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# Environmental Effects on Photosynthesis, Nitrogen-Use Efficiency, and Metabolite Pools in Leaves of Sun and Shade Plants[1]

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## ABSTRACT

**Effects of varying light intensity and nitrogen nutrition on photosynthetic physiology and biochemistry were examined in the sun plant *Phaseolus vulgaris* (common bean) and in the shade plant *Alocasia macrorrhiza* (Australian rainforest floor species). In both *Phaseolus* and *Alocasia*, the differing growth regimes produced large changes in photosynthetic capacity and composition of the photosynthetic apparatus. $CO_2$-saturated rates of photosynthesis were linearly related to leaf nitrogen (N) content in both species but photosynthesis per unit leaf N was markedly higher for *Phaseolus* than for *Alocasia*. Photosynthetic capacity was also higher in *Phaseolus* per unit ribulose 1,5-bisphosphate (RuBP) carboxylase (RuBPCase) protein. The leaf content of RuBPCase was linearly dependent on leaf N content in the two species. However, the proportion of leaf N which was RuBPCase was greater in *Phaseolus* than in *Alocasia* and was more sensitive to growth conditions, ranging from 6% of leaf N at low light to 20% at high light. In *Alocasia*, this range was much less, 6 to 11%. However, chlorophyll content was much more sensitive to light intensity in *Alocasia*. Thus, the RuBPCase/chlorophyll ratio was quite responsive to N availability and light intensity in both species (but for different reasons), ranging from 6 grams per gram for *Phaseolus* and 2 grams per gram for *Alocasia* at high leaf N and 1.5 gram per gram for *Phaseolus* and 0.5 gram per gram for *Alocasia* at low leaf N. These large changes in the proportions of components of the photosynthetic apparatus had marked effects on the sensitivity of these species to photoinhibition. These environmental effects also caused changes in the absolute levels of metabolites of the photosynthetic carbon reduction cycle. Concentrations of RuBP and P-glycerate were approximately 2-fold higher in high light-grown than low light-grown *Phaseolus* and *Alocasia* when expressed on a leaf area basis. However, if metabolite pool sizes are expressed on the basis of the RuBPCase catalytic site concentration, then they were little affected by the marked changes in leaf makeup. There appears to be fundamental differences between these species in the mechanism of sun-shade adaptation and N partitioning in the photosynthetic apparatus that result in significant differences in the N-use efficiency of photosynthesis between *Phaseolus* and *Alocasia* but similar RuBPCase:substrate:product ratios despite these differences.**

Many plant species exhibit acclimation of their photosynthetic apparatus to varying light intensity. This acclimation is characterized by significant alterations in the relative distribution of resources among the component parts of the photosynthetic apparatus (5). Light intensity also has a particularly dramatic effect on leaf N[3] content, often a limiting resource for plant growth (14). A majority of this N in $C_3$ plants is required for proteins involved in photosynthesis (10, 25) and photosynthetic capacity is known to be generally proportional to leaf N content (6). A significant portion of any change in leaf N content which may result from differences in light intensity during growth is the result of a change in the concentration of RuBPCase[3] (5), as RuBPCase represents approximately 20% of total N in leaves of well fertilized $C_3$ sun plants (12, 21). Changes in the activity of this enzyme are extremely well correlated with changes in photosynthetic capacity which occur with changes in the light intensity of growth (5), but it is unclear whether or not these changes represent an alteration in the relative proportion of total leaf N which has been allocated to this protein. Furthermore, it is important to understand how such changes affect the N-use efficiency of photosynthesis (8).

We hypothesize that if certain components of the leaf N budget predominate in the control of sun/shade acclimation, then redistribution of N among such key protein components of the photosynthetic apparatus may occur. Because large amounts of RuBPCase are required to support observed rates of photosynthesis in $C_3$ plants and it is often rate-limiting for photosynthesis, comparative studies of environmental effects on N-use efficiency of photosynthesis in sun and shade plants should begin by assessing the relative importance of RuBPCase in the N budget of leaves grown under different environmental conditions. We have examined the effect of varying light intensity during growth, in combination with varying N availability, on photosynthetic performance, leaf N, RuBPCase, Chl, and metabolite pools of leaves. We have chosen to study two species, one of which is considered to be a 'sun plant' (*Phaseolus vulgaris*, common bean) and one a 'shade plant' (*Alocasia macrorrhiza*, an Australian rain forest floor species). Although each exhibits substantial acclimation of photosynthesis to a wide range of light intensities, significant differences exist in photosynthetic characteristics and acclimation responses between these species which normally occupy opposite ends of the sun-shade spectrum. We have made use of the differing acclimation capacities and adaptive characteristics of these sun and shade species to address the question of how environmental and genetic factors interact to influence the

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[3] Abbreviations: RuBPCase, RuBP carboxylase/oxygenase, E.C. 4.1.1.39; A, net photosynthetic $CO_2$ assimilation; $C_i$, intercellular $CO_2$ partial pressure; RuBP, ribulose 1,5-bisphosphate; CABP, carboxyarabinitol bisphosphate; PGA, glycerate 3-phosphate.

composition of the photosynthetic apparatus and the N-use efficiency of photosynthesis.

## MATERIALS AND METHODS

*Phaseolus vulgaris* (L.) var Tendergreen (seeds from Northrup King) plants were grown in 4 L plastic pots in a compost:sand:perlite mixture (2:1:1 v:v:v) in a naturally illuminated greenhouse. The temperature was controlled at 27°C d, 15°C night, and RH was controlled at 60%. Plants were watered daily with modified Hoagland solution (16). 'High N' plants received a solution containing 10 mM $NO_3^-$, 6 mM $Cl^-$, 2 mM $SO_4^{2-}$, 1 mM $H_2PO_4^-$, 7 mM $K^+$, 5 mM $Ca^{2+}$, 2 mM $Mg^{2+}$, micronutrients, and FeEDTA. 'Low N' plants received 0.5 mM $NO_3^-$ (winter months) or 2 mM $NO_3^-$ (summer months), 3.5 mM $K^+$, 3 mM $Ca^{2+}$, 1.0 mM $Mg^{2+}$, 1 mM $H_2PO_4^-$, 6 mM $Cl^-$, and 2 mM $SO_4^-$.

Plants were grown throughout the year under either full sunlight or 50% of full sun obtained by placing plants under several layers of plastic screening. Summer plants received maximum light intensities of 1800 μmol quanta $m^{-2}$ $s^{-1}$, while winter plants received about 1000 to 1200 μmol quanta $m^{-2}$ $s^{-1}$. Plants were separated into four growth conditions for *Phaseolus*; (a) high light, high N; (b) high light, low N; (c) low light, high N; and (d) low light, low N.

*Alocasia macrorrhiza* (L.) G. Don was grown from seed collected in a rain forest area near Atherton, Queensland, Australia. Plants were maintained in Reno in either the greenhouse, where they received full sunlight, or in a growth chamber, where they received 20 μmol quanta $m^{-2}$ $s^{-1}$ under shade cloth. These plants were watered daily with Hoagland solution containing 10 mM $NO_3^-$.

Measurements of $CO_2$-saturated rates of photosynthesis were made at intercellular $CO_2$ partial pressures greater than 700 μ bar using a gas exchange system described by Sharkey *et al.* (24). Calculations of evaporation, conductance to gas exchange, $CO_2$ assimilation rate, and $C_i$ were made according to von Caemmerer and Farquhar (28). Leaf temperature was maintained at 25°C and the leaf to air vapor pressure difference at 10 mbar. Light intensity was 1000 to 1500 μmol quanta $m^{-2}$ $s^{-1}$ (depending upon the time of year plants were grown) for *Phaseolus* which was saturating for photosynthesis, and 500 μmol quanta $m^{-2}$ $s^{-1}$ for *Alocasia*. The data for *Phaseolus* in Figures 1 and 2 is a subset of a larger data set used for Figures 3 to 7, as $CO_2$-saturated rates of photosynthesis were determined for only that subset. The *Alocasia* data in Figures 1 and 2 was obtained subsequent to that shown in Figures 3 to 7.

A photosynthesis chamber in which a 6 $cm^2$ leaf disc could be rapidly frozen (<250 ms to 0°C) to stop metabolism (24) and split in half was used to make simultaneous measurements of photosynthesis, RuBPCase content, and metabolite pool sizes in leaves of *Phaseolus* and *Alocasia*. Each point in Figure 9 (A–C) represents an individual leaflet. Data for *Alocasia* in Table I represents the average of 3 to 4 samples at each $CO_2$ concentration.

The concentration of RuBPCase in leaves was determined by radio-labeling each catalytic site of the enzyme with $^{14}$C-CABP and precipitation of the enzyme-CABP complex with rabbit antibodies prepared against purified RuBPCase, as described by Evans and Seemann (12). It has been determined that no proteolysis of enzyme occurred during the incubation phase of this procedure (JR Seemann, unpublished data).

RuBP and PGA pool sizes were determined on half the leaf disc (3 $cm^2$) from the freeze-kill chamber. These discs were extracted in $HClO_4$ as described by Seemann and Sharkey (22). RuBP concentration was determined as $^{14}$C incorporation into acid stable counts using purified spinach RuBPCase. PGA concentration was determined spectrophotometrically by NADH oxidation coupled to the NAD-dependent glyceraldehyde 3-P dehydrogenase and α-glycerol-P dehydrogenase reactions.

Total leaf N was determined by colorimetric analysis using a Technicon Autoanalyzer, following digestion of the leaf sample in a sulfuric-selenous acid mixture at high temperature. This procedure is sensitive to all forms of organic N, of which 70 to 80% in a typical leaf is in proteins, 10% is in nucleic acids, 5 to 10% is in Chl and lipoproteins, and the remainder is mostly in free amino acids (7). Chl concentration was determined as described by Arnon (1).

Photoinhibition treatments were carried out on attached leaves inside a temperature-controlled (25°C leaf temperature), humidified gas exchange cuvette through which $N_2$ containing 2% $O_2$ was passed. The leaf was exposed to 2000 μmol quanta $m^{-2}$ $s^{-1}$. At indicated times, the chamber was briefly opened and a leaf disc (0.84 $cm^2$) punched from the leaf. This allowed the time course of photoinhibition to be determined on a single leaf. These leaf discs were dark-equilibrated in a light-tight microcuvette for 10 min, after which the microcuvette was attached to a fiber optic system and placed in liquid $N_2$. After 6 min at 77K, fluorescence kinetics were measured as described by Powles and Björkman (20). Fluorescence is reported as ln ($F_v/F_m$), where, $F_m$ = maximal fluorescence at 77K, $F_v = F_m - F_o$, and $F_o$ = initial fluorescence at 77K. Decay constants reported in Figure 8 are slopes of linear regressions.

## RESULTS

**Photosynthesis *versus* Leaf Nitrogen and RuBPCase.** We determined the $CO_2$-saturated rate of whole leaf photosynthesis in sun and shade leaves of *Phaseolus* grown at two levels of N nutrition, and in sun and shade grown *Alocasia*. The $CO_2$-saturated rate of photosynthesis represents the maximum biochemical capacity of a leaf to assimilate carbon. The $CO_2$ saturated rate of photosynthesis in *Phaseolus vulgaris* was linearly related to the leaf N content over the measured range of N contents (Fig. 1). A similar relationship has been seen in other species (6, 11, 14, 15). At very high leaf N contents, this relationship has been observed to become curvilinear (10). Assimilation was also linearly related to N in *Alocasia* grown at either high or low light (Fig. 1). Even in *Alocasia*, photosynthetic capacity and leaf N content were strongly stimulated by high light. The slope of the relationship between $CO_2$ saturated photosynthesis and leaf N was significantly higher in *Phaseolus* (0.33 mmol $CO_2$

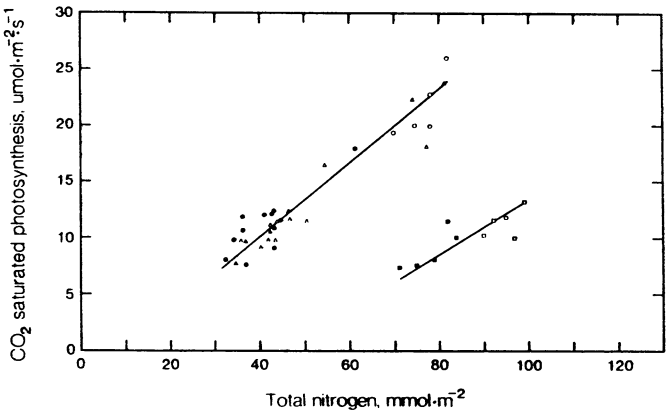


FIG. 1. Relationship between the $CO_2$-saturated rate of photosynthesis (μmol $CO_2$ $m^{-2}$ $s^{-1}$) and total N content (mmol $m^{-2}$) for leaves of *P. vulgaris* and *A. macrorrhiza*. Plants were grown under several different light and nitrogen regimes. *Phaseolus*: high light, high N (○); high light, low N (△); low light, high N (●); low light, low N (▲). *Alocasia*: high light, high N (□); low light, high N (■). See "Materials and Methods" for details of growth conditions. *Phaseolus*: $r = 0.96$, $y = 0.3289x - 3.161$; *Alocasia*: $r = 0.83$, $y = 0.2469x - 11.286$.

$mol^{-1}$ N $s^{-1}$) than in *Alocasia* (0.24 mmol $CO_2$ $mol^{-1}$ N $s^{-1}$). At any particular leaf N content, *Phaseolus* had a photosynthetic capacity 2 to 3 times higher than *Alocasia*.

In both species, growth at low light produced significant reductions in photosynthetic capacity (Fig. 1). In *Phaseolus*, growth at low N availability in high light also led to production of leaves with low photosynthetic rates and N contents about 50% of the time. Such leaves were similar to those of high N, low light-grown plants. Growth of low light plants at low N produced no additional change in plants of this species.

Photosynthetic capacity was also proportional to RuBPCase content in both *Phaseolus* and *Alocasia* (Fig. 2). However, *Phaseolus* had a higher photosynthetic capacity than *Alocasia* when expressed on the basis of RuBPCase content. At equal RuBPCase contents, the photosynthetic capacity at high $CO_2$ of *Phaseolus* was approximately 50% greater than in *Alocasia*.

**RuBPCase and Chl *versus* Leaf N.** The relationships between RuBPCase protein and total leaf N for the two species are shown in Figure 3. Over a 3-fold range of leaf N, the RuBPCase contents of *Phaseolus* and *Alocasia* were linear with N. The slope of this relationship in *Alocasia* was about one-third that of *Phaseolus*.

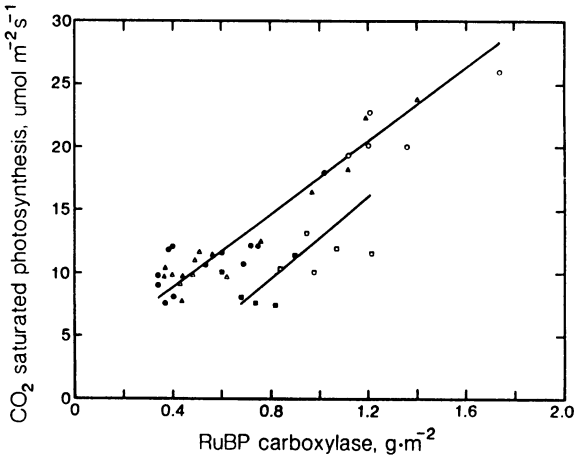


FIG. 2. Relationship between the $CO_2$-saturated rate of photosynthesis and the RuBPCase content of the same leaf. Symbols are as in Figure 1. *Phaseolus*: $r = 0.96$, $y = 14.44x + 3.26$; *Alocasia*: $r = 0.59$, $y = 18.23x - 5.65$.

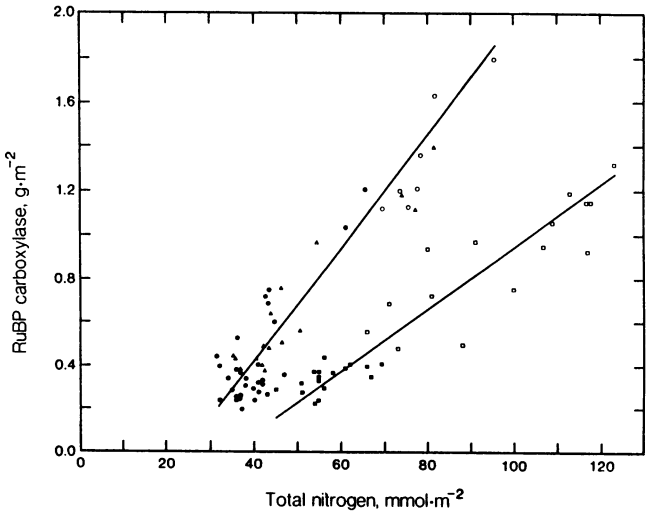


FIG. 3. Relationship between the RuBPCase content (g $m^{-2}$) and total N (mmol $m^{-2}$) for leaves of *P. vulgaris* and *A. macrorrhiza*. Species and growth conditions are as in Figure 1. *Phaseolus*: $r = 0.95$, $y = 0.026x - 0.637$; *Alocasia*: $r = 0.94$, $y = 0.144x - 0.4923$.

At any particular leaf N content, *Phaseolus* contained about twice the amount of RuBPCase as *Alocasia*. Growth at low light significantly reduced the RuBPCase content of leaves of both species. When grown at high light, *Alocasia* had 20% more N on a leaf area basis than *Phaseolus*. This was associated with the higher leaf specific weight of *Alocasia* than *Phaseolus* (data not shown). High light, low N *Phaseolus* had variable RuBPCase contents, ranging from being similar to low light plants to being similar to high light, high N plants. Low light, low N plants were similar to low light, high N plants.

The proportion of leaf N which was RuBPCase for the two species is shown in Figure 4. The curves are the transformed linear regressions from Figure 3. In plants grown under low light with high N, RuBPCase protein ranged from 6 to 20% of leaf N (Fig. 4) with the lowest values being obtained in winter grown plants (lowest light intensities). In plants grown in high light, RuBPCase protein comprised 11 to 23% of leaf N (with the lower values being low N-grown plants) and these proportions were correlated with leaf N. Leaves of shade-grown *Alocasia* had about 6% of leaf N as RuBPCase protein at 50 mmol N $m^{-2}$, and in sun-grown plants RuBPCase was about 11% of leaf N at 100 mmol N $m^{-2}$ (Fig. 4).

Light intensity had little effect on the Chl content of high N grown *Phaseolus* (Fig. 5). However, low light-grown plants had higher Chl contents per unit N, regardless of the N growth level, than did high light-grown plants. For each N growth level the data appear to fall on two lines. Growth at high light and low N had the largest effect on Chl content, sometimes reducing this value to as little as half that in other treatments. In *Alocasia* grown in the shade the Chl content was variable (Fig. 5), but much higher than in *Phaseolus* (up to 1 g $m^{-2}$ in shade-grown plants). In sun-grown *Alocasia* plants, Chl content was only about half that of plants grown in the shade but still significantly higher than in *Phaseolus*.

The species specific differences in RuBPCase and Chl contents and their different responses to environmental variation described above resulted in quite different relationships between the absolute amounts of these two components of the photosyn-

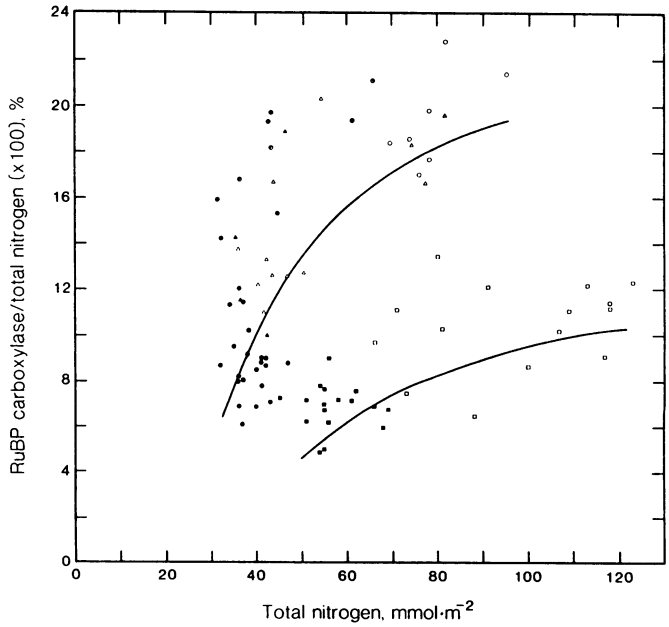


FIG. 4. Percentage of leaf total N which was RuBPCase shown as a function of leaf total N content for *P. vulgaris* and *A. macrorrhiza*. The value for RuBPCase on the *y*-axis was converted to N using 6.25 g protein $g^{-1}$ N and 14 g $mol^{-1}$ N. The curves are the transformed linear regressions from Figure 3. Species and growth conditions are as in Figure 1.

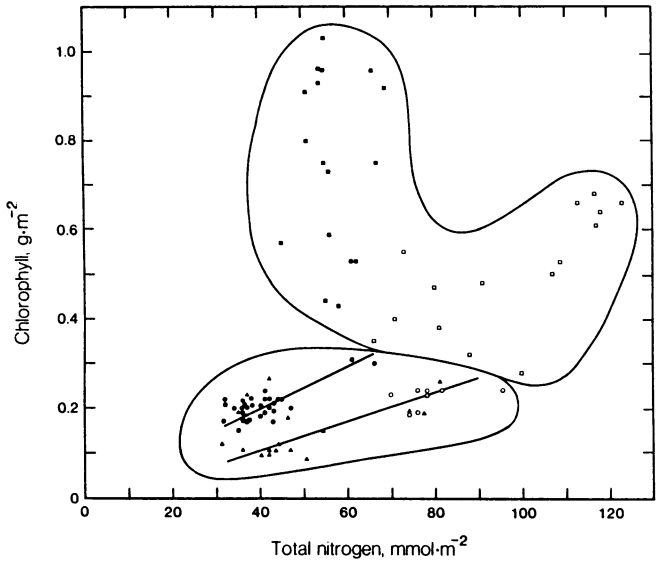


FIG. 5. Chl content of leaves of *Phaseolus* and *Alocasia* as a function of leaf total N content. Symbols are as in Figure 1.

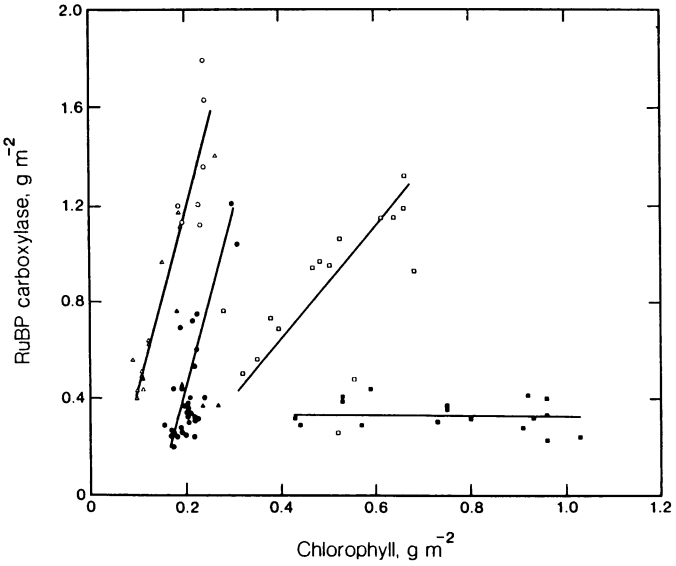


FIG. 6. Relationship between the RuBPCase and Chl ($a + b$) contents of leaves of *P. vulgaris* and *A. macrorrhiza*. Species and growth conditions are as in Figure 1.

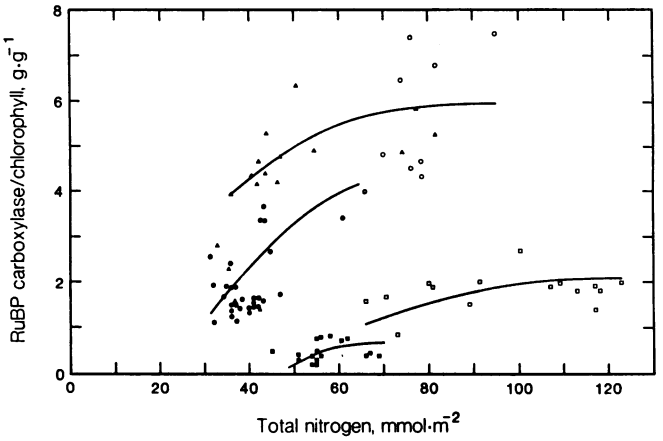


FIG. 7. Ratio of RuBPCase to Chl content (g $g^{-1}$) as a function of total N content (mmol $m^{-2}$) of leaves of *P. vulgaris* and *A. macrorrhiza*. Species and growth conditions are as in Figure 1.

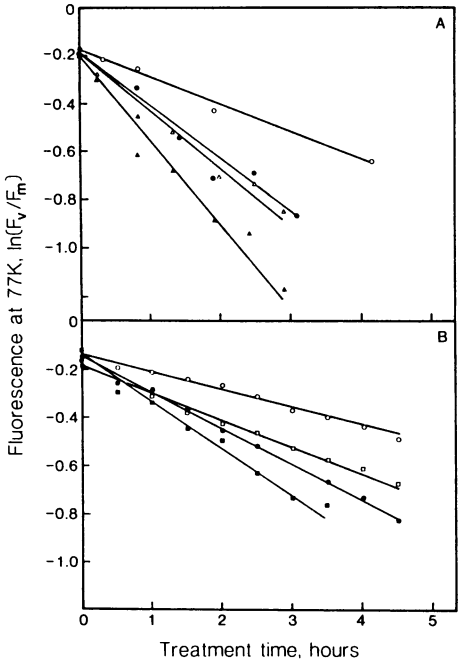


FIG. 8. Effect of a photoinhibitory treatment (2000 $\mu$mol quanta $m^{-2}$ $s^{-1}$, 2% $O_2$ in $N_2$) on the timecourse of 77K fluorescence decay. A, Leaves of winter-grown *P. vulgaris* grown under various environmental conditions. See "Materials and Methods" for details of measurements. Decay constants were ($h^{-1}$) high light, high N, 0.11; low light, high N, 0.22; high light, low N, 0.21; low light, low N, 0.32. B, The timecourse of a photoinhibitory treatment on ln ($F_v/F_m$) in summer-grown sun and shade *Alocasia* and *Phaseolus*. Decay constants: high light *Phaseolus*, 0.07; high light *Alocasia*, 0.10; low light *Phaseolus*, 0.14; low light *Alocasia*, 0.19. Symbols are as in Figure 1.

thetic apparatus (Fig. 6). Changes in light and N during growth promoted changes in RuBPCase content with only small changes in Chl content in both high light- and low light-grown *Phaseolus*, while exactly the opposite was true in low light-grown *Alocasia*. High light-grown *Alocasia* was intermediate. Figure 7 shows that the proportion of N invested in RuBPCase protein relative to Chl was significantly greater in *Phaseolus* than *Alocasia*; conversely, *Alocasia* allocated more N into Chl than *Phaseolus*. In both species, growth in high light with high levels of N-nutrition led to a higher ratio of RuBPCase protein to Chl. The Chl $a/b$ ratio also increased with increasing light intensity in both species (data not shown). These changes all suggest marked alterations in the relative proportions of chloroplast stromal activities and membrane bound activities in the treatments employed.

**Susceptibility to Photoinhibition.** Shade-grown plants are susceptible to light-dependent damage (photoinhibition) in bright light. This is exaggerated under conditions of $CO_2$ deprivation (2% $O_2$, zero $CO_2$), conditions which are also conducive to photoinhibition of sun-grown plants (20). Björkman (4) has concluded that a measure of leaf 77 K fluorescence, $F_v/F_m$, is linearly related to quantum yield when the primary photochemistry of leaves is damaged by photoinhibition. We measured 77 K fluorescence from punches of leaves held in high light and 20 mbar $O_2$ (2%) and zero $CO_2$. The results are plotted as ln ($F_v/F_m$) *versus* time, producing a linear relationship and allowing for determination of the rate constant of fluorescence decay. For *Phaseolus*, Figure 8A shows that shade-grown leaves were by far the most susceptible to photoinhibition, especially those supplied with low N. Leaves of plants grown in full sun with high N were

the least sensitive to these treatments, there being a 3-fold difference in the decay constant for the responses shown in Fig. 8A (see figure legend for values). Plants for which data is shown in Figure 8A were grown during the winter months.

Similar experiments were conducted with *Alocasia* grown either during the summer at full sunlight or at 20 μmol quanta $m^{-2}$ $s^{-1}$ (Fig. 8B). To allow direct comparison with *Phaseolus*, the photoinhibition experiments were repeated with bean plants grown under either full summer sun or 50% full sun. *Phaseolus* grown during the summer months (Fig. 8B) was more resistant to photoinhibition than plants grown during the winter (Fig. 8A) at both high light and shade conditions. High light-grown *Alocasia* was intermediate to high light and shade-grown *Phaseolus* in susceptibility to photoinhibition (Fig. 8B). Shade-grown *Alocasia* showed the greatest susceptibility to photoinhibition for plants grown at this time of year.

**Metabolite Pool Sizes during Photosynthesis.** The relationship between the large changes in the makeup of the photosynthetic apparatus (Figs. 3–7) and changes in leaf photosynthetic properties (Figs. 1, 2, 8) was further explored by analysis of metabolite pool sizes in leaves under conditions of steady state photosynthesis. For *Phaseolus*, winter grown plants with maximum rates of photosynthesis somewhat lower than those in Figure 1 were used. Low light-grown plants used for Figure 9 had levels of N, RuBPCase, and Chl which were 55, 37, and 95%, respectively, of the high light-grown plants used for the same figure.

The response of intact leaf photosynthesis is shown in Figure 9A. High light-grown plants had initial slopes and $CO_2$-saturated rates of photosynthesis about 2.5 times higher than low light-grown plants. Metabolite pool sizes as a function of $C_i$ were determined for these bean plants. At very low $C_i$, the pool size of RuBP was low, and then increased with $C_i$ to a peak at the $CO_2$ compensation point and then declined to a plateau at about 400 μbar (Fig. 9B). The pattern was the same in low light- and high light-grown plants, but the pool size was about 2-fold higher in high light-grown plants at any particular $CO_2$ partial pressure. The pool of PGA rose steadily with increasing $C_i$ to a plateau at about 400 μbar (Fig. 9C). Again the pool was 2-fold higher in high light-grown plants when expressed on a leaf area basis. These patterns in high light-grown *Phaseolus* are similar to those observed previously (2, 18). However, when metabolite pool sizes are expressed relative to the leaf content of RuBPCase catalytic sites, they are seen to be much less affected by the light intensity during growth (Table I). The ratio of the RuBP pool size to the RuBPCase catalytic site concentration (mol $mol^{-1}$) was approximately the same at either ambient or high $CO_2$ under both growth conditions, as was the ratio of the PGA pool to the RuBPCase catalytic site content.

Similar experiments were conducted with *Alocasia* grown at both high and low light. The results are summarized in Table I. On a leaf area basis, high light-grown *Alocasia* contained 1.4 to 2 times more RuBP than the low light-grown individuals. When expressed as a function of the RuBPCase catalytic site content, however, the RuBP pool sizes were comparable to *Phaseolus*. Values of 3 to 4 mol RuBP $mol^{-1}$ RuBPCase catalytic sites at ambient $CO_2$ and 1 to 2 mol $mol^{-1}$ at high $CO_2$ are similar to previously measured values and are likely to be rate saturating and rate limiting for photosynthesis, respectively (2, 22, 27). A similar result was found for PGA, although the *Alocasia* PGA pool size was lower than in *Phaseolus*.

## DISCUSSION

Our data relate to several important questions which underlie the way plants respond to the availability of light and nutrients during growth. The results provide quantitative analyses of leaf nitrogen budgets in relation to sun-shade responses and their dependence on N-nutrition. Our measurements of RuBPCase

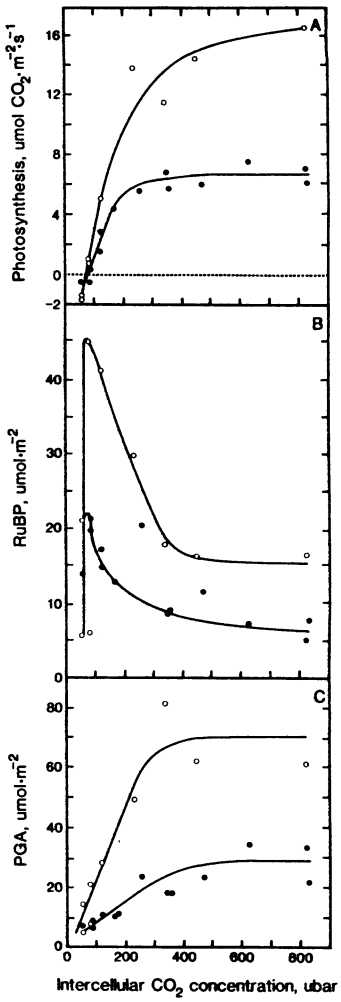


FIG. 9. A, Net photosynthetic $CO_2$ assimilation (A) as a function of intercellular $CO_2$ partial pressure ($C_i$) for leaves of *P. vulgaris* grown at either high or low light intensity. Each point represents a separate leaf. After the steady state rate was achieved, the leaf was freeze-clamped under those conditions and stored in liquid $N_2$ until analyzed for RuBP and PGA concentration. High light-grown plants (○); low light-grown plants (●). B, The concentration of RuBP (μmol $m^{-2}$) for the freeze-clamped leaves in (A) plotted as a function of $C_i$. C, The concentration of PGA (μmol $m^{-2}$) for the freeze-clamped leaves in (A) plotted as a function of $C_i$.

Table I. *RuBP and PGA Pool Sizes in Phaseolus and Alocasia Expressed as a Function of the RuBP Catalytic Site Content of the Same Leaf*

Numbers in parentheses are the same metabolite content on a leaf area basis for *Alocasia*. Data for *Phaseolus* corresponds to plants and data used for Figure 9.

| Species | Growth Treatment | Ambient $CO_2$ | | High $CO_2$ | |
|---|---|---|---|---|---|
| | | RuBP[a] | PGA[a] | RuBP[a] | PGA[a] |
| *Phaseolus* | High light | 3.1 | 4.9 | 1.6 | 7.2 |
| | Low light | 3.3 | 4.4 | 1.7 | 10.8 |
| *Alocasia* | High light | 2.8 (57[b]) | 1.3 (27[b]) | 1.2 (28[b]) | NA[c] |
| | Low light | 3.9 (42[b]) | 1.3 (12) | 1.2 (14[b]) | 3.4 (38) |

[a] Mol metabolite · $mol^{-1}$ CABP binding sites. [b] μmol · $m^{-2}$. [c] Not available.

protein contents and total leaf N establish the significant role of this key protein in leaf N budgets during shade-sun acclimation, previously inferred on the basis of changes in enzyme activity (5). Medina (17) measured fraction-1 protein in extracts of *Atriplex* leaves from plants grown in 22 mM $NO_3^-$ and 1 mM $NO_3^-$ under high light (100% sunlight) and shade (35%). He found that fraction-1 protein (presumably RuBPCase protein) declined from 16% of total leaf N in high-light, high-N to 6% in high-light, low-N and 8% in the shade, high-N treatments. These values compare well with the range of 6 to 20% of leaf N for RuBPCase protein in our experiments with *Phaseolus* (Fig. 4). In *Alocasia*, shade-grown plants contained about the same proportion of leaf N as RuBPCase, but in sun-grown plants this proportion did not increase above 13%. The absolute amounts of RuBPCase protein on an area basis in *Alocasia* were similar to those in *Phaseolus* (Fig. 3) so the lower fraction of total leaf N as RuBPCase was due to higher relative contents of other nitrogenous compounds. The markedly lower N-use efficiency of photosynthesis in *Alocasia* compared with *Phaseolus* (Fig. 1) is thus a partial consequence of a significant N investment in compounds of the leaf other than RuBPCase. However, it is also clear that *Alocasia* has a significantly lower rate of $CO_2$-saturated photosynthesis than does *Phaseolus* at equal RuBPCase concentrations (Fig. 2). This should also contribute significantly to the lower N-use efficiency of photosynthesis observed in *Alocasia*. The basis for this difference in the efficiency of RuBPCase is unknown.

One of the most striking relationships in our data is the ratio between RuBPCase protein and Chl (Fig. 7). In *Phaseolus* this ratio was well correlated with light and nutrient conditions during growth. Changes in this ratio in *Phaseolus* were largely the result of changes in the leaf content of RuBPCase protein (Fig. 6). The RuBPCase:Chl ratio ranged from 1.0 g RuBPCase $g^{-1}$ Chl in low N, shade-grown plants to 7.5 in high N, sun-grown plants. This corresponds well with 7.9 in high N, high light *Atriplex*, 2.0 in high N, low light or low N, high light *Atriplex* (17) and 6 g $g^{-1}$ seen in desert annuals growing in Death Valley, California (23). Terashima and Inoue (26), using SDS-PAGE methods for RuBPCase quantification, found ratios of 14.1 in palisade (sun) chloroplasts and 6.8 in mesophyll (shade) chloroplasts of spinach leaves. Although the same trend in the ratio of RuBPCase to Chl was evident in *Alocasia* in response to shade and sun, ratios were much lower (0.2 to 2.0) because of much higher Chl contents (Fig. 6).

These data indicate that there are significant genotypically determined differences between herbaceous species from open habitats (such as *Phaseolus*) and those from closed understory environments (such as *Alocasia*) as far as the responsiveness of the photosynthetic apparatus to sun-shade conditions is concerned. Both appear to effect substantial increases in RuBPCase protein in response to growth in bright light. In *Alocasia* this change was associated with a marked increase in Chl content, in contrast to *Phaseolus* (Fig. 6). The implications of these changes for the more limited capacity for photosynthetic acclimation in *Alocasia* compared with *Phaseolus* remains to be determined, and will depend on quantitative analyses of chloroplast membrane protein-pigment complexes. It is tempting to suggest that a large part of the much higher leaf N concentration at equivalent RuBPCase protein concentration in *Alocasia* may be so associated.

That changes in the ratio of RuBPCase protein to Chl during sun-shade acclimation in *Phaseolus* are reflected in sensitivity to photoinhibition is clearly evident from our experiments. Shade-grown, low N plants with a low ratio of RuBPCase to Chl are much more susceptible to photoinhibition than others (Fig. 8, A and B). *Alocasia*, with even lower ratios of RuBPCase to Chl, was also quite sensitive. However, it is interesting to note that all winter-grown *Phaseolus* treatments with the exception of high light, high nitrogen were at least as susceptible to photoinhibition as shade-grown *Alocasia*. This surprising tolerance to high light in *Alocasia* may be related to its capacity to effectively use sunflecks for much of its carbon gain and to tolerate suddenly appearing gaps in the tropical forest overstory (RW Pearcy, personal communication).

Our data indicate that the extensive acclimation of photosynthesis from shade to sun which is evident in *Phaseolus* (Figs. 1, 2, 9) and many other herbaceous species (5) is dominated by the ability to increase the proportion of leaf N dedicated to RuBPCase protein. It is not surprising therefore that limiting N nutrition limits acclimation in these plants and others (13, 19). Acclimation from shade to sun also depends on increases in other carbon reduction cycle enzymes and in electron transport and other photochemical activities (3, 9). Despite these complex readjustments of the photosynthetic apparatus which occur during light acclimation in both *Phaseolus* and *Alocasia*, the acclimation process functions in such a way that a relatively constant relationship between the levels of carboxylation substrate (RuBP), carboxylation enzyme (RuBPCase), and product (PGA) is maintained (Table I). Further studies are needed to evaluate in more detail what appear to be alternative accommodations of photosynthesis in shade and sun in *Phaseolus* and *Alocasia*.

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