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