Mooney, H. A., and S. L. Gulmon. 1979. Environmental and evolutionary constraints on the photosynthetic characteristics of higher plants. Pp. 316–337 *in* O. T. Solbrig, S. Jain, G. B. Johnson, and P. H. Raven (eds.), Topics in plant population biology. Columbia University Press, New York.

Nobel, P. S., and P. W. Jordan. 1983. Transpiration analysis of desert species: resistances and capacitances for a C_3, C_4, and a CAM plant. Journal of Experimental Biology 34:1379–1391.

Orians, G. H., and O. T. Solbrig. 1977. A cost-income model of leaves and roots with special reference to arid and semi-arid areas. American Naturalist 111:677–690.

Raschke, K. 1979. Movements of stomata. Pp. 383–441 *in* W. Haupt and M. E. Feinleib (eds.), Physiology of movements, vol. 7, Encyclopedia of plant physiology, new series. Springer-Verlag, New York.

Schaffer, W. M. 1977. Some observations on the evolution of reproductive rate and competitive ability in flowering plants. Theoretical Population Biology 11:90–104.

Schaffer, W. M., R. S. Inouye, and T. S. Whittam. 1982. The dynamics of optimal energy allocation for an annual plant in a seasonal environment. American Naturalist 120:787–815.

Schulze, E.-D., and A. E. Hall. 1982. Stomatal responses, water loss and CO_2 assimilation rates of plants in contrasting environments. Pp. 181–230 *in* O. L. Lange, P. S. Nobel, C. B. Osmond, and H. Ziegler (eds.), Physiological plant ecology II, vol. 12B, Encyclopedia of plant physiology, new series. Springer-Verlag, New York.

Sugiyama, T., M. Miyuno, and M. Hayashi. 1984. Partitioning of nitrogen among ribulose-1,5-biphosphate carboxylase/oxygenase, phosphoenolpyruvate carboxylase, and pyruvate orthophosphate dikinase as related to biomass productivity in maize seedlings. Plant Physiology 77:665–669.

Tyree, M. T., M. A. Dixon, E. L. Tyree, and R. Johnson. 1984. Ultrasonic acoustic emission from the sapwood of cedar and hemlock: an examination of three hypotheses regarding cavitations. Plant Physiology 75:988–992.

1 The photosynthesis–nitrogen relationship in wild plants

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Worldwide, nitrogen is one of the mineral nutrients most limiting to plant growth. Though it is the most abundant element in the atmosphere, nitrogen becomes available to plants largely through the recycling of organic matter or through the energetically expensive reduction of dinitrogen gas (see Chapter 10). Nitrogen is easily lost from ecosystems by leaching or conversion to N_2 gas by denitrifying bacteria.

At the system level, the importance of nitrogen is underscored by the sensitivity of managed and natural ecosystems to nitrogen fertilization. Agricultural grain yield is highly correlated with the level of nitrogen application. This trend extends across a wide variety of crops and farming systems and includes the record yields of several crops (Ritchie 1980). Natural ecosystems respond to nitrogen fertilization with increased productivity or changes in species composition or both (Lee et al. 1983).

Nitrogen is a limiting resource in many ecosystems, but levels of leaf nitrogen reflect the relative partitioning among multiple sinks, as well as total availability. From an evolutionary perspective, the problem of nitrogen limitation has two components. How should a plant allocate a given nitrogen pool between reproduction, leaves, roots, and stems for the maximization of fitness? And, in any ecological setting, how big should the nitrogen pool be? Answering these questions requires knowledge of the costs of nitrogen acquisition and the benefits of alternative nitrogen deployment patterns. Mooney and Gulmon (1979, 1982) have established a conceptual framework for analyzing the costs and benefits of nitrogen acquisition and deployment, but the specific shapes of the cost and benefit functions remain somewhat conjectural. The relationship between photosynthesis and leaf nitrogen is one of the most important benefit functions, because photosynthesis provides the energy and structural substrates necessary for reproduction, growth, or foraging for additional nitrogen. Here we shall explore aspects of this benefit function, examining the generality, the mechanism, and some of the implications of the relationship between the photosynthesis and the nitrogen content of leaves.

Photosynthesis–nitrogen relationships are intrinsically complex, because photosynthesis represents the integrated operation of a series of processes sensitive to environmental factors as well as leaf physiology and structure. The ecologically relevant measure of photosynthesis is time-integrated photosynthesis in the natural environment, but this index reveals little about leaf function. Maximum photosynthetic capacity under optimum conditions may not always scale simply with time-integrated CO_2 fixation, but measurements of photosynthetic capacity are important for three reasons. They indicate the maximum rates of CO_2 fixation that occur in nature and also the maximum possible benefits from a given investment in photosynthetic machinery. In addition, if constructing and maintaining photosynthetic machinery is expensive, photosynthetic capacities should be tuned to the constraints of the environment, and unusable capacity should be trimmed by natural selection. Thus, photosynthetic capacity provides a useful starting point for analyzing the relationship between photosynthesis and leaf nitrogen.

All of the biochemical and photobiological processes of photosynthesis require nitrogenous compounds. In addition to the proteins (typically 16% nitrogen) that catalyze the reactions of CO_2 fixation and the regeneration of the CO_2 acceptor, photosynthesis requires reducing equivalents (NADPH) and ATP, produced by light-driven electron-transport and proton-transport reactions. Nitrogenous compounds that provide the basis for these reactions include chlorophyll (6% nitrogen), chlorophyll proteins, electron-transport proteins, and the ATP-synthesizing enzyme. The nitrogen investment in many of these compounds is not precisely known, but the proportion of the total leaf nitrogen allocated to photosynthetic reactions is undoubtedly large. As a first approximation, the commitment of leaf nitrogen to photosynthesis in C_3 plants is given by the 75% of the leaf nitrogen that can be recovered from the chloroplasts (Stocking and Ongun 1962), minus the probably small proportion of the chloroplast nitrogen invested in the reactions of nitrite reduction and amino acid synthesis, plus the nitrogen incorporated in the peroxisome and mitochondrial enzymes that recycle the products of photorespiration, plus the nitrogen in that fraction of the nucleic acids and protein-synthesizing machinery necessary to produce and recycle the components of the photosynthetic reactions. These compounds may account for well over three-quarters of the total leaf nitrogen.

A number of studies have reported correlations between some measure of photosynthetic capacity and (a) total leaf nitrogen, (b) the nitrogen content of some protein fraction, or (c) the activities of particular enzymes,

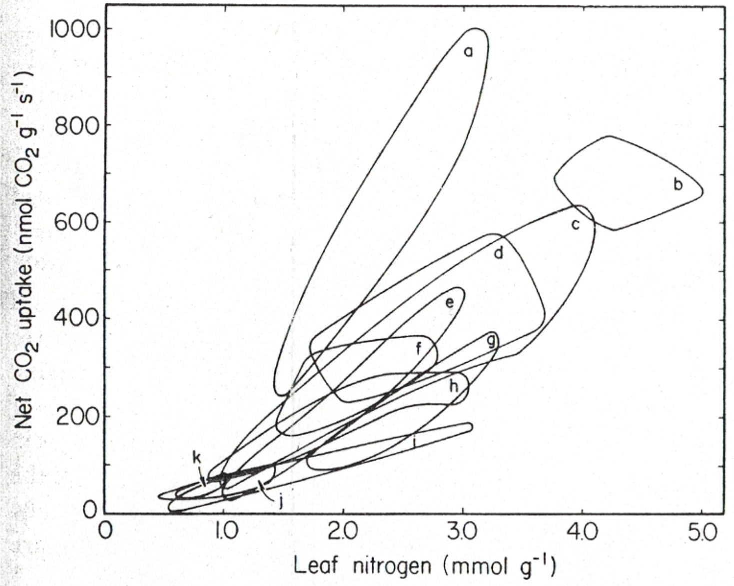

Figure 1.1. A_{max}–N relationships for a wide variety of plant species. Each outline circumscribes all of the data reported in each study. The study species and the experimental conditions are described in Table 1.1. Included are data from 33 C_3 species and 11 C_4 species. Some of the studies present data from naturally grown plants, whereas others report data from plants grown in controlled environments. The experimental variables manipulated to introduce variations in A_{max} and N include nitrogen availability, light availability, and leaf age. Those in group a, with the greatest A_{max} per unit of N, are the only C_4 data in this summary.

Nátr (1975), Yoshida and Coronel (1976), Björkman (1981), and Hesketh et al. (1983) have recently reviewed many of these studies. We approach the photosynthesis–nitrogen relationship in a broader context, examining general trends that cut across taxonomic groups, habitats, and life forms.

To provide an overview of the generality in the photosynthesis–nitrogen relationship, we have summarized a series of studies reporting photosynthetic capacity and leaf nitrogen content on the basis of leaf dry weight (Figure 1.1). In each of these studies, photosynthetic capacity (A_{max}) is the photosynthetic rate measured under saturating light intensity, optimum temperature, relatively high humidity, and the CO_2 concentration typical of normal air. Leaf nitrogen (N) is that measured on the same or matched leaves by Kjeldahl analysis. This procedure is sensitive to all forms of organic nitrogen, of which 70–80% in a typical leaf is in proteins, 10% is in nucleic acids, 5–10% is in chlorophyll and lipoproteins, and the remainder is mostly in free amino acids (Chapin and Kedrowski 1983). For the data summarized in Figure 1.1, correlation coefficients between A_{max} and N range from 0.51 to 0.97, with a median value of 0.90 (Table 1.1). The slopes of linear-regression equations expressing A_{max} as a function of N vary substantially (Table 1.1), but data from all but one of the studies cluster around a single straight line. The outlying data in Figure 1.1 are the results of Pearcy et al. (1982) and represent the only C_4 plants included in the figure. The general comparison provided here confirms the taxonomically limited conclusions of Brown (1978), Bolton and Brown (1980), Schmitt and Edwards (1981), Brown and Wilson (1983), and Wilson and Brown (1983) that for a given investment in leaf nitrogen, C_4 plants tend to realize a higher A_{max} than do C_3 plants. To the extent that environmental conditions permit leaves to operate near A_{max}, the higher A_{max} per unit of leaf nitrogen in C_4 species suggests that selection may favor these plants in nitrogen-limited habitats. This hypothesis is not supported by much of the available data (Pearcy and Ehleringer 1984), but deserves further study. Possible explanations for this difference between C_3 and C_4 plants are discussed by Brown (1978) and Raven and Glidewell (1981).

The fact that all the C_3 plants in Figure 1.1 cluster tightly around a single straight line suggests a fundamental relationship that is relatively insensitive to differences among species or growth conditions. Included in the figure are data from herbs, shrubs, and trees, from evergreens and deciduous species, from leaves ranging in age from a few weeks to more than three years, from plants growing naturally in the field, and from plants maintained under a variety of greenhouse and growth-chamber conditions.

In analyzing these and other reports of $A_{max}-N$ relationships, we consider examples in which both A_{max} and N are presented on the basis of leaf dry weight or leaf area, but we largely ignore the substantial literature in which A_{max} is presented as an area-based quantity and N is presented on a leaf-weight basis. Because leaf-specific weight (LSW) or weight per unit of leaf area tends to change in response to variation in nutrient availability (Loveless 1961; Gulmon and Chu 1981) or variation in light intensity during growth (Björkman 1981), the results of studies reporting A_{max} on an area basis and N on a weight basis can be very confusing.

A survey: the photosynthesis–nitrogen relationship in natural vegetation

For a detailed examination of the $A_{max}-N$ relationship, we restrict our attention to naturally growing C_3 plants. The emphasis on C_3 plants is

able 1.1. *Summary of selected studies reporting a relationship between photosynthetic capacity at light saturation (A_{max}) and leaf organic nitrogen (N), both expressed r unit of leaf weight*

eference	Species	Equation[a]	*r*	*n*	Notes	Label in Figure 1.1
örkman and Holmgren (1963)	*Solidago virgaurea*	$A_{max} = -255 + 195 \cdot N$	0.93	12	Twelve clones grown in growth chambers at high light	*g*
ooney et al. (1978)	6 *Eucalyptus* species	$A_{max} = -9.5 + 66.2 \cdot N$	0.97	18	One-year-old nursery-grown plants and 2-month-old phytotron-grown plants	*i*
eld (1981)	*Lepechinia calycina*	$A_{max} = -45 + 161 \cdot N$	0.93	15	Leaf age and shade varied: irrigated plants grown outside	
ulmon and Chu (1981)	*Diplacus aurantiacus*	$A_{max} = -82 + 164 \cdot N$	0.96	23	Growth-chamber plants under several light and nitrogen treatments	*c*
edina (1981)	*Nicotiana glauca* and *Eucalyptus camaldulensis*	$A_{max} = -123 + 175 \cdot N$	0.96	32	Excised branches of naturally growing plants	*e*
ooney et al. (1981)	5 species of Death Valley annuals	$A_{max} = 171 + 80 \cdot N$	0.64	20	Leaf age series on naturally growing plants	*d*
ooney et al. (1981)	4 species of old-field annuals	$A_{max} = 12 + 123 \cdot N$	0.78	21	Leaf age series on naturally growing plants	*f*
arcy et al. (1982)	11 *Euphorbia* species (C_4)	$A_{max} = -180 + 361 \cdot N$	0.90	11	Greenhouse-grown plants: each A_{max} and N represents mean of several leaves	*a*
ooney et al. (1983)	6 fynbos species	$A_{max} = -47 + 113 \cdot N$	0.77	13	One-year-old leaves on naturally growing plants	*k*
eld and Mooney (1983)	*Lepechinia calycina*	$A_{max} = 8 + 93.8 \cdot N$	0.89	27	Leaf age series on naturally growing plants	*h*
eld et al. (1983)	5 species of California evergreens	$A_{max} = 14 + 28.6 \cdot N$	0.51	53	Leaf age series on naturally growing plants	*j*
ooney (unpublished)	*Raphanus sativus*	n.s.	0.04	5	Growth-chamber-grown plants	*b*

is in nmol CO_2 $g^{-1}s^{-1}$ and N is in mmol g^{-1}

in the interest of brevity. The focus on naturally growing plants arises from the hypothesis that an A_{max}–N relationship reflects evolutionary responses to natural habitats and may be altered by growing plants under modified nutrient, water, light, or CO_2 availability. Here we shall consider A_{max}, leaf nitrogen, LSW, and stomatal conductance from four studies. Collectively, these studies reported data from 137 leaves of 21 plant species, including trees, shrubs, and herbs from environments varying widely in nitrogen availability and productivity. Five species are winter annuals of Death Valley, California; four are summer annuals of central Illinois (all described by Mooney et al. 1981); one is a drought-deciduous shrub of the California chaparral (Field and Mooney 1983); five are evergreen shrubs and trees from coastal central California (Field et al. 1983); six are shrubs from South African mountain fynbos (Mooney et al. 1983). In this combined data set, which we refer to as the VINE (vegetation in natural environments) survey, maximum leaf durations vary 50-fold, LSW varies by a factor of 5, and A_{max} varies more than 20-fold in measurements based on a standardized protocol and instrumentation. The desert annuals have some of the highest photosynthetic capacities of any C_3 plants (Mooney et al. 1976), and the evergreen sclerophylls have some of the lowest (Mooney and Gulmon 1979). The VINE survey does not include plants that grow under deep shade, and none of the species has a symbiotic nitrogen-fixing microorganism.

In order to explore the widest range of naturally occurring nitrogen levels, we include measurements from leaves of different ages, ranging from the youngest fully expanded leaf to the oldest nonnecrotic leaf. Leaf nitrogen declines with increasing leaf age in essentially all plants and provides a completely natural source of intraspecies (and intraplant) variation. Though leaf aging is a complex process, many aspects of the gradual changes in leaf physiology following full expansion are consistent with the interpretation that the dominant phenomenon of leaf aging is nitrogen mobilization and export (Field and Mooney 1983), a process that may increase whole-plant photosynthesis when old leaves are increasingly shaded by younger leaves (Field 1983).

Over the 137 leaves in the VINE survey, A_{max} is highly correlated with leaf N. This conclusion applies when both parameters are expressed on the basis of either leaf dry weight (Figure 1.2) or leaf area (Figure 1.3). As observed in other studies (Gulmon and Chu 1981; Medina 1981; Field and Mooney 1983), the correlation coefficient is higher for weight-based measurements ($r = 0.92$) than for area-based measurements ($r = 0.53$). Subsets within the survey, however, show similar weight-based and area-based [illegible] species (annuals and deciduous perennials), the correlation coefficients are similar for area-based ($r = 0.86$) and weight-based ($r = 0.84$) measurements. For the 11 species of evergreen sclerophylls, the area-based ($r = 0.51$) and weight-based ($r = 0.50$) correlations are also similar. The greater variance in A_{max}–N relationships for sclerophylls than for nonsclerophylls has been reported earlier (Medina 1981; Field et al. 1983) and is an important element in the continuing discussion of the implications of sclerophylly. We shall review aspects of this discussion when we consider nitrogen-use efficiency and the causal basis of the A_{max}–N relationship.

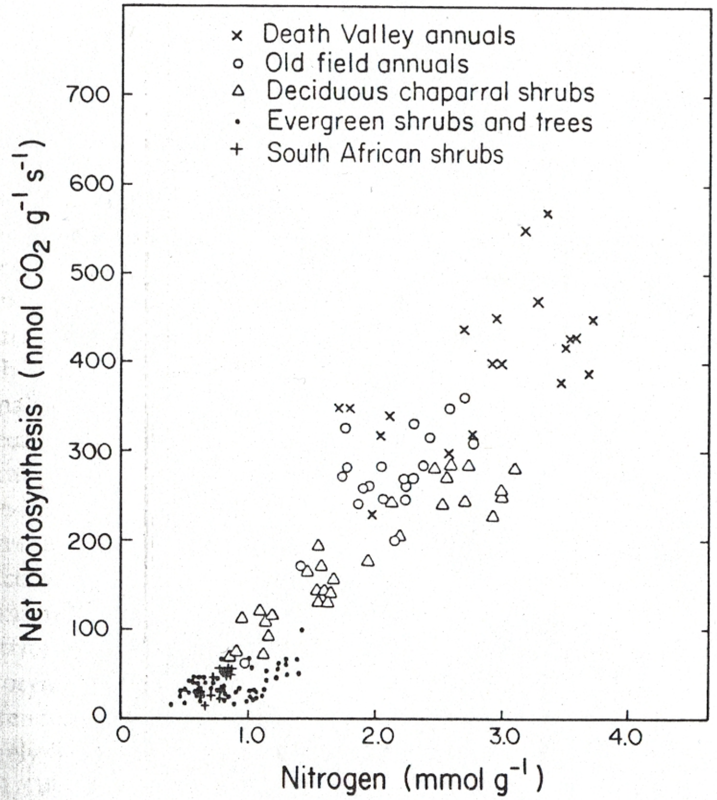

Figure 1.2. A_{max}–N relationships for the VINE survey, representing 21 species grown under natural conditions. A_{max} was measured in the field. N was determined with a micro-Kjeldahl assay on the same leaves used for the photosynthesis measurements. A_{max} and N are both expressed on a leaf-weight basis. $A_{max} = -76.1 + 149 \cdot N$, $n = 137$, $r = 0.92$, $P < 0.001$.

If we focus on the weight-based A_{max}–N relationship, the correlation is striking for both its linearity and its limited scatter (Figure 1.2). The limited scatter suggests that over a wide diversity of C_3 plants, photosyn

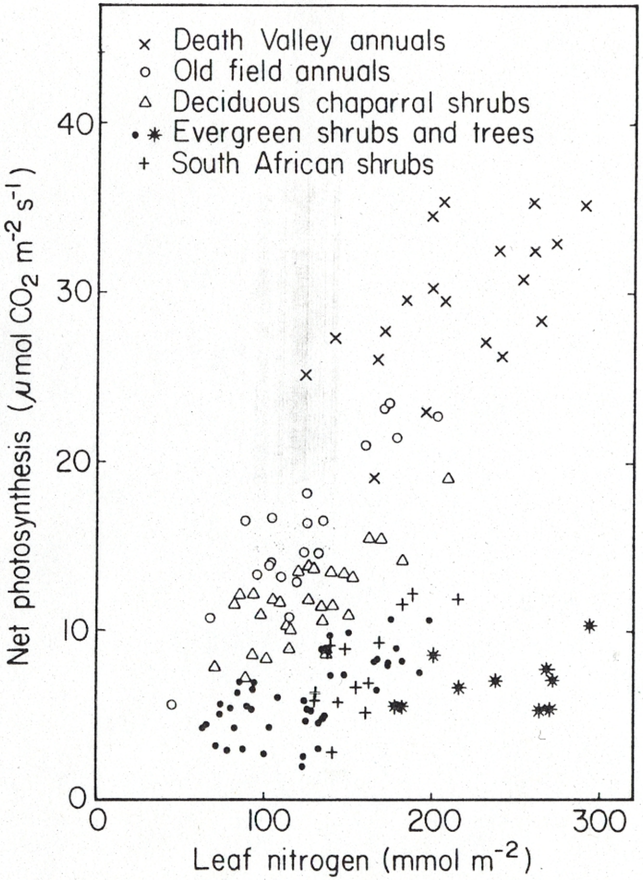

Figure 1.3. $A_{max}-N$ relationships for the VINE survey, with A_{max} and N expressed on a leaf-area basis. Data from *Prunus ilicifolia* are marked by asterisks. $A_{max} = 0.342 + 0.0814 \cdot N$, $n = 137$, $r = 0.53$, $P < 0.001$.

thetic capacity is strongly regulated by leaf nitrogen, without large effects due to habitat, growth form, or interspecies differences. The linearity, a feature not observed in some single-species analyses (Evans 1983), indicates important constraints on the relationship between nitrogen-based limitations and other limitations to A_{max}. We shall address both the linearity and the scatter in considering the causal basis of the $A_{max}-N$ relationship.

The area-based $A_{max}-N$ relationship (Figure 1.3) is somewhat confusing. The general rankings of species groups are similar for the weight-based and area-based analyses, but with one important exception. Some of the evergreen sclerophylls have high N per unit area, but low A_{max} (Figure

1.3). The most striking example of these species is *Prunus ilicifolia*, a shrub of the California chaparral. This species is a special case in that its leaves contain cyanogenic glucosides. These nitrogen-containing secondary compounds almost certainly play no direct functional role in determining A_{max} and may represent allocation of nitrogen away from compounds functionally related to A_{max} and toward defense. We shall assess the possibility that sclerophylls, in general, allocate proportionally less nitrogen to photosynthesis than do nonsclerophylls when we consider nitrogen-use efficiency. Some of the ecological factors controlling the allocation of nitrogen to defensive compounds have been considered by Bryant et al. (1983) and by Gulmon and Mooney (Chapter 20).

Which basis for expressing the $A_{max}-N$ relationship is more significant functionally? Compelling arguments can be used to support either expression. Because light capture and CO_2 exchange with the atmosphere are intrinsically area-based phenomena, the area-based analysis provides a resource-harvesting framework for understanding the $A_{max}-N$ relationship. On the other hand, a weight-based analysis yields more information on the economics of nitrogen and carbon allocation. Each expression gives important information, and the sources of the differences between them can contribute to the elucidation of the functional and ecological controls on photosynthetic capacity and leaf nitrogen.

Differences between the weight-based and area-based relationships result largely from variation in LSW (leaf weight/leaf area). In the VINE survey, LSW varies inversely with the weight-based measure of leaf nitrogen (Figure 1.4). The evergreen sclerophylls have the highest LSWs and also the survey's lowest photosynthetic capacities, either weight-based or area-based. The Death Valley annuals have the highest photosynthetic capacities on both measurement bases and a narrow range of LSW, somewhat above the lowest values. Converting from a weight-based (Figure 1.2) to an area-based (Figure 1.3) $A_{max}-N$ relationship requires only multiplying each value for A_{max} and N by the LSW for that leaf. The consequence of the inverse relationship between LSW and N per unit weight is to increase small values and decrease large values of area-based A_{max} and N, relative to the weight-based parameters. Thus, the transformation from a weight-based to an area-based analysis tends to compress the total range of variation in A_{max} and N, and to increase the variability among the leaves with the highest LSWs.

The $A_{max}-N$ relationship is not fundamentally changed by the choice of measurement basis (as long as A_{max} and N are expressed in the same units), but the choice of units does alter the prominence of various segments of

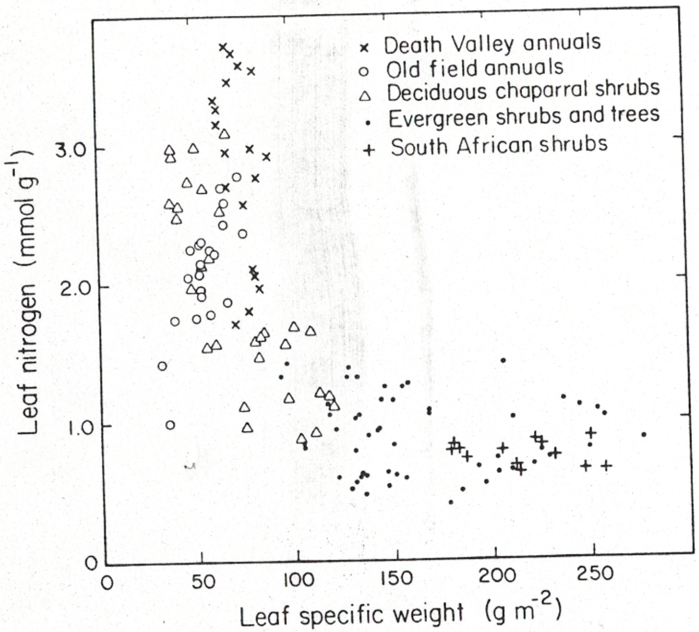

Figure 1.4. Relationship between leaf nitrogen per unit of leaf weight (N) and leaf-specific weight (LSW) for the VINE survey.

lated, and A_{max} per unit of leaf nitrogen is lower for the evergreen sclerophylls than for the other species.

Nitrogen-use efficiency

To simplify discussion of the A_{max} realized for a given level of N, it is useful to eliminate the confounding variable LSW and consider the ratio of A_{max} to N, which we term potential photosynthetic nitrogen-use efficiency (PPNUE). As long as A_{max} and N are both expressed on the same basis, PPNUE is independent of the measurement basis. We can view PPNUE as an index of potential performance under defined conditions that allows direct comparison among species. PPNUE is not an ecologically complete definition of nitrogen-use efficiency, but it is an important component of a more general, ecological definition, as provided by Rundel (1982) or Vitousek (1982). In addition to photosynthesis or growth per unit of nitrogen in tissue, these more complete definitions account for the critical roles in nitrogen-use efficiency played by leaf duration, nitrogen recovery from leaves, and whole-plant patterns of nitrogen allocation

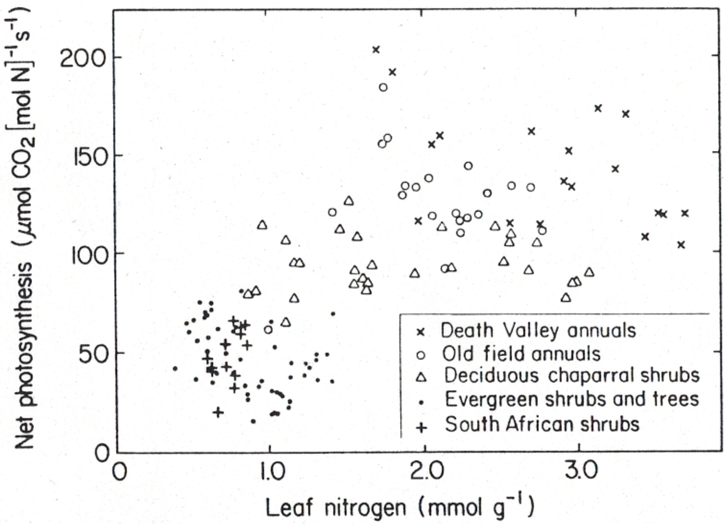

Figure 1.5. Relationship between photosynthetic capacity per unit of leaf nitrogen (PPNUE) and the weight-based measure of leaf nitrogen for the VINE survey.

interpretation of nitrogen-use efficiency. Its potential utility for approaching broader questions about nitrogen and growth will be increased as this index is modified to reflect long-term photosynthesis under natural conditions, and as photosynthetic data are combined with other components of nitrogen-use efficiency.

For the VINE survey, the analysis of PPNUE does provide several insights into the A_{max}–N relationship. PPNUE is lowest in the plants of lowest A_{max}, and increases with A_{max}. From values of less than 30 μmol CO$_2$ [mol N]$^{-1}$ s^{-1} for leaves of less than 1 mmol N g^{-1}, PPNUE rises to a plateau at around 125 μmol CO$_2$ [mol N]$^{-1}$ s^{-1} for leaves with nitrogen contents above about 2 mmol g^{-1} (Figure 1.5). Reviewing the data of Medina (1981) and others, Grubb (1984) also reached the conclusion that PPNUE is positively related to A_{max}. In the VINE survey, the relationship can be seen more precisely as a staircase of nearly constant PPNUE within major species types (annuals, deciduous shrubs, evergreen sclerophylls) and stepwise increases between them (Table 1.2). Although not in subgroups within the VINE survey, an increase in PPNUE with increasing N per unit weight is suggested by some studies on individual species (e.g., Gulmon and Chu 1981).

Several hypotheses may potentially explain the increase in PPNUE with increasing A_{max}. Observing that A_{max} and leaf longevity tend to be inversely

Table 1.2. *Potential photosynthetic nitrogen-use efficiency (PPNUE) and nitrogen per unit of leaf weight (N/wt) for the species groups in the VINE survey*[a]

Species group	PPNUE (μmol CO_2[mol N]$^{-1}$ s^{-1})	N/wt (mmol g^{-1})	PPNUE vs. N/wt r	$p <$
Death Valley annuals	142 a	2.9 a	−0.58	0.01
Old-field annuals	130 a	2.2 b	0.31	n.s.
Drought-deciduous shrubs	95 b	1.9 b	0.07	n.s.
California evergreens	46 c	0.9 c	−0.42	0.01
South African shrubs	49 c	0.8 c	0.58	0.05

[a] Within a column, values followed by different letters are significantly different ($p < 0.001$). Values followed by the same letter are not significantly different ($p > 0.05$). In two of the three cases where the correlation between PPNUE and N/wt is significant, the correlation coefficient is negative.

the prerequisites for longevity constrain the options for A_{max}. From this, we can derive two hypotheses. First, Medina (1981) suggested that PPNUE is low in evergreen sclerophylls because the thick or impermeable cell walls required for longevity impose large resistances to CO_2 diffusion. Second, differences in PPNUE may result from differences in nitrogen allocation. Specifically, leaves with high A_{max} may invest a larger proportion of the leaf nitrogen in the primary carboxylating enzyme of C_3 plants, ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), and leaves with low A_{max} may invest a larger proportion of the leaf nitrogen in compounds required for longevity.

The data from the VINE survey do not provide a direct test of these two hypotheses for the relationship between PPNUE and N or A_{max}, but they do suggest two modifications of the second hypothesis that do not require assumptions about the prerequisites for leaf longevity. For an essentially linear weight-based A_{max}–N relationship, we can write

$$A_{max} = mN + b$$

or

$$\text{PPNUE} = A_{max}/N = m + b/N$$

If the y intercept (b) is positive, PPNUE decreases with increasing N, and if b is negative, PPNUE increases with increasing N, reaching a maximum value of m, the slope of the A_{max}–N relationship. This algebraic formulation has two implications. First, PPNUE will increase with N whenever, at least in extrapolation, some minimum nitrogen level greater than zero is required for a positive A_{max}. Because photosynthesis is only one of many nitrogen-requiring metabolic processes in leaf cells, it is intuitively reasonable that some sort of a nitrogen threshold for photosynthetic competence (A_{max} just greater than zero) does exist, and that this threshold value forces the increase in PPNUE with increasing A_{max} or N. If this hypothesis is correct, low PPNUE may result directly from low leaf nitrogen and is not necessarily mechanistically related to features required to increase leaf longevities.

In the VINE survey, the y intercept in the weight-based A_{max}–N relationship is negative. The regression equation suggests that a nitrogen concentration of about 0.5 mmol N g^{-1} is required for an A_{max} just equal to zero. While this value provides a first approximation of the nitrogen content at minimum photosynthetic competence, it must be considered a very rough approximation, because there is little reason to expect a constant threshold value of nitrogen independent of species or leaf type. Consistent with this caveat, extrapolated values for N at $A_{max} = 0$ vary widely among studies (data not shown).

A second implication also concerns the nitrogen invested in reactions unrelated to photosynthesis. When a high LSW dilutes a given A_{max} over a large amount of leaf mass, the nitrogen invested in reactions of intermediary metabolism, biosynthesis, and maintenance of ion gradients may not be diluted precisely in parallel. Because cell viability, independent of photosynthesis, requires nitrogen in proteins and nucleic acids, the nitrogen requirement for reactions not related to photosynthesis may scale positively with LSW. To the extent that this is the case, low PPNUE and high LSW are functionally related, because the proportion of the total leaf nitrogen invested in photosynthetic machinery decreases as LSW increases. This hypothesis not only explains the low PPNUE of evergreen sclerophylls but also is consistent with the pattern in the area-based A_{max}–N relationship (Figure 1.3), where some evergreens contain more nitrogen than would be predicted from the regression with A_{max}.

A third hypothesis is that low PPNUE results from inefficient allocation of nitrogen among photosynthetic compounds, such that some compounds are present in large excess, while the rate-limiting compounds are underrepresented for lack of nitrogen investment. Because photosynthesis is a very complex process, the problems of efficient allocation may be substantial. It is also possible that efficient nitrogen allocation under natural conditions is not efficient under the conditions employed for measurement of A_{max}. For example, shade plants invest large quantities of nitrogen in light-harvesting pigments and proteins, but make only small investments in Rubisco and other CO_2-processing

This nitrogen allocation pattern may be efficient under low light but inefficient under saturating light. Much work remains to be done in assessing the relationship between A_{max} and the photosynthesis realized in nature.

A final hypothesis to explain the variation in PPNUE is that the kinetic properties of the photosynthesis-limiting nitrogenous compounds are not constant across species and that those plants with the highest PPNUEs have the most active or efficient rate-limiting enzymes.

To date, relatively little evidence is relevant to discriminating among the hypotheses that potentially explain the variation in PPNUE. No analysis based solely on interpretation of gas-exchange experiments can distinguish effects of cell-wall resistance to CO_2 diffusion from effects of nitrogen investment in nonphotosynthetic compounds, or from effects of variation in enzyme or allocation efficiency. However, the available information generally does not support the hypothesis that low PPNUE in sclerophylls results from unusually large internal resistances to CO_2 diffusion.

Three lines of evidence support this position. First, internal resistances should, in general, decrease as the ratio of the internal cell surface area to leaf area (S_i/S_l) increases (Nobel et al. 1975; Raven and Glidewell 1981). Though it is not always the case (Longstreth and Nobel 1980), the S_i/S_l ratio increases with LSW, if cell size and shape are constant. Thus, leaves with high LSWs should typically have low internal resistances. Second, the suggestion that extensive cell-wall development in sclerophylls imposes large limitations to CO_2 diffusion requires that these leaves contain an unusually large proportion of their biomass in cell walls. However, Merino et al. (1984) report that the biomass allocation to cell-wall components in the sclerophyllous chaparral shrub *Heteromeles arbutifolia* is 39.7% (46.7% including ash), comparable to the 38.1% (47.3% including ash) allocated to cell walls in the deciduous shrub *Lepechinia calycina*, and to the 41.4% (47.9% including ash) allocated to cell walls in the deciduous shrub *Diplacus aurantiacus*, after removal of the external resin in the latter species. All these values for leaves of perennials are lower than the 51.0% (56.0% including ash) of the total biomass allocated to cell-wall constituents in young corn plants (Penning de Vries et al. 1974). Neither of these lines of evidence addresses the permeability of the cell walls, but a third does.

Farquhar et al. (1982) argue that because diffusion and the biochemistry of carboxylation discriminate differently against the naturally occurring carbon isotope ^{13}C, then the isotopic composition of plants should reflect the relative importance of limitations to photosynthesis by diffusion and biochemical factors. In experiments where diffusional limitations are controlled, large limitations by diffusion result in high ratios of $^{13}C/^{12}C$. Small diffusional limitations lead to low ratios (Farquhar et al. 1982). For a broad survey of C_3 species, the mean $^{13}C/^{12}C$ ratio is 27.8 parts per thousand less than that in the standard limestone, with a range of 21 to 36 (Troughton et al. 1974). For the five species of California evergreens studied by Field et al. (1983), the mean ratio is 27.9 parts per thousand less than that in the standard limestone (unpublished data). The similarity between the mean values for C_3 plants and for evergreen sclerophylls indicates that the proportional limitations to photosynthesis imposed by diffusion are similar for the two groups. None of this evidence is conclusive in itself, but it all indicates that evergreen sclerophylls do not have proportionally large cell-wall resistances to CO_2 diffusion. The hypotheses favored by elimination are that PPNUE is low in the sclerophylls because proportionally less of their nitrogen is allocated to photosynthetic reactions, or that the sclerophylls have inefficient photosynthetic machinery. We shall review additional evidence concerning these hypotheses in the following section on the causal basis of the $A_{max}-N$ relationship, but quantification of the relative importance of these hypotheses awaits further research.

Correlation or cause

Variation in leaf nitrogen can clearly explain much of the variation in photosynthetic capacity, across a wide variety of plant communities. From a purely predictive viewpoint, the correlation should prove a useful tool in the preliminary characterization of photosynthetic capacities. To understand the ecological controls on photosynthetic capacity, we need to move beyond the correlation to an assessment of cause and effect. This assessment warrants analysis at two levels. At the proximate level, establishing the functional basis of the $A_{max}-N$ relationship will provide the foundation from which to approach a wide variety of ecological questions. How will particular plants or communities respond to changes in nitrogen availability? If nitrogen uptake is increased at the expense of additional allocation of carbon to roots, will the increased photosynthesis more than offset the costs of root maintenance and construction? At the ultimate level, important questions concern the control of nitrogen investment and the efficacy of that control. What controls nitrogen levels? How do actual nitrogen investments compare with the optimal investments for the maximization of growth, yield, or fitness? Here, we concentrate primarily on the proximate analysis of cause in the $A_{max}-N$ relationship, providing a basis for future work at a variety of levels

The A_{max}–N relationship suggests that N determines A_{max}, but it does not establish causation. At least three kinds of functional relationships are consistent with the correlation. Nitrogen levels may determine A_{max}; N may be controlled in response to A_{max}; or both N and A_{max} may be regulated by some other factor or factors. To summarize the evidence relevant to each of these hypotheses, we need to state them more precisely.

Hypothesis 1. Photosynthetic capacity is limited by one or more processes that operate at rates determined by their nitrogen contents. As a direct assignment of cause and effect, this hypothesis focuses attention on the physiological basis of photosynthesis. Net CO_2 fixation is potentially limited by CO_2 transport through gaseous or liquid media, by enzymatic reduction of CO_2, or by light-driven generation of NADPH and ATP. Within each process, limiting steps may operate at rates determined by nitrogen levels.

Hypothesis 1 is compatible with the possibility that A_{max} is limited by multiple nitrogenous compounds or by a combination of nitrogenous and nonnitrogenous factors. If multiple nitrogenous compounds limit A_{max}, nitrogen is efficiently distributed among the limiting factors only when all nitrogen redistributions decrease A_{max}. To the extent that inefficient nitrogen distributions decrease A_{max}, hypothesis 1 does require a relatively efficient distribution of nitrogen among limiting compounds. If a combination of nitrogenous and nonnitrogenous factors limit A_{max}, a tight A_{max}–N relationship is still possible, but it requires that the nonnitrogenous limitations be either small or a constant proportion of the total limitation to A_{max}.

Hypothesis 2. Independent of the factors that actually limit photosynthetic capacity, leaf nitrogen content is regulated to reflect photosynthetic capacity. This hypothesis emphasizes potential intraplant competition for nitrogen allocation. To the extent that the whole plant is nitrogen-limited, excess capacity in any enzyme or other nitrogenous compound represents an inefficiency, a diversion of nitrogen away from a rate-limiting step in the same leaf or away from investment in some other part of the plant where the nitrogen could contribute to growth and reproduction. With the maximum efficiency of nitrogen allocation, the level of every nonlimiting nitrogenous compound should be adjusted downward to the lowest activity still above rate limitation. At this level, the distinction between the functional constraint postulated in hypothesis 1 and an adjustment for efficient allocation fades. Hypothesis 2 does not

nitrogenous compounds. If nonnitrogenous factors limit A_{max}, excess capacity in nitrogenous compounds should still be trimmed.

Hypothesis 3. The correlation between photosynthetic capacity and leaf nitrogen is not the result of a functional relationship but arises because both parameters are sensitive to or are controlled by other leaf parameters. This possibility, a null hypothesis with respect to the functional relationships suggested in hypotheses 1 and 2, should be accepted if A_{max} and N are invariant when expressed on the basis of some fundamental unit but are changed in concert when expressed on the basis of some derived unit. For example, if A_{max} and N were intrinsically area-based parameters, and if they were constants on a leaf-area basis, variation in LSW would transform these constants into a perfect correlation between the weight-based measures of A_{max} and N. Such a correlation could reflect nothing more than the differential utilization of leaves for storage, or differential allocation to defensive compounds. In the VINE survey, A_{max} and N both varied over at least a fivefold range, independent of the measurement basis. The magnitude of this variation militates against the acceptance of the null hypothesis but does not eliminate the possibility that some components of the A_{max}–N relationship result from spurious or secondary correlations.

Quantifying the importance of secondary effects resulting from potentially confounding variables like LSW, S_i/S_l, or leaf age is partially amenable to multiple-correlation analysis (e.g., Field and Mooney 1983), but the fundamental nature of the A_{max}–N relationship is too poorly known to allow much confidence in an analysis based on linear or simple curvilinear responses.

To evaluate the relative importance of each of these three hypotheses, we combine an analysis of the VINE survey with results from a variety of studies that have probed the biochemistry of photosynthesis in one or a few species. Because the data required for definitive interpretations of several aspects of the A_{max}–N relationship are not yet available, many of our comments are speculative and are intended as much to identify unanswered questions as to integrate existing information.

Deciding among the three hypotheses

The three hypotheses postulate that the A_{max}–N relationship results from substantially different mechanisms. Though the mechanisms among them is more a problem of quantifying relative importance than of rejecting falsifiable alternatives.

Nitrogen-based limits to A_{max}

Summarizing photosynthesis as an aggregate of processes performing CO_2 transport, carboxylation, CO_2 reduction, and generation of reducing equivalents and ATP, we can search for nitrogen-based rate limitation in each of these processes. Among these, the best-studied candidate for limiting A_{max} in C_3 plants is the primary carboxylating enzyme in C_3 plants and the ultimate CO_2-fixing enzyme in all plants, ribulose-1,5-bisphosphate carboxylate/oxygenase (Rubisco) (Lorimer 1981). Circumstantial evidence that Rubisco limits A_{max} comes from its extreme abundance [it often constitutes over 40% of the leaf soluble protein (Collatz et al. 1979; Wittenbach 1979; Friedrich and Huffaker 1980) and 15–33% of the total leaf protein (Collatz et al. 1979; Seemann et al. 1981; Somerville et al. 1982)] and its low catalytic activity. Each of the eight active sites in a very large enzyme (molecular weight = 550,000) (Jensen and Bahr 1977) catalyzes only about 2 carboxylations (Badger and Collatz 1977), or 0.4 oxygenation per second (Farquhar et al. 1980). The combination of low catalytic activity and competitive inhibition by oxygen suggests that the high levels of Rubisco reflect nitrogen allocation to reduce rate limitation.

This circumstantial evidence is reinforced by a strong correlation between A_{max} and the total activity of Rubisco. Summarizing several studies of sun and shade plants grown under a variety of light regimens, Björkman (1981) found a correlation coefficient of 0.96 between A_{max} and Rubisco activity. This correlation is both general and robust. A_{max} and Rubisco activity vary in parallel in experimental treatments involving light intensity during growth (Björkman 1968), nitrogen availability (Medina 1971; Wong 1979), partial defoliation (Wareing et al. 1968; Neales et al. 1971), ploidy series (Randall et al. 1977; Molin et al. 1982), or leaf aging (Wittenbach 1979; Friedrich and Huffaker 1980).

The correlation between A_{max} and Rubisco activity is very strong, but some evidence indicates variation in the kinetic parameters of Rubisco, which could translate into variation in activity per unit of nitrogen invested in the enzyme. The CO_2 concentration required for half saturation of the enzyme varies twofold among C_3 grasses and over fourfold between C_3 and C_4 grasses (Yeoh et al. 1980). The rate of product formation per active site is about 70% greater in Rubisco from spinach than in that from soybean, and the difference in in vitro activities is reflected in measurements of whole-leaf photosynthesis (Seemann and Berry 1982). The specific activity

(Wittenbach 1979; Friedrich and Huffaker 1980) or strongly decreasing (Hall et al. 1978). Though the kinetic parameters of Rubiscos from C_3 plants vary substantially, the enzyme's relative specificity for CO_2 and O_2 is nearly constant (Jordan and Ogren 1981). At this point, it appears that Rubisco activity per unit of invested nitrogen is not constant, but the variability is probably minor enough to appear as limited scatter in the VINE survey.

Some studies have indicated that Rubisco is not fully activated in vivo (Perchorowicz et al. 1980; Perchorowicz and Jensen 1983), but the evidence for incomplete activation is compelling only under conditions much different from those used for the determination of A_{max}. von Caemmerer and Farquhar (1981), Seemann et al. (1981), and Seemann and Berry (1982) all found that the activity of Rubisco is consistent with in vivo photosynthetic rates only if one assumes full activation.

While relatively strong and very general, the evidence for a functional limitation of A_{max} by Rubisco activity is largely correlative and subject to the interpretation that Rubisco varies either in response to A_{max} or in response to other factors that control A_{max}. The studies indicating that Rubisco activity is barely sufficient to account for observed photosynthetic rates begin to move beyond correlation but focus on measurements under low CO_2 concentrations, where Rubisco is most likely to be rate-limiting. One other line of evidence indicating that Rubisco activity, rather than other nitrogenous compounds, controls A_{max} comes from studies in which Rubisco was not a constant proportion of total protein and in which A_{max} varied more strongly with Rubisco than with protein (Medina 1971; Wittenbach 1983).

Although Rubisco clearly limits photosynthesis under some conditions, variation in Rubisco alone cannot account for the A_{max}–N relationship. Rubisco does constitute a substantial proportion of the total leaf protein, but the variation in nitrogen is much too large to be due to changes in one or a few enzymes. In the Death Valley annuals, Rubisco represents about 18% of the total leaf protein (Seemann et al. 1981). Eliminating all of the nitrogen in the Rubisco still leaves nitrogen contents more than an order of magnitude higher than the highest levels measured in some of the California evergreens (Figure 1.2). Levels of many nitrogenous compounds must be changing in concert.

Several examples document correlations between Rubisco and total nitrogen. In wheat, Rubisco activity scales linearly with total N (Evans 1983). In nine species of Death Valley annuals (Seemann et al. 1981), in soybean and spinach (Seemann and Berry 1982), and in aging leaves of and wheat (Wittenbach 1979), Rubisco constitutes a relatively constant proportion of the total leaf protein. The proportion of total nitrogen in Rubisco appears to decrease late in leaf senescence (Wittenbach 1979; Friedrich and Huffaker 1980; Wittenbach et al. 1980) and may be sensitive to some kinds of source–sink manipulations (Wittenbach 1983).

The correlation between Rubisco and N or protein suggests three interpretations: Levels of other nitrogenous compounds may be adjusted to reflect the limitation of A_{max} by Rubisco, levels of Rubisco may be adjusted to reflect the limitation of A_{max} by other nitrogenous compounds, or A_{max} may be colimited by Rubisco and other nitrogenous compounds. Each of these possibilities is consistent with hypothesis 2: adjustment of nitrogen investment for maximum efficiency of nitrogen allocation. The third possibility represents the merger of functional limitation (hypothesis 1) with efficient investment (hypothesis 2).

Photosynthesis may be limited by Rubisco activity, but it may also be limited by CO_2 transport, CO_2 reduction capacity, or light-driven generation of reducing equivalents and ATP. Rate limitation by CO_2 transport is discussed by Cowan (Chapter 5), who demonstrates that low allocation of N to carbonic anhydrase (the enzyme that catalyzes the interconversion of CO_2 and bicarbonate) can depress A_{max} through effects on CO_2 transport. Rate limitation by either CO_2 reduction or the light reactions can be summarized under effects on regeneration of the CO_2 acceptor, ribulose-1,5-bisphosphate (RuBP) (Farquhar et al. 1980).

Evidence for limitation of A_{max} by RuBP regeneration is beginning to accumulate. The investment of nitrogen in the components of RuBP regeneration is substantial, probably greater than the investment in Rubisco (Kirk and Tilney-Bassett 1978). RuBP regeneration (Evans 1983) and electron-transport capacity (von Caemmerer and Farquhar 1981) are strongly correlated with Rubisco activity. Further, the total concentration of electron-transport components, as indicated by chlorophyll concentration, changes with A_{max} under treatments where rate limitation by Rubisco is unlikely (Terry 1983). Farquhar et al. (1980) summarize a great deal of biochemical information in a model that predicts that photosynthesis is limited by RuBP regeneration at CO_2 concentrations above some critical value. von Caemmerer and Farquhar (1981) argue that nitrogen is efficiently allocated between Rubisco and the components of RuBP regeneration when that critical CO_2 concentration is adjusted to the level at which the leaf normally operates. At this point, A_{max} is colimited by Rubisco and RuBP regeneration.

In summary, strong evidence supports the hypothesis that A_{max} is some-

nitrogenous compounds responsible for RuBP regeneration are certainly adjusted in concert with levels of Rubisco and may be important limitations to A_{max}.

Limitations by physical factors

In C_3 plants, photosynthesis is almost always limited by CO_2 diffusion as well as biochemical factors. Because C_3 plants are typically not CO_2-saturated in normal air (Pearcy and Ehleringer 1984), every step in the diffusion pathway from the bulk atmosphere to the sites of carboxylation in the chloroplasts represents a concentration drop that decreases photosynthesis. The concentration drop across the stomata is typically large, often about 100 μmol mol^{-1} or nearly one-third of the total CO_2 concentration (Wong et al. 1979; Sharkey et al. 1982). Farquhar and Sharkey (1982) provide a simple and elegant method for calculating the proportional limitations to photosynthesis due to effects of stomata. The concentration changes across other components of the diffusion pathway are more difficult to measure, but diffusion limitations imposed by the boundary layer, by the pathway from the substomatal cavity to the photosynthesizing cells (Parkhurst, Chapter 7 in this volume, but see Sharkey et al. 1982 for conflicting evidence), and by liquid-phase transport into the chloroplasts (Cowan, Chapter 5 in this volume; Nobel et al. 1975; Evans 1983) may all present substantial limitations to A_{max} under some circumstances.

These diffusional limitations must be balanced by at least one biochemical limitation. Photosynthetic capacity is completely limited by diffusion only when the CO_2 concentration at the sites of carboxylation drops below the CO_2 compensation point at which respiratory CO_2 evolution equals photosynthetic CO_2 uptake. Though many models have assumed these low CO_2 concentrations at the carboxylation sites, recent evidence indicates that the activity of the carboxylase is sufficient to support observed photosynthetic rates only if the CO_2 concentration at the carboxylation sites is substantially higher (von Caemmerer and Farquhar 1981; Seemann and Berry 1982; Evans 1983).

Each of the segments in the CO_2 diffusion pathway limits photosynthetic capacity, but with proportional magnitudes sensitive to other diffusional and biochemical limitations. In C_4 plants, which may be CO_2-saturated in normal air (Pearcy and Ehleringer 1984), there is no conceptual requirement for multiple limitations to photosynthetic capacity.

Stomatal limitations. Stomatal limitations to A_{max} are generally smaller than predicted by the linear analyses used for the last 25 years larger (Comstock and Ehleringer 1984). These relatively large diffusional limitations could affect the $A_{max}-N$ relationship in three ways. If stomatal conductances were constant as biochemical limitations changed, the $A_{max}-N$ relationship would curve downward at high levels of N, as stomatal limitation prevented large gains from increased nitrogen investment. If stomatal limitations were large but variable, they should add scatter to the $A_{max}-N$ relationship. If stomatal conductance scales with biochemical limitations, establishing a proportionally constant stomatal limitation, the $A_{max}-N$ relationship should have little scatter and should be linear or some other shape set by the response of the biochemistry to changes in nitrogen investment.

An accurate assessment of stomatal limitation requires an analysis of the CO_2 response of photosynthesis (Farquhar and Sharkey 1982), data not available for the VINE survey. At a slightly less sophisticated level, a proportionally constant stomatal limitation is indicated by a linear relationship between A_{max} and stomatal conductance (g). In the VINE survey, A_{max} and g are highly correlated ($r = 0.77$), even though the Illinois annuals tend to have high g values in relation to A_{max} (Figure 1.6).

The scatter in the $A_{max}-g$ relationship suggests some variation in stomatal limitation to A_{max}. The five species of California evergreens in the VINE survey provide evidence for this. Field et al. (1983) found that the species with a higher A_{max} for a given N (a higher PPNUE) tended to be from wetter sites than species with lower PPNUE. Much of their difference in PPNUE values is attributable to higher stomatal conductance in the species of the wetter sites, and these differences generate scatter in the $A_{max}-N$ relationship.

The mechanisms responsible for maintaining nearly constant limitations by g are not well known. Mooney and Gulmon (1979) argued that investments in photosynthetic machinery should be reduced when drought limits g, a postulate supported by evidence from Ehleringer (1983), who reported that N declines with increasing drought in the desert annual *Amaranthus palmeri.* Alternatively, the value of g may be regulated by the value of A_{max}, as determined by biochemical factors. Some of the strongest evidence for control of g by biochemical capacity comes from Wong et al. (1979), who demonstrated that stomatal conductance in C_3 and C_4 species is somehow regulated by photosynthetic capacity under a wide variety of treatments, including nutrient availability during growth, nutrient withdrawal, water stress, and chemical inhibition of photosynthesis. This type of coordination tends to eliminate scatter or curvilinearity in the $A_{max}-N$ relationship that could result from variable stomatal

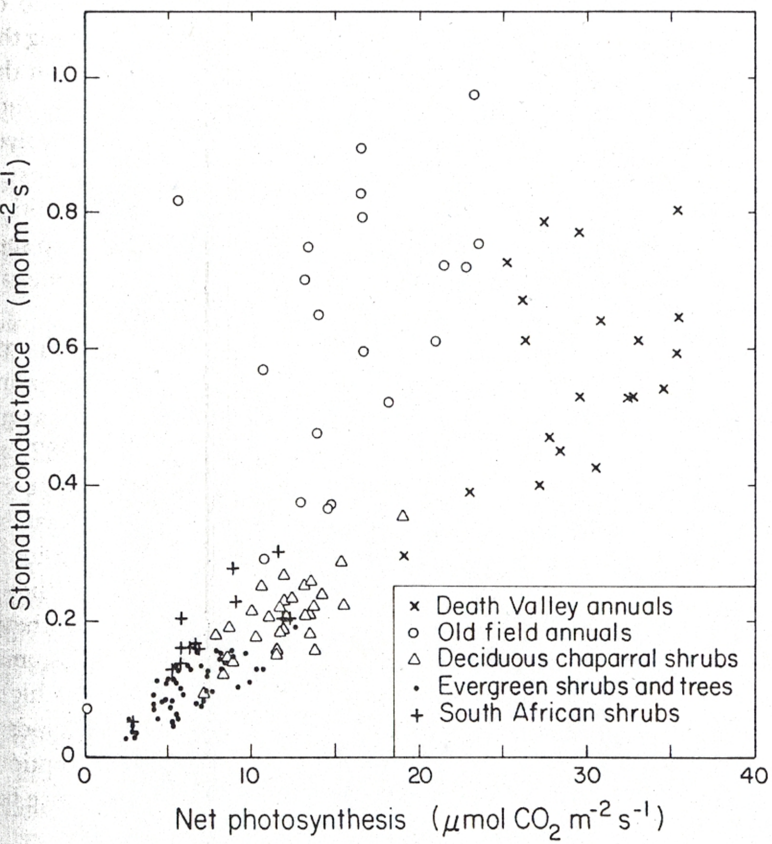

Figure 1.6. Relationship between photosynthetic capacity per unit of leaf area (A_{max}) and stomatal conductance (g) for the VINE survey. Stomatal conductances were measured simultaneously with photosynthetic capacity. $g = 0.0119 + 0.0215 \cdot A_{max}$, $n = 135$, $r = 0.77$, $P < 0.001$.

Other physical limitations. Nobel et al. (1975), Raven and Glidewell (1981), and Cowan (Chapter 5) have calculated that A_{max} typically includes a substantial limitation caused by resistance to CO_2 diffusion between the surfaces of the photosynthesizing cells and the sites of carboxylation. To the extent that this resistance is influenced by the activity of carbonic anhydrase (Cowan, Chapter 5), it acts as both a nitrogenous limitation and a physical limitation to A_{max}. Experimentally, it is very difficult to separate liquid-phase diffusion resistance from biochemical limitations. Nobel and associates demonstrated strong correlations between a measure of photosynthetic capacity and the ratio of cell surface area to leaf area (S_i/S_l) (Nobel et al. 1975; Nobel 1976, 1977, 1980), indicating that A_{max} may be limited by the internal surface area available for diffusion. However, as Björkman (1981) pointed out, the ratio of internal volume to leaf area also tends to increase with S_i/S_l, admitting the possibility that increases in A_{max} are more closely tied to increases in the amount of biochemical machinery than to increases in internal surface area. One promising approach to separating these limitations involves comparison of in vitro biochemical characteristics with in vivo photosynthetic rates. Knowing the in vitro kinetic properties of a rate-limiting enzyme, it is possible to calculate the CO_2 concentration required to support observed photosynthetic rates. From this CO_2 concentration, one can calculate the physical limitations to in vivo CO_2 diffusion.

This approach has been best developed for Rubisco. From experiments at low CO_2 concentrations, under which photosynthetic rates can be more accurately related to in vitro Rubisco kinetics, von Caemmerer and Farquhar (1981), Seemann et al. (1981), and Seemann and Berry (1982) all concluded that Rubisco activity is barely sufficient to support observed photosynthetic rates. In these studies, in vivo photosynthetic rates can be explained only by assuming no drop in CO_2 concentration across the liquid phase. Using a similar analysis, however, Evans (1983) found that photosynthesis was less than expected in leaves with the highest photosynthetic capacities, suggesting a drop in CO_2 concentration between the substomatal cavity and the sites of carboxylation. Evans's (1983) conclusion highlights the possibility that liquid-phase diffusion resistance usually imposes relatively small limitations to A_{max} but that when leaves develop exceptionally high biochemical capacities, limitations by liquid-phase resistance become important.

A general, quantitative summary of the magnitude of limitations by liquid-phase diffusion resistance is not yet possible. The very high correlation in the $A_{max}-N$ relationship is consistent with a minor limitation. If the limitation were large but constant, we would expect downward curvature at the upper end of the $A_{max}-N$ relationship, as the diffusional limitation increasingly constrained the gains of additional nitrogen investment. This kind of curvature is apparent in some single-species $A_{max}-N$ relationships (Evans 1983), suggesting that the liquid-phase diffusion resistance is less variable within species than among species. Finally, the $A_{max}-N$ relationship is consistent with large liquid-phase diffusion limitations, if the magnitude of those limitations scales linearly with the limitations imposed by biochemical factors. Unfortunately, the interface of biochemical and liquid-phase diffusional limitations to A_{max} has not been well studied. Correlations between S_i/S_l and A_{max} suggest linear scaling but have not been reinforced by independent measures of biochemical limits to A_{max}.

Integration and conclusions

In C_3 plants, A_{max} is typically limited by biochemical and diffusional factors, with the majority of the total limitation caused by biochemical factors. The biochemical limitations are imposed by nitrogen-containing compounds, and the biochemical capacity for photosynthesis scales with the nitrogen investment in these compounds. Of the nitrogenous limits, limitation by Rubisco is the best-documented case, but limitation by compounds involved in RuBP regeneration and by carbonic anhydrase is also likely. At this point, we cannot distinguish broad colimitation of A_{max} by nitrogenous factors from limitation by one or a few nitrogenous compounds and adjustment to near limitation in others. In variable natural environments, this distinction may be somewhat artificial, as changes in the environment alter the balance among limiting factors.

The mechanisms responsible for adjusting levels of nitrogenous compounds are not generally known. Schmidt and Mishkind (1983) demonstrated that the required 1 : 1 ratio between large and small subunits of Rubisco is maintained by rapid degradation of excess small subunits. They further suggested that similar proteolytic systems could establish and preserve an appropriate balance among a wide variety of photosynthetic compounds by degrading any components not integrated into a functional pathway. Little is known about the ways that environmental signals specifying the appropriate balance among photosynthetic compounds are transduced into controlling mechanisms.

The integrated balance among nitrogenous limitations to A_{max} is, in general, paralleled by a scaling between nitrogenous and diffusional limitations. Neither the mechanisms nor the ecological priorities for this scaling are well known. Is nitrogen investment curtailed when diffusional limitations become too large, or is nitrogen the primary control? This question has no single answer, and priority among multiple constraints is probably sensitive to a variety of environmental and phylogenetic factors (Mooney and Gulmon 1979). When constraints on water loss, the risk of herbivory, or limited light availability decrease the gains expected from high N, plants generally forgo those investments. In these cases, N is the major determinant of A_{max}, but it is not the primary environmental constraint. In other cases, limited N availability may act as both the major limitation to A_{max} and the major environmental constraint. An important implication of the adjustment of biochemical limitations in response to environmental constraints is that A_{max} is not an ecologically meaningless physiological parameter, but is an index of integrated natural constraints

Much remains to be learned about the mechanistic basis and the ecological implications of the A_{max}–N relationship. Mechanistically, the distribution of quantitative limitations is still unknown, as is the nature of the controls on the relative levels of limiting factors. At the interface between mechanisms and implications, much work needs to be done on the sensitivity of the nature and magnitude of the limitations to changes in the environment. Ecologically, it is of great interest to quantify the relationship between A_{max} and photosynthesis under natural conditions and to use the A_{max}–N relationship for cost–benefit analyses of plant structure and function.

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References

Badger, M. R., and G. J. Collatz. 1977. Studies on the kinetic mechanism of ribulose-1,5-bisphosphate carboxylase and oxygenase reactions, with particular reference to the effect of temperature on kinetic parameters. Carnegie Inst. Washington Yearbook 76:355–361.

Björkman, O. 1968. Carboxydismutase activity in shade-adapted and sun-adapted species of higher plants. Physiol. Planta. 21:1–10.

– 1981. Responses to different quantum flux densities. Pp. 57–107 *in* O. L. Lange, P. S. Nobel, C. B. Osmond, and H. Ziegler (eds.), Physiological plant ecology, I, vol. 12A, encyclopedia of plant physiology, new series. Springer-Verlag, Berlin.

Björkman, O., and P. Holmgren. 1963. Adaptability of the photosynthetic apparatus to light intensity in ecotypes from exposed and shaded habitats. Physiol. Planta. 16:889–914.

Bolton, J. K., and R. H. Brown. 1980. Photosynthesis of grass species differing in carbon dioxide fixation pathways. V. Response of *Panicum maximum, Panicum miliodes,* and tall fescue *(Festuca arundinacea)* to nitrogen nutrition. Plant Physiol. 66:97–100.

Brown, R. H. 1978. A difference in N use efficiency in C_3 and C_4 plants and its implications in adaptation and evolution. Crop Sci. 18:93–98.

Brown, R. H., and J. R. Wilson. 1983. Nitrogen response in *Panicum* species differing in CO_2 fixation pathways. II. CO_2 exchange characteristics. Crop Sci. 23:1154–1159.

Bryant, J. P., F. S. Chapin II, and D. R. Klein. 1983. Carbon/nutrient balance of boreal plants in relation to vertebrate herbivory. Oikos 40:357–368.

Chapin, F. S., III. 1980. The mineral nutrition of wild plants. Ann. Rev. Ecol.

Chapin, F. S., III, and R. A. Kedrowski. 1983. Seasonal changes in nitrogen and phosphorus fractions and autumn retranslocation in evergreen and deciduous taiga trees. Ecology 64:376–391.

Collatz, G. J., M. Badger, C. Smith, and J. A. Berry. 1979. A radioimmune assay for RuP_2 carboxylase protein. Carnegie Inst. Washington Yearbook 78:171–175.

Comstock, J., and J. Ehleringer. 1984. Photosynthetic responses to slowly decreasing leaf water potentials in *Encelia frutescens.* Oecologia 61:241–248.

Ehleringer, J. 1983. Ecophysiology of *Amaranthus palmeri,* a sonoran desert summer annual. Oecologia 57:107–112.

Evans, J. R. 1983. Nitrogen and photosynthesis in the flag leaf of wheat *(Triticum aestivum* L.). Plant Physiol. 72:297–302.

Farquhar, G. D., M. C. Ball, S. von Caemmerer, and Z. Rosskandik. 1982. Effect of salinity and humidity on $\delta^{13}C$ value of halophytes – evidence for diffusional isotope fractionation determined by the ratio of intracellular/atmospheric partial pressure of CO_2 under different environmental conditions. Oecologia 52:121–124.

Farquhar, G. D., M. H. O'Leary, and J. A. Berry. 1982. On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. Aust. J. Plant Physiol. 9:121–137.

Farquhar, G. D., and T. D. Sharkey. 1982. Stomatal conductance and photosynthesis. Ann. Rev. Plant Physiol. 33:317–345.

Farquhar, G. D., S. von Caemmerer, and J. A. Berry. 1980. A biochemical model of photosynthetic CO_2 assimilation in the leaves of C_3 species. Planta 149:78–90.

Field, C. 1981. Leaf age effects on the carbon gain of individual leaves in relation to microsite. Pp. 41–50 *in* N. S. Margaris and H. A. Mooney (eds.), Components of productivity of Mediterranean-climate regions – basic and applied aspects. Dr. W. Junk, The Hague.

– 1983. Allocating leaf nitrogen for the maximization of carbon gain: Leaf age as a control on the allocation program. Oecologia 56:341–347.

Field, C., J. Merino, and H. A. Mooney. 1983. Compromises between water-use efficiency and nitrogen-use efficiency in five species of California evergreens. Oecologia 60:384–389.

Field, C., and H. A. Mooney. 1983. Leaf age and seasonal effects on light, water, and nitrogen use efficiency in a California shrub. Oecologia 56:348–355.

Friedrich, J. W., and R. C. Huffaker. 1980. Photosynthesis, leaf resistances, and ribulose-1,5-bisphosphate carboxylase degradation in senescing barley leaves. Plant Physiol. 65:1103–1107.

Gulmon, S. L., and C. C. Chu. 1981. The effects of light and nitrogen on photosynthesis, leaf characteristics, and dry matter allocation in the chaparral shrub, *Diplacus aurantiacus.* Oecologia 49:207–212.

Hall, N. P., A. J. Keys, and M. J. Merrett. 1978. Ribulose-1,5-diphosphate carboxylase protein during flag leaf senescence. J. Exp. Bot. 29:31–37.

Hesketh, J. D., E. M. Larson, A. J. Gordon, and D. B. Peters. 1983. Internal factors influencing photosynthesis and respiration. Pp. 381–411 *in* J. E. Dale and F. L. Milthorpe (eds.), The growth and functioning of leaves. Cambridge University Press.

Jensen, R. G., and J. T. Bahr. 1977. Ribulose-1,5-bisphosphate carboxylase-oxygenase. Ann. Rev. Plant Physiol. 28:379–400.

Jordan, D. B., and W. L. Ogren. 1981. Species variation in the specificity of ribulose bisphosphate carboxylase/oxygenase. Nature 291:513–515.

Kirk, J. T. O., and R. A. E. Tilney-Bassett. 1978. The plastids, ed. 2. Elsevier, Amsterdam.

Lee, J. A., R. Harmer, and R. Ignaciuk. 1983. Nitrogen as a limiting factor in plant communities. Pp. 95–112 *in* J. A. Lee, S. McNeill, and I. H. Rorison, (eds.), Nitrogen as an ecological factor. Blackwell, Oxford.

Longstreth, D. J., and P. S. Nobel. 1980. Nutrient influences on leaf photosynthesis: effects of nitrogen, phosphorus, and potassium for *Gossypium hirsutum* L. Plant Physiol. 65:541–543.

Lorimer, G. H. (1981). The carboxylation and oxygenation of ribulose 1,5-bisphosphate: the primary events in photosynthesis and photorespiration. Ann. Rev. Plant Physiol. 32:49–83.

Loveless, A. R. 1961. A nutritional interpretation of sclerophylly based on differences in the chemical composition of sclerophyllous and mesophytic leaves. Ann. Bot. 25:168–183.

Medina, E. 1971. Effect of nitrogen supply and light intensity during growth on the photosynthetic capacity and carboxydismutase activity of leaves of *Atriplex hastata* ssp. *hastata*. Carnegie Inst. Washington Yearbook 70:551–559.

– 1981. Nitrogen content, leaf structure and photosynthesis in higher plants: a report to the UNEP study group on photosynthesis and bioproductivity. IVIC, Caracas, Venezuela.

Merino, J., C. Field, and H. A. Mooney. 1984. Construction and maintenance costs of Mediterranean-climate evergreen and deciduous leaves. II. Biochemical pathway analysis. Oecologia Plantarum 4:211–229.

Molin, W. T., S. P. Meyers, G. R. Baer, and L. E. Schrader. 1982. Ploidy effects in isogenic populations of alfalfa. II. Photosynthesis, chloroplast number, ribulose-1,5-bisphosphate carboxylase, chlorophyll, and DNA in protoplasts. Plant Physiol. 70:1710–1714.

Mooney, H. A., J. Ehleringer, and J. A. Berry. 1976. High photosynthetic capacity of a winter annual in Death Valley. Science 194:322–324.

Mooney, H. A., P. J. Ferrar, and R. O. Slatyer. 1978. Photosynthetic capacity and carbon allocation patterns in diverse growth forms of *Eucalyptus*. Oecologia 36:103–111.

Mooney, H. A., C. Field, S. L. Gulmon, and F. A. Bazzaz. 1981. Photosynthetic capacity in relation to leaf position in desert versus old-field annuals. Oecologia 50:109–112.

Mooney, H. A., C. Field, S. L. Gulmon, P. Rundel, and F. J. Kruger. 1983. Photosynthetic characteristics of South African sclerophylls. Oecologia 58:398–401.

Mooney, H. A., and S. L. Gulmon. 1979. Environmental and evolutionary constraints on the photosynthetic characteristics of higher plants. Pp. 316–337 *in* O. T. Solbrig, S. Jain, G. B. Johnson, and P. H. Raven (eds.), Topics in plant population biology. Columbia University Press, New York.

– 1982. Constraints on leaf structure and function in reference to herbivory.

Nátr, L. 1975. Influence of mineral nutrition on photosynthesis and the use of assimilates. Pp. 537–556 *in* J. P. Cooper (ed.), Photosynthesis and productivity in different environments. Cambridge University Press.

Neales, T. F., K. J. Treharne, and P. F. Wareing. 1971. A relationship between net photosynthesis, diffusive resistance and carboxylating enzyme activity in bean leaves. Pp. 89–96 *in* M. S. Hatch, C. B. Osmond, and R. O. Slatyer (eds.), Photosynthesis and photorespiration. New York: Wiley-Interscience, New York.

Nobel, P. S. 1976. Photosynthetic rates of sun versus shade leaves of *Hyptus emoryi* Torr. Plant Physiol. 58:218–223.

– 1977. Internal leaf area and cellular CO_2 resistance: photosynthetic implications of variations with growth conditions and plant species. Physiol. Planta. 40:137–144.

– 1980. Leaf anatomy and water-use efficiency. Pp. 43–55 *in* N. C. Turner and P. J. Kramer (eds.), Adaptation of plants to water and high temperature stress. Wiley-Interscience, New York.

Nobel, P. S., L. J. Zaragoza, and W. K. Smith. 1975. Relation between mesophyll surface area, photosynthetic rate, and illumination during development for leaves of *Plectranthus parviflorus* Henckel. Plant Physiol. 55:1067–1070.

Pearcy, R. W., and J. Ehleringer. 1984. Comparative ecophysiology of C_3 and C_4 plants. Plant Cell Env. 7:1–13.

Pearcy, R. W., K. Osteryoung, and D. Randall. 1982. Carbon dioxide exchange characteristics of C_4 Hawaiian *Euphorbia* species native to diverse habitats. Oecologia 55:333–341.

Penning de Vries, F. W. T., A. H. M. Brunsting, and H. H. Van Laar. 1974. Products, requirements and efficiency of biosynthesis: a quantitative approach. J. Theor. Biol. 45:339–377.

Perchorowicz, J. T., and R. G. Jensen. 1983. Photosynthesis and activation of ribulose bisphosphate carboxylase in wheat seedlings. Plant Physiol. 71:955–960.

Perchorowicz, J. T., D. A. Raynes, and R. G. Jensen. 1981. Light limitation of photosynthesis and activation of ribulose bisphosphate carboxylase in wheat seedlings. Proc. Natl. Acad. Sci. USA 78:2985–2989.

Randall, D. D., C. J. Nelson, and K. H. Asay. 1977. Ribulose bisphosphate carboxylase. Altered genetic expression in tall fescue. Plant Physiol. 59:38–41.

Raven, J. A., and S. M. Glidewell. 1981. Processes limiting photosynthetic conductance. Pp. 109–136 *in* C. B. Johnson (ed.), Physiological processes limiting plant productivity. Butterworth, London.

Ritchie, J. T. 1980. Plant stress research and crop production: the challenge ahead. Pp. 21–29 *in* N. C. Turner and P. J. Kramer (eds.), Adaptation of plants to water and high temperature stress. Wiley-Interscience, New York.

Rundel, P. W. 1982. Nitrogen utilization efficiencies in Mediterranean-climate shrubs of California and Chile. Oecologia 55:409–413.

Schmidt, G. D., and M. L. Mishkind. 1983. Rapid degradation of unassembled ribulose 1,5-bisphosphate carboxylase small subunits in chloroplasts. Proc. Natl. Acad. Sci. USA 80:2632–2636.

Schmitt, M. R., and G. E. Edwards. 1981. Photosynthetic capacity and nitrogen thesis. J. Exp. Bot. 32:459–466.

Seemann, J. R., and J. A. Berry. 1982. Interspecific differences in the kinetic properties of RuBP carboxylase protein. Carnegie Inst. Washington Yearbook 81:78–83.

Seemann, J. R., J. M. Tepperman, and J. A. Berry. 1981. The relationship between photosynthetic performance and the levels and kinetic properties of RuBP carboxylase-oxygenase from desert winter annuals. Carnegie Inst. Washington Yearbook 80:67–72.

Sharkey, T. D., K. Imai, G. D. Farquhar, and I. R. Cowan. 1982. A direct confirmation of the standard method of estimating intercellular partial pressure of CO_2. Plant Physiol. 69:657–659.

Somerville, C. R., A. R. Portis, and W. L. Ogren. 1982. A mutant of *Arabidopsis thalina* which lacks activation of RuBP carboxylase in vivo. Plant Physiol. 70:381–387.

Stocking, C. R., and A. Ongun. 1962. The intracellular distribution of some metallic elements in leaves. Am. J. Bot. 49:284–289.

Terry, N. 1983. Limiting factors in photosynthesis. IV. Iron stress-mediated changes in light-harvesting and electron transport capacity and its effects on photosynthesis *in vivo*. Plant Physiol. 71:855–860.

Troughton, J. H., K. A. Card, and C. H. Hendy. 1974. Photosynthetic pathways and carbon isotope discrimination by plants. Carnegie Inst. Washington Yearbook. 73:768–780.

Vitousek, P. 1982. Nutrient cycling and nutrient use efficiency. Am. Nat. 119:553–572.

von Caemmerer, S., and G. G. Farquhar. 1981. Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. Planta 153:376–387.

Wareing, P. F., M. M. Khalifa, and K. J. Treharne. 1968. Rate-limiting processes in photosynthesis at saturating light intensities. Nature 220:453–457.

Wilson, J. R., and R. H. Brown. 1983. Nitrogen response of *Panicum* species differing in CO_2 fixation pathways. I. Growth analysis and carbohydrate accumulation. Crop Sci. 23:1148–1153.

Wittenbach, V. A. 1979. Ribulose bisphosphate carboxylase and proteolytic activity in wheat leaves from anthesis through senescence. Plant Physiol. 64:884–887.

– 1983. Effect of pod removal on leaf photosynthesis and soluble protein composition of field-grown soybeans. Plant Physiol. 73:121–124.

Wittenbach, V. A., R. C. Ackerson, R. T. Giaquinta, and R. R. Hebert. 1980. Changes in photosynthesis, ribulose bisphosphate carboxylase, proteolytic activity, and ultrastructure of soybean leaves during senescence. Crop Sci. 20:225–231.

Wong. S. C. 1979. Elevated atmospheric partial pressure of CO_2 and plant growth. I. Interactions of nitrogen nutrition and photosynthetic capacity in C_3 and C_4 plants. Oecologia 44:68–74.

Wong, S. C., I. R. Cowan, and G. D. Farquhar. 1979. Stomatal conductance correlates with photosynthetic capacity. Nature 282:424–426.

Yeoh, H.-H., M. R. Badger, and L. Watson. 1980. Variations in $K_m(CO_2)$ of ribulose-1,5-bisphosphate carboxylase among grasses. Plant Physiol. 66:1110–1112.

Yoshida, S., and V. Coronel. 1976. Nitrogen nutrition, leaf resistance, and leaf photosynthetic rate of the rice plant. Soil Sci. Plant Nutr. 22:207–211.

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