For each species, light acclimation of photosynthesis had little effect on the rate of photosynthesis per unit Rubisco protein or the light response of Rubisco carbamylation and CA1P metabolism. In contrast, photosynthesis per unit Rubisco protein was significantly higher in *G. max* than in *A. macrorrhiza*, due in part to a lower total (fully carbamylated) molar activity (activity per unit enzyme) of *A. macrorrhiza* Rubisco than that of *G. max*. Comparison of the light response of Rubisco regulatory mechanisms between *G. max* and *A. macrorrhiza* indicated some degree of adaptation, such that carbamylation was higher and CA1P levels lower at lower PFDs in the shade species than the sun species. However, this adjustment was not sufficient for Rubisco in low light grown *A. macrorrhiza* to be fully active at the growth PFD. Photosynthesis in *A. macrorrhiza* appeared to become RuBP regeneration-limited at lower PFDs than *G. max*, and this was probably the determinant of the light saturated rate of photosynthesis in the shade species. The low efficiency of Rubisco use in *A. macrorrhiza* was a major contributing factor to its five- to sixfold lower photosynthetic NUE than *G. max*. Shade species such as *A. macrorrhiza* appear to make far from maximal use of Rubisco protein N.**

Plant species are typically genetically predisposed (adapted) for growth over a specific range of PFD.[2] These so-called sun or shade plants may also possess the capacity to respond to differences in PFD within the PFD range which they are adapted to grow (acclimation). Both adaptation and acclimation to different PFDs involve numerous changes in the morphology, physiology, and biochemistry of the plant, including photosynthesis (for review, see ref. 2). Adaptation/acclimation of the photosynthetic apparatus involves changes in the levels of carbon reduction cycle enzymes, electron transport components, and proteins and pigments associated with light harvesting. This adaptation/acclimation is often characterized by a redistribution of resources among these components of the photosynthetic apparatus, and is dominated by the capacity of the plant to change the proportion of leaf N dedicated to Rubisco protein (for review, see ref. 6). Since as much as 20 to 25% of total N in a leaf may be contained in Rubisco (5), changes in the activity and/or regulation of this enzyme associated with light adaptation/acclimation could have a considerable impact on photosynthetic NUE.

Rubisco activity is light-dependent, both because production of the substrate RuBP is dependent upon ATP and NADPH production, and because mechanisms for the control of this enzyme's activity are linked to PFD (for review, see refs. 9 and 11). These mechanisms, carbamylation-decarbamylation, Rubisco activase, and CA1P metabolism, affect the efficiency of Rubisco use. At low PFDs, where the capacity for RuBP regeneration typically limits photosynthesis, the efficiency of Rubisco use is potentially low, as evidenced by the fact that the activity of the enzyme is generally reduced by these regulatory mechanisms to match the reduced capacity for RuBP regeneration (3, 8). Plants which grow at low PFD might be expected to produce less Rubisco per unit area than plants growing at high PFD, and regulate its activity in such a way that it was fully active at lower PFDs than plants growing at high PFDs. In this paper, a hypothesis is proposed concerning how plants which grow at different PFDs might adjust the regulatory characteristics of Rubisco in order to maximize photosynthetic NUE. To test this hypothesis, the photosynthetic characteristics and Rubisco regulatory properties of two species which are adapted to grow at substantially different PFDs were examined. *Glycine max* (soybean) is adapted for growth at relatively high PFDs. *Alocasia macrorrhiza* (an Australian tropical understory species) is generally considered to be adapted for growth at low PFDs. The results reported in this paper demonstrate that each species possesses a significant capacity for acclimation of photosynthesis to different PFDs within the bounds of the PFD ranges to which they are adapted. Photosynthetic NUE of both species was conserved during light acclimation, primarily through adjustments in the level of Rubisco protein. However, photosyn-

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[2] Abbreviations: PFD, photon flux density; CA1P, 2-carboxyarabinitol 1-phosphate; $C_i$, intercellular $CO_2$ partial pressure; N, total nitrogen; NUE, nitrogen use efficiency; RuBP, ribulose 1,5-bisphosphate; Rubisco, RuBP carboxylase (EC 4.1.1.39).

thetic NUE was significantly lower in *A. macrorrhiza* than in *G. max*, primarily due to a reduction in the efficiency of Rubisco use for photosynthesis, despite the fact that apparently adaptive differences in the light response of Rubisco regulation were observed. This lower efficiency (lower whole leaf $CO_2$ assimilation per unit Rubisco protein) in *A. macrorrhiza* was the result of both a failure of Rubisco regulatory mechanisms to produce full enzyme activity at the lowest growth PFDs, and an apparently lower specific activity of the enzyme than that of *G. max*. Measurement of the RuBP pool size in both species indicated that RuBP regeneration capacity may limit the full potential activity of Rubisco from being expressed in *A. macrorrhiza*, even at PFDs which can produce full activity of this enzyme *in vivo*.

## MATERIALS AND METHODS

### Plant Growth

*Glycine max* var Williams was grown from seed in a naturally illuminated greenhouse at 27/18°C and 60% RH. Plants received either 1000 to 1500 μmol quanta $m^{-2}$ $s^{-1}$ or 250 to 500 μmol quanta $m^{-2}$ $s^{-1}$, the latter PFD range provided by growing plants within enclosures surrounded by plastic screen. *Alocasia macrorrhiza* (L.) G. Don was grown from seed originally collected in a rain forest area near Atherton, Queensland, Australia, by Dr. Robert Pearcy (University of California, Davis). Plants were maintained in either a growth room, where they received 20 to 50 μmol quanta $m^{-2}$ $s^{-1}$ from fluorescent growth lamps, or in the greenhouse, where they received natural illumination of 250 to 500 μmol quanta $m^{-2}$ $s^{-1}$ under plastic screens. All plants received modified Hoagland solution containing 10 mM $NO^-_3$ daily (12).

### Photosynthesis and Rubisco Measurements

Measurements of $CO_2$ assimilation rates of attached, intact leaves were made using a gas exchange system described by Kobza and Seemann (8). Leaf temperature was maintained at approximately 25°C and the leaf to air vapor pressure difference at 10 mbar. Light was provided by a fiber optic illuminator (Schott KL 1500), and PFD was determined with a LiCor quantum sensor (model LI185B). Calculations of evaporation, conductance to gas exchange, $CO_2$ assimilation rate, and $C_i$ were made according to Caemmerer and Farquhar (4). Leaves were allowed to equilibrate in the gas exchange cuvette at a particular PFD for approximately 30 min before the photosynthetic rate was determined. A photosynthesis cuvette was used in which an 8 $cm^2$ leaf disc could be rapidly frozen to stop metabolism (<250 ms to 0°C) and split in half, allowing measurements of photosynthesis, Rubisco content, Rubisco carbamylation state, Rubisco molar activity, RuBP pool size, and Chl content to be made on the same leaf. Samples were stored in liquid nitrogen until processing. Each data point in Figures 2 through 5 represent the mean ± SE of three to six samples.

Measurement of Rubisco carbamylation, molar activity, and content were made using half of the frozen leaf sample, as described by Kobza and Seemann (8). The carbamylation percentage (activation state) of Rubisco is the initial activity (substrate-saturated activity of rapidly extracted enzyme) divided by the fully carbamylated activity (total activity) (×100). It should be noted that the carbamylation percentage does not include any catalytic sites bound with a tight binding inhibitor such as CA1P, since those sites have no activity in either the initial or total assays.

The total molar activity (activity per unit enzyme) of Rubisco was obtained by determining the enzyme content in the extract by $^{14}C$-labeled 2-carboxyarabinitol 1,5-bisphosphate binding, as described by Kobza and Seemann (8). The molar activity of Rubisco (mol $CO_2 \cdot mol^{-1}$ Rubisco $\cdot s^{-1}$) was calculated by dividing the total activity (fully carbamylated activity) by the Rubisco content. This value is dependent on the concentration of tight-binding inhibitors (*e.g.* CA1P) in the leaf and not on the carbamylation state (8).

RuBP assays were carried out with $HClO_4$ acid extracts of the other half of the leaf disc, as described by Kobza and Seemann (8). Chl concentration was determined according to Arnon (1) on an aliquot of the initial leaf Rubisco extract taken prior to centrifugation.

## RESULTS AND DISCUSSION

### Model for Sun/Shade Adaptation/Acclimation

This section presents a model for sun/shade (high light/low light) adaptation/acclimation of photosynthesis and Rubisco regulation. The data presented in Figure 1 are hypothetical, but reflect what is already known about light adaptation/acclimation of photosynthesis and the effects of changing PFD on Rubisco activity. Figure 1A illustrates the light response of photosynthetic $CO_2$ assimilation for hypothetical sun and shade species (or sun and shade leaves of one species). The sun plant/leaf (adapted or acclimated for growth at high PFD) is postulated to have a light-saturated photosynthetic rate (on a leaf area basis) that is twice that of the shade plant/leaf (adapted or acclimated for growth at low PFD). The PFD at which photosynthesis is light saturated in the sun plant/leaf is also twice that of the shade plant/leaf. The sun plant/leaf would then be expected to contain twice the amount of Rubisco protein per unit leaf area as the shade plant/leaf in order to support the twofold higher rate of photosynthesis. These relative levels of Rubisco protein assume that Rubisco activity per unit enzyme is equal between the sun and shade species/leaves, that there is no light regulation of Rubisco activity, and that the rate of photosynthesis is limited by Rubisco activity. The rates of photosynthesis on a leaf area basis (Fig. 1A) can then be expressed on the basis of the Rubisco content (mol $CO_2$ fixed $\cdot mol^{-1}$ Rubisco $\cdot s^{-1}$) (Fig. 1B). Maximal rates of photosynthesis per unit Rubisco would then be equal in the two plants/leaves, but the shade plant/leaf would achieve that maximal rate at half the PFD as the sun plant/leaf.

Light regulation of Rubisco activity can now be imposed upon these hypothetical sun and shade plants/leaves. Evidence indicates that the extent of Rubisco carbamylation and CA1P metabolism parallel the rate of photosynthesis in sun plants such as *Phaseolus vulgaris, Beta vulgaris,* and *Spinacea oleracea* (8). A hypothetical relationship between PFD and Rubisco carbamylation for a sun plant/leaf is illustrated by

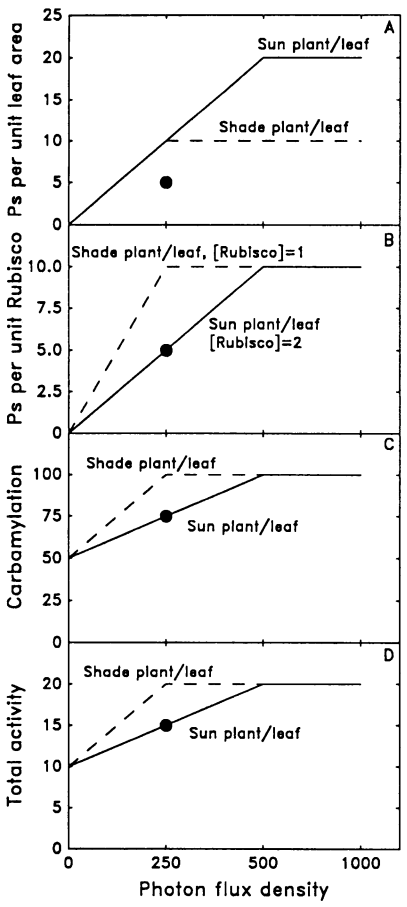


**Figure 1.** Hypothetical responses of sun (solid lines) and shade (dashed lines) plants/leaves to PFD ($\mu$mol quanta $m^{-2}$ $s^{-1}$). A, Photosynthesis per unit leaf area; B, photosynthesis per unit Rubisco protein, assuming the sun plant/leaf has twice as much Rubisco per unit leaf area (2 units) as the shade plant/leaf (1 unit); C, the percent carbamylation of Rubisco in these plants; D, the total (fully carbamylated) molar activity of Rubisco (mol $CO_2 \cdot mol^{-1}$ Rubisco $\cdot s^{-1}$) in these plants. In panels C and D, the solid circles indicate the percent carbamylation and molar activity of Rubisco in the shade plant/leaf if the regulatory characteristics of its Rubisco were the same as in the sun plant/leaf. The consequences of such regulation on whole leaf photosynthesis for the shade plant/leaf are shown by the solid circles in panels A and B.

the solid line in Figure 1C. At a PFD which is light-saturating for photosynthesis, Rubisco is fully carbamylated. The hypothetical relationship between PFD and the total molar activity of Rubisco, which is a quantitative measure of the CA1P pool size (lower total activity corresponds to greater CA1P pool size; see ref. 8), is illustrated for a sun plant/leaf by the solid line in Figure 1D. The total activity of Rubisco also reaches its maximal level at the same PFD where the rate of photosynthesis saturates. If the assumption is made that Rubisco is used with equal effectiveness in photosynthesis (constant photosynthesis/unit Rubisco) in both sun and shade plants/leaves, then there should be a quantitative change in the light response of regulation of Rubisco activity by these two regulatory mechanisms in the shade plant/leaf. This change is necessary in order that Rubisco be fully active in the shade plant/leaf at a lower PFD then it would be in the sun plant/leaf. In other words, both the carbamylation state and total activity of Rubisco should be maximal at the PFD at which photosynthesis is light saturated (or vice versa). Such a light response of Rubisco regulation is illustrated by the dashed lines in Figure 1, C and D. In this hypothetical example, Rubisco in the shade plant/leaf is fully active at one-half the PFD at which both full carbamylation and the maximum molar activity (all CA1P metabolized) is achieved in the sun plant/leaf. This adjustment of Rubisco regulation would allow the shade plant/leaf to use half as much Rubisco to achieve half the photosynthetic rate of the sun plant/leaf. Alternatively, if the Rubisco in the shade plant/leaf retained the regulatory characteristics of the Rubisco in the sun plant/leaf, the carbamylation state and total activity of Rubisco at the light saturation point for photosynthesis would be those indicated by the solid circles on the sun plant/leaf Rubisco responses in Figure 1, C and D. The resultant 50% lower Rubisco activity (25% reduction associated with each of the two regulatory mechanisms) of the same amount of Rubisco would cause the shade plant/leaf to achieve only half the photosynthetic rate per unit area and per unit Rubisco (solid circles in Fig. 1, A and B) as it would with the adjusted Rubisco regulatory characteristics.

The effect of each of these two regulatory options on photosynthetic NUE can then be approximated. The assumption is made that 10% of the N in the hypothetical shade plant/leaf is Rubisco protein (similar to *A. macrorrhiza*; 12), and that this plant/leaf exists in a habitat with a maximum incident PFD of 250 $\mu$mol quanta $m^{-2}$ $s^{-1}$. In the hypothetical plant that can acclimate (or has adapted) Rubisco regulatory characteristic to lower PFDs, all 10% of that protein N would be in active Rubisco at 250 $\mu$mol quanta $m^{-2}$ $s^{-1}$. In contrast, the hypothetical plant/leaf which cannot acclimate Rubisco regulation would have 5% of its total leaf N (one-half of its Rubisco) inactive at 250 $\mu$mol quanta $m^{-2}$ $s^{-1}$. At greater Rubisco N/Total N ratios, the reduction in NUE would be proportionately greater. A shade plant/leaf growing at less than 250 $\mu$mol quanta $m^{-2}$ $s^{-1}$ with no further adjustment of Rubisco regulatory characteristics would also suffer a reduction in photosynthetic NUE.

This model for light acclimation of photosynthesis is compared in the following sections to data for the sun species *Glycine max* and the shade species *Alocasia macrorrhiza*, each grown at two different PFDs.

### Photosynthesis per Unit Leaf Area

The response of photosynthetic $CO_2$ assimilation per unit leaf area by *G. max* grown at either high (1000–1500 $\mu$mol quanta $m^{-2}$ $s^{-1}$) or low (250–500 $\mu$mol quanta $m^{-2}$ $s^{-1}$) PFD is shown in Figure 2A. In both the low light and high light grown plants, photosynthesis was light saturated at approximately 500 $\mu$mol quanta $m^{-2}$ $s^{-1}$. The light saturated rate of photosynthesis was approximately 40% higher in the high light than low light grown *G. max* (Table I). $C_i$ was similar in plants from the two growth treatments (Table II).

In *A. macrorrhiza*, the pattern of photosynthetic acclimation was qualitatively similar to that in *G. max*, although rates of $CO_2$ assimilation and the PFD required for saturation of

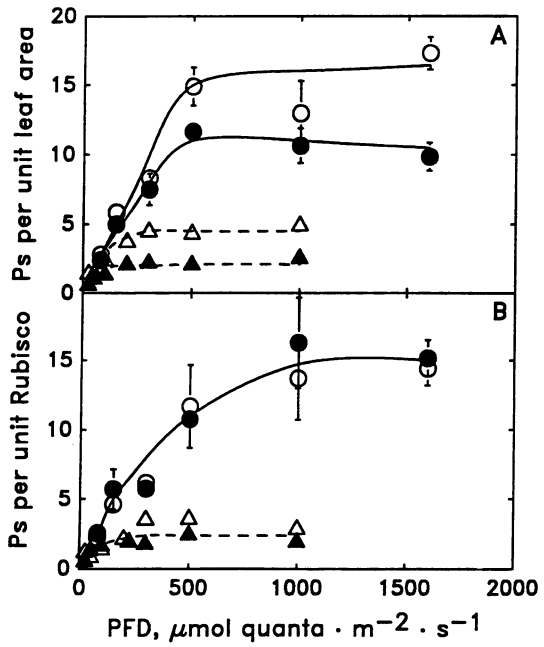


**Figure 2.** A, Photosynthesis per unit leaf area ($\mu$mol $CO_2 \cdot m^{-2} \cdot s^{-1}$) as a function of PFD for *G. max* and *A. macrorrhiza* grown at either high or low PFD. Leaves were equilibrated at the indicated PFD for approximately 30 min before the rate of photosynthesis was recorded and the leaf freeze clamped. B, Photosynthesis per unit Rubisco protein in the same leaf area (mol $CO_2 \cdot mol^{-1}$ Rubisco $\cdot s^{-1}$). Open symbols, high light grown plants; closed symbols, low light plants; solid lines, *G. max*; dashed lines, *A. macrorrhiza*. See "Materials and Methods" section for details of growth conditions.

photosynthesis were substantially lower (Fig. 2A). Photosynthesis was light saturated at approximately 50 to 150 $\mu$mol quanta $m^{-2}$ $s^{-1}$ in plants grown at low PFD (20–50 $\mu$mol quanta $m^{-2}$ $s^{-1}$), and approximately 200 to 300 $\mu$mol quanta $m^{-2}$ $s^{-1}$ in individuals of this species grown at high light (250–500 $\mu$mol quanta $m^{-2}$ $s^{-1}$). At light saturation the rate of $CO_2$ assimilation was approximately 50% greater in high light than low light grown *A. macrorrhiza*. This capacity for photosynthetic light acclimation in *A. macrorrhiza* is similar to that reported for these species by Seemann *et al.* (12) and Sims and Pearcy (14). $C_i$ was similar between high and low light grown *A. macrorrhiza*, but about 10 to 18% lower than the $C_i$ in high and low light grown *G. max* (Table II). Light saturated photosynthetic rates of high and low light grown *G. max* were approximately 70% greater than those of high and low light grown *A. macrorrhiza*, respectively (Table I).

### Photosynthesis per Unit Rubisco

For each species, high light grown plants had more Rubisco per unit leaf area than low light grown plants (45% more in *G. max*, 25% more in *A. macrorrhiza*; see Table II). High and low light grown *A. macrorrhiza* had approximately 5 to 15% more Rubisco protein per unit leaf area then high and low light grown *G. max*, respectively. If rates of photosynthesis per unit leaf area for *G. max* and *A. macrorrhiza* are divided by the Rubisco content per unit area for that same leaf (Fig. 2B), the efficiency of photosynthesis per unit Rubisco can then be assessed. Light acclimation of photosynthesis in both the sun plant *G. max* and the shade plant *A. macrorrhiza* resulted in relatively similar rates of $CO_2$ assimilation per unit Rubisco protein between high and low light grown individuals of each species and close to the same efficiency of Rubisco use. This result is consistent with the original hypothesis, and is to be expected if Rubisco activity limits photosynthetic capacity under both growth PFDs.

Photosynthesis per unit Rubisco was substantially different between the two species, however, not consistent with the original hypothesis. At light saturation *G. max* had rates of photosynthesis per unit of Rubisco protein approximately 80% higher than *A. macrorrhiza* (Table I). The approximately 30 $\mu$bar difference in $C_i$ between these species (Table II) would only account for a small part of this difference (14). This difference between sun and shade species in Rubisco efficiency in photosynthesis is similar to that reported by Seemann *et al.* (12), who compared $CO_2$-saturated rates of photosynthesis to the Rubisco content in *Phaseolus vulgaris* and *A. macrorrhiza*, both grown at a range of PFDs and nitrogen availabilities. They found an approximate two-fold difference in $CO_2$ saturated photosynthesis per unit Rubisco protein between those two species. The greater difference reported here may

**Table I.** *Light Saturated Photosynthetic Rates, Rubisco Contents, and Photosynthetic NUE of High and Low Light-Grown G. max and A. macrorrhiza*

Photosynthetic rates and Rubisco contents are from plants measured and freeze-clamped at light saturation (1600 $\mu$mol quanta $m^{-2}$ $s^{-1}$ for *G. max* and 1000 $\mu$mol quanta $m^{-2}$ $s^{-1}$ for *A. macrorrhiza*). Data in this table thus represents a subset of that in Table II. Leaf nitrogen contents were calculated from the linear regressions for Rubisco *versus* leaf nitrogen in Figure 3 of Seemann *et al.* (12). N data for *P. vulgaris* was used for *G. max*. The ratio of Rubisco nitrogen to total nitrogen was calculated assuming 6.25 g N/g Rubisco and 14 g N/mol.

| Species | Growth PFD | $CO_2$ uptake | Rubisco | Ps/Rub | N | Ps/N × $10^{-3}$ | Rub N/ Tot N |
|---|---|---|---|---|---|---|---|
| | $\mu$mol/m²/s¹ | | $\mu$mol/m² | $s^{-1}$ | mmol/m² | $s^{-1}$ | % |
| *G. max* | 1000 ($n$ = 7) | 17.3 ± 1.2 | 1.26 ± 0.15 | 13.7 | 51 | 0.34 | 15.5 |
| | 250 ($n$ = 6) | 9.8 ± 1.0 | 0.69 ± 0.11 | 14.2 | 39 | 0.25 | 11.1 |
| *A. macrorrhiza* | 250 ($n$ = 4) | 5.1 ± 0.4 | 1.73 ± 0.15 | 3.0 | 100 | 0.05 | 10.9 |
| | 50 ($n$ = 3) | 2.7 ± 0.2 | 1.29 ± 0.02 | 2.1 | 83 | 0.03 | 9.8 |

**Table II.** *Growth Conditions, Rubisco and Chl Contents, and Intercellular $CO_2$ Partial Pressures ($C_i$) for G. max and A. macrorrhiza Grown at High or Low PFDs*

Values are the mean ± SE

| Species | Growth PFD | $C_i$ | Rubisco | Chlorophyll | Rubisco/Chl | Chl A/B |
|---|---|---|---|---|---|---|
| | $\mu mol/m^2/s^1$ | $\mu bar$ | $\mu mol/m^2$ | $\mu g/cm^2$ | g/g | |
| *G. max* | High | 236 ± 4 | 1.31 ± 0.07 | 18.8 ± 0.8 | 3.83 | 3.91 ± 0.06 |
| | (1000) | $n = 31$ | $n = 30$ | $n = 21$ | | $n = 21$ |
| | Low | 233 ± 4 | 0.96 ± 0.07 | 20.5 ± 1.0 | 2.58 | 3.48 ± 0.05 |
| | (250) | $n = 29$ | $n = 27$ | $n = 19$ | | $n = 19$ |
| *A. macrorrhiza* | High | 212 ± 8 | 1.53 ± 0.07 | 32.6 ± 2.5 | 2.58 | 3.55 ± 0.05 |
| | (250) | $n = 23$ | $n = 23$ | $n = 16$ | | $n = 16$ |
| | Low | 192 ± 11 | 1.02 ± 0.06 | 55.3 ± 2.5 | 1.01 | 3.20 ± 0.05 |
| | (50) | $n = 19$ | $n = 19$ | $n = 17$ | | $n = 17$ |

be the result of not only the difference in $C_i$, but also the somewhat lower rates of photosynthesis for *A. macrorrhiza* than have been observed in some other studies (12, 14), although not all (13).

### Rubisco Carbamylation

The steady state values of Rubisco carbamylation in leaves of both high and low light grown *G. max* and *A. macrorrhiza* (associated with the photosynthetic rates shown in Fig. 2) are shown in Figure 3A. The relationship between carbamylation and PFD was similar for both high and low light grown plants of each species. In *G. max* Rubisco carbamylation increased in a curvilinear fashion from 40 to 50% at low PFDs to 85 to 95% at 1000 $\mu$mol quanta $m^{-2}$ $s^{-1}$, with a PFD for light saturation of approximately 500 $\mu$mol quanta $m^{-2}$ $s^{-1}$, similar to that for photosynthesis (Fig. 2A). In this instance, acclimation of photosynthesis did not involve changes in the light response of Rubisco carbamylation, as originally hypothesized. Whether growth of *G. max* at PFDs lower than 250 to 500 $\mu$mol quanta $m^{-2}$ $s^{-1}$ would have resulted in such a response is not known.

In contrast, in *A. macrorrhiza* there is an indication that carbamylation saturated at a somewhat lower PFD (150–200 $\mu$mol quanta $m^{-2}$ $s^{-1}$) than in *G. max*, as hypothesized. This apparent reduction in the PFD required for full carbamylation of Rubisco in *A. macrorrhiza* could allow individuals of this species growing at 250 $\mu$mol quanta $m^{-2}$ $s^{-1}$ to make more efficient use of their Rubisco by having less enzyme which is more active. However, it is clear that *A. macrorrhiza* growing at 20 to 50 $\mu$mol quanta $m^{-2}$ $s^{-1}$ would have contained Rubisco that was only approximately 60% carbamylated at this PFD, indicating that *A. macrorrhiza* could not adjust the light response of Rubisco carbamylation to make the most efficient use of Rubisco at these very low PFDs. This result may reflect the capacity of Rubisco activase. The fact that steady state photosynthesis did not increase above 150 to 200 $\mu$mol quanta $m^{-2}$ $s^{-1}$ in low light grown *A. macrorrhiza*, even though Rubisco carbamylation increased, indicates that photosynthesis was rate limited by some other factor than the carboxylation capacity of Rubisco (see below). A step increase in PFD (*e.g.* a sunfleck) sufficient to promote full carbamylation of Rubisco might cause a transient increase in photosynthesis until RuBP regeneration capacity became rate limiting.

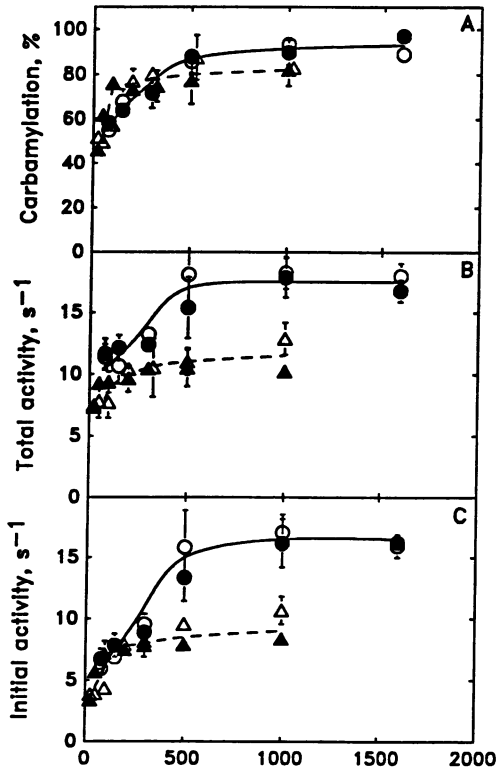


**Figure 3.** A, Carbamylation state of Rubisco (percent) as a function of PFD in leaves of *G. max* and *A. macrorrhiza* used for the determination of photosynthetic rate in Figure 2. See "Materials and Methods" section for details concerning the measurement of carbamylation state. B, The total (fully carbamylated) molar activity of Rubisco (mol $CO_2 \cdot mol^{-1}$ Rubisco $\cdot s^{-1}$) as a function of PFD in leaves used for the determination of photosynthesis in Figure 2. C, The initial activity of Rubisco as a function of PFD. Symbols and lines are as in Figure 2.

### Rubisco Total Activity

Changes in the total (fully carbamylated) molar activity of Rubisco (activity per unit Rubisco protein) have been related to changes in the leaf content of the naturally occurring tight binding inhibitor of Rubisco catalysis, CA1P (11). CA1P binds stoichiometrically to Rubisco catalytic sites such that, for example, a 50% reduction in the fully carbamylated molar activity of Rubisco is the result of a CA1P pool size of 0.5 mol CA1P $\cdot mol^{-1}$ Rubisco catalytic sites (illustrated in Fig.

1D). This compound is produced by both *G. max* (15) and *A. macrorrhiza* (10).

In both *G. max* and *A. macrorrhiza*, Rubisco total activity was low at low PFD (Fig. 3B), and increased as PFD increased. In *G. max* the total activity reached its maximum (approximately 17.5 $s^{-1}$) at approximately 500 μmol quanta $m^{-2}$ $s^{-1}$, similar to the PFD at which full carbamylation occurred (Fig. 3A) and photosynthesis became light saturated (Fig. 2A). There were no significant differences in the light response of the total molar activity of Rubisco (CA1P metabolism) between high and low light grown individuals of this species. In *A. macrorrhiza* the light response of Rubisco total activity was also similar between high and low light grown individuals of this species, but both qualitatively and quantitatively different than *G. max*. Rubisco total activity in *A. macrorrhiza* saturated at a lower PFD than *G. max*, about 200 μmol quanta $m^{-2}$ $s^{-1}$. Thus, *A. macrorrhiza* growing at 250 μmol quanta $m^{-2}$ $s^{-1}$ would have degraded all CA1P bound to Rubisco, and the total activity of the enzyme would be maximal. This difference between *G. max* and *A. macrorrhiza* in the light response of Rubisco regulation is consistent with the original hypothesis. However, *A. macrorrhiza* growing at 20 to 50 μmol quanta $m^{-2}$ $s^{-1}$ would contain inactive Rubisco resulting from CA1P bound to the enzyme at this PFD. Again, a step increase in PFD (*e.g.* a sunfleck) could transiently increase photosynthesis by promoting rapid CA1P degradation.

At high PFD, the total molar activity of Rubisco should reflect the maximum specific activity of the enzyme, since all CA1P should be degraded above approximately 500 μmol quanta $m^{-2}$ $s^{-1}$ (8, 10). The maximum molar activity of Rubisco from *A. macrorrhiza* (approximately 11.5 $s^{-1}$) was significantly lower than that of *G. max* (approximately 17.5 $s^{-1}$) (Fig. 3B), when leaves of both species were extracted and assayed under identical conditions. Seemann *et al.* (10) reported somewhat higher values for the maximum total activity of Rubisco from *A. macrorrhiza*, but values for *G. max* were not obtained at the same time for direct comparison. The lower maximum total activity of *A. macrorrhiza* Rubisco as compared to Rubisco from *G. max* suggests that there exist intrinsic differences in the catalytic activity per unit protein of this enzyme between these species. Differences in the specific activity of Rubisco between species have been observed previously and have been related to differences in whole leaf photosynthetic $CO_2$ assimilation (for review, see ref. 5). The approximately 35% lower total activity of Rubisco in *A. macrorrhiza* as compared to *G. max* indicates that the shade species would have to produce 35% more Rubisco per unit leaf area than the sun species in order to have an equal rate of photosynthesis, all else remaining the same. Whether this characteristic of Rubisco is a general phenomenon among plant species genetically adapted to growth at low light remains to be determined.

### Rubisco Initial Activity

The initial activity of Rubisco is the result of both carbamylation and CA1P effects on its activity, and the light response of this activity is shown in Figure 3C. The light response of Rubisco initial activity was similar for high and low light grown individuals of each species, but substantially different between *G. max* and *A. macrorrhiza*. This activity was light saturated at a substantially lower PFD in *A. macrorrhiza* (approximately 200 μmol quanta $m^{-2}$ $s^{-1}$) than in *G. max* (approximately 500 μmol quanta $m^{-2}$ $s^{-1}$). This result suggests a genetically based difference in the light regulation of Rubisco between these two species, and is in agreement with the hypothesis concerning efficient use of Rubisco for photosynthesis.

These differences between *G. max* and *A. macrorrhiza* in Rubisco regulation and activity result in significant differences in the relationship between photosynthesis per unit Rubisco protein (from Fig. 2B) and the initial activity of Rubisco (Fig. 4). There was a significant linear relationship between these two parameters for each species, as has been demonstrated for a number of other species (3, 8). However, the slope of the relationship for *G. max* (1.12) was approximately threefold higher than that for *A. macrorrhiza* (0.35).

### RuBP Pool Sizes

Measurement of the RuBP pool size in high and low light grown *G. max* and *A. macrorrhiza* indicated little difference in the amount of this compound per unit leaf area between light treatments of a single species (Fig. 5). The shape of the response of the RuBP pool size to PFD was similar in both species. However, the RuBP pool size in *A. macrorrhiza* was significantly lower than in *G. max* at all PFDs. Since Rubisco levels per unit leaf area were somewhat higher in *A. macrorrhiza* than *G. max* (Tables I and II), the RuBP content expressed on a Rubisco catalytic site basis was approximately twofold higher in *G. max* than *A. macrorrhiza*. This lower capacity for RuBP production relative to the Rubisco content in *A. macrorrhiza* indicates that the photosynthetic capacity of *A. macrorrhiza* may have been limited by RuBP regeneration capacity at higher PFDs, particularly in the low light grown plants. This apparent limitation was likely responsible for determining the light saturation point of photosynthesis for low light grown *A. macrorrhiza*, since higher PFDs which increased Rubisco initial activity did not increase photosynthesis.

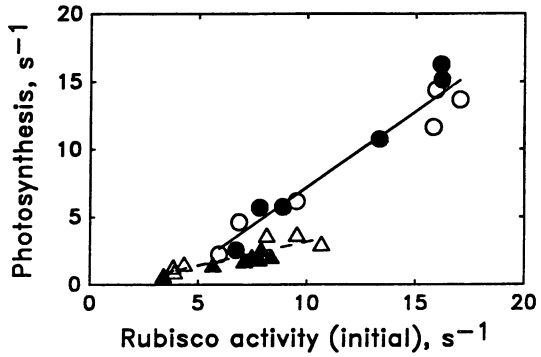


**Figure 4.** Relationship between photosynthesis per unit Rubisco protein (from Fig. 2B) and the initial activity of Rubisco in the same leaf (from Fig. 3C) for *G. max* and *A. macrorrhiza*. Symbols and lines are as in Figure 2. The linear regressions are: *G. max*, $y = 1.121 \times - 4.011$, $r = 0.97$; *A. macrorrhiza*, $y = 0.349 \times -0.314$, $r = 0.85$.

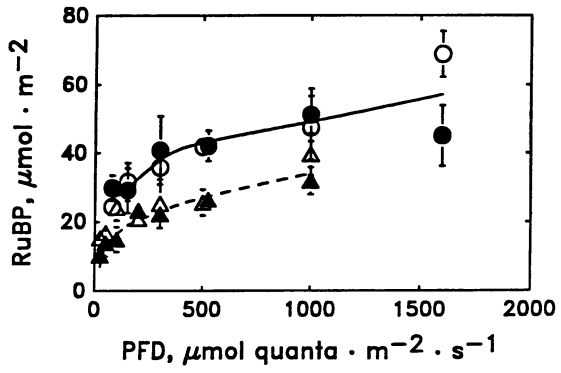


**Figure 5.** RuBP pool size as a function of PFD for *G. max* and *A. macrorrhiza*, in the same leaves used for Figures 2, 3, and 4. Symbols and lines are as in Figure 2.

### Nitrogen Allocation and Photosynthetic NUE

Approximately 50% of leaf nitrogen is associated with proteins involved in photosynthesis (6). This protein nitrogen is found in one of two major pools, either thylakoid membrane-bound proteins involved in the light reactions for photosynthesis, or soluble proteins associated with the photosynthetic carbon reduction cycle. Leaf Chl and Rubisco content can serve as indicators of protein nitrogen content in the membrane bound and soluble protein pools, respectively (5). Chl content of leaves of *G. max* was relatively unaffected by growth at different PFDs (Table II), similar to *Phaseolus vulgaris* (12). In *A. macrorrhiza*, Chl content was approximately 40% higher in high light grown than low light grown plants, and from 40 to 60% higher than in *G. max* (Table II). The Rubisco/Chl ratio, an indicator of the relative investment in stromal *versus* thylakoid proteins, was significantly lower in *A. macrorrhiza* than *G. max*, and lower in low light grown than in high light grown individuals of both species (Table II). This result indicates a greater relative investment of leaf N at low light into light harvesting components of the photosynthetic apparatus than into Rubisco protein, which declined from 15 to 10% of total leaf N between high light grown *G. max* and low light grown *A. macrorrhiza* (Table I). Such reductions in the partitioning of leaf total N to Rubisco are characteristic of adaptation and acclimation to low light (2, 6). Chl *A/B* ratios were lower in *A. macrorrhiza* than *G. max*, and lower in low light grown individuals of each species than in high light grown individuals (Table II), consistent with adaptation and acclimation to growth at low light, respectively.

Comparison of photosynthetic NUE (photosynthesis at light saturation per unit leaf N) between *G. max* and *A. macrorrhiza* (Table I) indicates that NUE was slightly higher in high light than low light grown plants of the same species, but approximately six- to sevenfold higher in *G. max* than in *A. macrorrhiza*. Seemann *et al.* (12) found a two- to threefold difference between *P. vulgaris* and *A. macrorrhiza* calculated using $CO_2$-saturated rates of photosynthesis. The lower efficiency of Rubisco use in photosynthesis in *A. macrorrhiza* than *G. max* was a major contributor to the shade species' significantly lower photosynthetic NUE than the sun species.

## CONCLUSIONS

Species such as *G. max* and *A. macrorrhiza* appear to be genetically adapted for growth in a particular range of PFDs (high and low, respectively). Within this range, each possesses the capacity to acclimate the photosynthetic apparatus for growth at different PFDs. This adaptation/acclimation process appears to involve some change in the light response of Rubisco regulatory mechanisms between these two species, consistent with the original hypothesis for sun and shade plants. Rubisco in the shade species (*A. macrorrhiza*) can reach full activity at lower PFDs than the enzyme in the sun species (*G. max*), and thus *A. macrorrhiza* requires less enzyme than if its Rubisco were regulated as it is in *G. max*. However, this apparently adaptive response of *A. macrorrhiza* was not sufficient for this species to make the most efficient use of Rubisco protein when growing at very low PFDs, since a portion of it remained inactive due to decarbamylation and the presence of CA1P. This unused Rubisco capacity in *A. macrorrhiza* growing at very low PFDs may be important during transient sunflecks which promote full carbamylation and complete CA1P degradation. The lower apparent total activity (specific activity ) of *A. macrorrhiza* Rubisco than that of *G. max* contributes to the significantly lower photosynthetic NUE in *A. macrorrhiza* relative to *G. max*, which is typical of shade species in general (5). This apparent inefficiency of N use in shade plants seems to support the suggestion by Evans (5) that nitrogen may not be the limiting factor for growth in low light environments, as it often is in high light ones (7).

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## LITERATURE CITED

1. **Arnon DI** (1949) Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant Physiol **24:** 1–15
2. **Björkman O** (1981) Responses to different quantum flux densities. *In* OL Lange, PS Nobel, CB Osmond, H Zeigler, eds, Encyclopedia of Plant Physiology (New Series), Vol 12A. Springer-Verlag, Berlin, pp 57–107
3. **Caemmerer S von, Edmondson DL** (1986) The relationship between steady-state gas exchange, in vivo $RuP_2$ carboxylase activity and some carbon reduction cycle intermediates in *Raphanus sativus*. Aust J Plant Physiol **13:** 669–688
4. **Caemmerer S von, Farquhar GD** (1981) Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. Planta **153:** 376–387
5. **Evans JR** (1989) Photosynthesis and nitrogen relationships in leaves of $C_3$ plants. Oecologia **78:** 9–19
6. **Evans JR, Seemann JR** (1989) The allocation of protein nitrogen in the photosynthetic apparatus: costs, consequences and control. *In* WR Briggs, ed, Photosynthesis. Alan R Liss, Inc (in press)
7. **Field C, Mooney HA** (1986) The photosynthesis-nitrogen relationship in wild plants. *In* TJ Givnish, ed, On the Economy of Plant Form and Function. Cambridge University Press, London, pp 25–55
8. **Kobza J, Seemann JR** (1988) Mechanisms for the light regulation of ribulose-1,5-bisphosphate carboxylase activity and photosynthesis in intact leaves. Proc Natl Acad Sci USA **85:** 3815–3819

9. **Salvucci ME** (1989) Regulation of Rubisco activity *in vivo*. Physiol Plantarum (in press)
10. **Seemann JR, Kirschbaum MUF, Sharkey TD, Pearcy RW** (1988) Regulation of ribulose-1,5-bisphosphate carboxylase activity in *Alocasia macrorrhiza* in response to step changes in irradiance. Plant Physiol **88:** 148–152
11. **Seemann JR, Kobza J, Moore Bd** (1989) Metabolism of 2-carboxyarabinitol 1-phosphate and regulation of ribulose-1,5-bisphosphate carboxylase activity. Photosynth Res (in press)
12. **Seemann JR, Sharkey TD, Wang J, Osmond CB** (1987) Environmental effects on photosynthesis, nitrogen-use-efficiency, and metabolite pools in leaves of sun and shade plants. Plant Physiol **84:** 796–802
13. **Sharkey TD, Seemann JR, Pearcy RW** (1986) Contribution of metabolites of photosynthesis to postillumination $CO_2$ assimilation in response to lightflecks. Plant Physiol **82:** 1063–1068
14. **Sims DA, Pearcy RW** (1989) Photosynthetic characteristics of a tropical understory herb, *Alocasia macrorrhiza*, and a related crop species, *Colocasia esculenta* grown in contrasting light environments. Oecologia **79:** 53–59
15. **Vu JCV, Allen LH, Bowes G** (1984) Dark/light modulation of ribulose bisphosphate carboxylase activity in plants from different photosynthetic categories. Plant Physiol **76:** 843–845