

*Minireview*

## **Photosynthetic acclimation in trees to rising atmospheric CO<sub>2</sub>: A broader perspective\***

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### **Abstract**

Analysis of leaf-level photosynthetic responses of 39 tree species grown in elevated concentrations of atmospheric CO<sub>2</sub> indicated an average photosynthetic enhancement of 44% when measured at the growth [CO<sub>2</sub>]. When photosynthesis was measured at a common ambient [CO<sub>2</sub>], photosynthesis of plants grown at elevated [CO<sub>2</sub>] was reduced, on average, 21% relative to ambient-grown trees, but variability was high. The evidence linking photosynthetic acclimation in trees with changes at the biochemical level is examined, along with anatomical and morphological changes in trees that impact leaf- and canopy-level photosynthetic response to CO<sub>2</sub> enrichment. Nutrient limitations and variations in sink strength appear to influence photosynthetic acclimation, but the evidence in trees for one predominant factor controlling acclimation is lacking. Regardless of the mechanisms that underlie photosynthetic acclimation, it is doubtful that this response will be complete. A new focus on adjustments to rising [CO<sub>2</sub>] at canopy, stand, and forest scales is needed to predict ecosystem response to a changing environment.

**Abbreviations:**  $A/C_i$  – photosynthesis as a function of internal [CO<sub>2</sub>];  $J_{\max}$  – maximum rate of electron transport; Rubisco – ribulose-1,5-bisphosphate carboxylase/oxygenase;  $V_{c_{\max}}$  – maximum rate of carboxylation

### **1. Introduction**

As global atmospheric CO<sub>2</sub> concentrations continue to rise throughout the next century, from a pre-industrial level of approximately 280  $\mu\text{L L}^{-1}$  to a projected 530 to 600  $\mu\text{L L}^{-1}$  by the middle of the next century (Watson et al. 1990), the responses of plants to this changing atmosphere become of considerable interest. Apart from whatever climate changes may occur in association with rising CO<sub>2</sub> concentrations, there will be direct effects of ever-increasing concentrations of

CO<sub>2</sub> on plant productivity and on carbon exchange rates between terrestrial vegetation and the atmosphere. The first step in whatever response or adjustment to rising [CO<sub>2</sub>] a plant may exhibit is at the leaf level, potentially leading to a suite of changes in physiology, growth, and morphology. For this reason, the effect of CO<sub>2</sub> enrichment on photosynthesis of individual leaves has been a focus of much of the research on vegetation responses to a changing atmosphere. There has been a particular emphasis on biochemical and physiological adjustments ('ac-

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climation') that alter the response of photosynthesis to  $\text{CO}_2$  concentration over time. This review will consider the evidence for photosynthetic acclimation in trees and discuss how changes at higher levels of organization may be just as important as adjustments in leaf biochemistry or physiology.

### *Some terminology*

What is meant by photosynthetic 'acclimation'? The term has been used in the elevated  $[\text{CO}_2]$  literature in several different ways, ranging from a general conversational usage to a much more specific physiological meaning. Webster's Dictionary defines acclimation as any physiological adjustment by an organism to environmental change, in this case, rising atmospheric  $[\text{CO}_2]$ . The more general usage is reflected in the review by Eamus and Jarvis (1989), who wrote of non-acclimated, acclimating and acclimated trees, which had been exposed to the higher  $[\text{CO}_2]$  for various lengths of time. In this usage, any biochemical or physiological changes that result from growth in elevated  $[\text{CO}_2]$  could be considered to be acclimation responses. This review will discuss changes at several levels of organization that occur in response to growth at elevated  $[\text{CO}_2]$  if they affect photosynthesis.

Somewhat more specifically, a physiological acclimation is often expected to be in a direction that improves fitness in the changed environment, as in adjustment of the temperature optimum of an enzyme in an animal raised at a higher temperature. Whether or not photosynthetic acclimation to elevated  $[\text{CO}_2]$  results in improved fitness remains to be seen. A sudden increase in atmospheric  $[\text{CO}_2]$  increases net photosynthesis because  $\text{CO}_2$  is the substrate for photosynthesis, and because higher concentrations of  $\text{CO}_2$  result in more favorable competition with  $\text{O}_2$  for ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), the primary enzyme of the photosynthetic pathway. With continued exposure to elevated  $[\text{CO}_2]$ , however, does the initial stimulation of photosynthesis decline? If so, this might be a positive adaptation, potentially resulting in optimized use and allocation of resources (e.g., nitrogen) while providing the same amount of carbon fixation in the new higher  $[\text{CO}_2]$ . This type of decrease in

photosynthetic rate has often been reported in crop and tree species (see reviews by Cure and Acock 1986, Stitt 1991, Bowes 1991, Mousseau and Saugier 1992), either as partial or occasionally as 'complete' acclimation. The acclimation is 'complete' in the sense that photosynthesis in plants grown at elevated  $[\text{CO}_2]$  has decreased to the same rate as in plants grown in current ambient  $[\text{CO}_2]$ , not necessarily in the sense that a final equilibrium has been reached. Such declines in photosynthetic rate or capacity have also been referred to as downward (or down-) regulation, photosynthetic inhibition, or homeostasis, although each of these terms may have as many unwarranted connotations as has the term acclimation. Whatever terminology is chosen, declines in photosynthesis are commonly, but not universally, reported, and apparent 'upward' regulation of photosynthesis has also been observed. It is, however, the more commonly reported downward adjustment of photosynthetic enhancement that is most often referred to as 'photosynthetic acclimation' to elevated  $[\text{CO}_2]$ , and this review will, with some reservations, follow that convention. The extent, magnitude, environmental controls and biochemical basis of this downward adjustment are central to the prediction of global response to rising  $[\text{CO}_2]$ , and these questions will be examined here in that context.

### *Why trees?*

It is particularly important to examine the possibility of photosynthetic acclimation in trees. Forests occupy a significant portion of the land surface, and forest trees contribute up to 70% of terrestrial carbon fixation (Waring and Schlesinger 1985), making them a major sink for  $\text{CO}_2$  on a global scale. The responses of trees to rising  $[\text{CO}_2]$  are thus of interest for reasons of predicting not only forest productivity, but also forest interactions and feedbacks with the atmosphere. These feedbacks include the contributions of forests to local climate and hydrology, as well as their role in the carbon cycle. If photosynthesis is stimulated by higher atmospheric  $[\text{CO}_2]$  and if sustained increased growth follows this, then the potential of forests to fix and sequester increasing amounts of atmospheric carbon becomes important in predicting future atmospheric  $\text{CO}_2$ .

concentrations. If, on the other hand, complete photosynthetic acclimation occurs, then there might be no change in productivity or carbon sequestration.

In addition to their impact in the global carbon cycle, there are some major differences between trees and annual herbaceous plants that may affect long-term photosynthetic responses to  $[\text{CO}_2]$ . Although similar in basic biochemistry and physiology to other  $\text{C}_3$  plants, the perennial nature of trees provides an opportunity for continuing physiological adjustments and feedbacks, either in direct response to  $[\text{CO}_2]$ , or as a secondary response, e.g., to changes in foliar N concentrations. Trees differ from annual crops also in the longevity of individual leaves, e.g., in conifers, and in their large and sustained carbon storage capacity and sink strength. These differences could form the basis for either biochemical or morphological adjustments occurring over longer time scales.

What scales are important in considering photosynthetic acclimation in trees? Biochemical changes in response to growth at elevated  $[\text{CO}_2]$  have been observed in trees just as they have in herbaceous plants, and this scale may be the appropriate one to elucidate the mechanisms of photosynthetic adjustments. Integration to the leaf level is necessary, however, to take into account stomatal responses, changes in leaf mass to area ratio and leaf anatomy, and changes in related physiological parameters. To predict forest-level photosynthesis, growth and productivity, or global-level atmospheric feedbacks, however, leaf-level acclimation of photosynthesis must be linked to acclimation responses at higher scales. Changes in leaf size or number, branching patterns, root-to-shoot ratios, and phenology must be considered when scaling up to the whole plant level. Moving to the forest stand level requires additional considerations, such as the effects of  $[\text{CO}_2]$  on photosynthesis and growth after canopy closure, when most of the canopy is in partial shade. Will growth of a tree or a stand be simply accelerated, or will standing biomass be increased? Effects of elevated  $[\text{CO}_2]$  on decomposition and nutrient cycling through the system may also be important at the stand level. Most of these larger scale questions are outside the scope of this review, but they remain relevant to the issues driving the study of photo-

synthetic acclimation in trees. Again, because global issues drive the interest in tree responses, the most relevant time scales are years or decades, rather than photosynthetic responses or acclimation over a period of hours or days. There are major technical limitations in working with large trees over long time spans, and because of that much of the work on photosynthetic acclimation has been done on seedlings, with studies only recently progressing to longer term field studies on older trees, or using mature tissues. Likewise, the long-term adjustments of photosynthesis are influenced by variables including nutritional status and sink strength, which have been studied in both the growth chamber and the field, and we will address the influences of these factors on the acclimation response.

## 2. Evidence for photosynthetic acclimation in trees – techniques and observations

Short-term increases in the  $\text{CO}_2$  concentration surrounding a healthy  $\text{C}_3$  plant can be expected to stimulate photosynthetic rates. Although the responses most relevant to the global carbon cycle are those of trees exposed to a gradually increasing mean  $[\text{CO}_2]$ , the only realistic approach consists of abruptly increasing the  $\text{CO}_2$  concentration surrounding the plant, or growing the plant from seed in elevated  $[\text{CO}_2]$ . Photosynthetic acclimation is then evaluated by comparing the photosynthetic rates of the plant growing in the elevated  $[\text{CO}_2]$  with rates of a plant remaining at current ambient  $[\text{CO}_2]$ . This technique, and variations thereof, has generated a great deal of information on the photosynthetic response of trees to elevated  $[\text{CO}_2]$  in the last 25 years. Drawing upon this information we begin to assess the potential for photosynthetic acclimation in response to atmospheric  $\text{CO}_2$  enrichment.

### *Gas-exchange techniques for assessing photosynthetic acclimation*

Probably the most convenient technique for assessing the acclimation response of plants grown at elevated  $[\text{CO}_2]$  is to compare rates of net photosynthesis for leaves measured at their

respective growth  $[\text{CO}_2]$ . While this comparison may be the relevant one for predicting assimilation rates in rising  $[\text{CO}_2]$ , application of this approach requires caution, in that only broad generalizations about photosynthetic acclimation can be made. Because photosynthesis more often than not increases with atmospheric  $\text{CO}_2$  enrichment, in many instances this approach yields little information on acclimation *per se* if rates remain higher in elevated  $[\text{CO}_2]$ . On the other hand, if photosynthesis at elevated  $[\text{CO}_2]$  is equal to or lower than that of ambient-grown plants then information from this technique can be of greater value, enabling one to infer complete photosynthetic acclimation. Such a response suggests that plants grown at elevated  $\text{CO}_2$  have, for whatever reason, either lost or reduced their capacity to use additional  $\text{CO}_2$  in support of photosynthetic processes. When using this technique it is important to realize that no mechanistic interpretation for the acclimation response can be made.

Another technique used in assessing photosynthetic acclimation is to measure rates of net photosynthesis after plants grown at ambient and elevated  $[\text{CO}_2]$  have been transferred to a similar ambient  $[\text{CO}_2]$  atmosphere. Switching experiments such as these are based on the premise that if growth at elevated  $[\text{CO}_2]$  has altered the photosynthetic capacity of a plant to acquire carbon, then this should be most evident when the confounding effects of differing  $\text{CO}_2$  concentrations between treatments have been removed. If rates of net photosynthesis are comparable between leaves grown at elevated and ambient  $[\text{CO}_2]$  then an acclimation response can generally be dismissed. Should differences between treatments be observed, with photosynthetic rates for plants grown at elevated  $[\text{CO}_2]$  being either greater or lower than those of the ambient-grown controls, then upward or, more likely, downward regulation can be inferred. This technique offers convenience (and therefore the possibility of a large sample size) coupled with the fact that results are easily interpretable. However, as with measurements made at the growth  $[\text{CO}_2]$ , no mechanistic interpretation for the acclimation response is possible.

Perhaps the most often used technique for assessing photosynthetic acclimation and for

identifying the mechanisms which contribute to this response is that of constructing  $A/C_i$  curves (see Harley et al. 1992). Using this approach, relationships are established between photosynthesis ( $A$ ) and intercellular  $[\text{CO}_2]$  ( $C_i$ ). These curves can be analyzed based on well-tested equations which describe how photosynthesis is regulated by i) the amount, activity, and kinetic properties of Rubisco, ii) the rate of ribulose-1,5-bisphosphate regeneration, and iii) the rate of triose phosphate utilization (Farquhar et al. 1980, Sharkey 1985, Harley and Sharkey 1991). Developing  $A/C_i$  curves for plants grown at elevated and ambient  $[\text{CO}_2]$  is not easily accomplished, however, nor is their interpretation, which requires the recognition that metabolic changes associated with acclimation are reflected not only in rates of photosynthesis, but also in the shape of the  $A/C_i$  curve. As internal  $[\text{CO}_2]$  rises, for example, the rate of photosynthesis increases in a near-linear manner, followed by a curvilinear region, and then a plateau where net photosynthesis is either insensitive or inversely-sensitive to further increases in  $\text{CO}_2$  (Stitt 1991). In the initial linear region, photosynthetic rates are limited by the amount, activity, and kinetic properties of Rubisco, while in the curvilinear and plateau regions rates of photosynthesis are limited by light harvesting, electron transport to NADPH, synthesis of ATP, Calvin cycle capacity, and by limits imposed through end-product synthesis. This latter limitation occurs because phosphorylated intermediates produced in photosynthesis are not converted rapidly enough into non-phosphorylated compounds, and as a result pools of inorganic phosphorus in the cytosol and chloroplast become depleted. Photosynthesis eventually decreases in response to limits imposed by end-product synthesis because inorganic phosphorus is required in the chloroplast for ATP synthesis (see Stitt 1991). Clearly, being able to relate the results of controlled  $\text{CO}_2$ -exposure studies to our understanding of photosynthetic metabolism and how it is regulated is a strength of this technique.

#### *Experimental evidence for photosynthetic acclimation in trees*

All three gas-exchange techniques described above have been used to investigate the occur-

rence of photosynthetic acclimation in trees exposed to atmospheric  $\text{CO}_2$  enrichment, and the mechanisms responsible. In this section, we have compiled several data bases, each of which contains information on the use of a given gas-exchange technique to study photosynthetic acclimation in trees. In compiling each data base, we took certain liberties that may or may not reflect the true intent of the original analysis. For example, Fig. 1 contains all observations that relate to photosynthesis at ambient and elevated  $[\text{CO}_2]$ , regardless of length of exposure, pot size, nutritional status, method of  $\text{CO}_2$  exposure, or stage of plant development, and including all elevated growth  $\text{CO}_2$  concentrations. If we were able to extract observations from a published table, figure, histogram, or personal communication, it was included in Fig. 1. Tables 1 and 2 pool this information further by presenting simply a species-specific mean for each study regardless of what treatments might have been imposed on the plants or when rates of photosynthesis were measured. Furthermore, the ratios pre-

sented do not indicate whether the photosynthetic differences in the original reference were statistically significant. While this type of analysis tends to ignore subtle differences between studies, it does offer the hope that in so doing, the 'big picture' might become clearer and, as a result, that generalities might appear that would otherwise remain obscured. Readers are urged to consult the original reference for details and for the authors' interpretation of their results.

Controlled-exposure studies that report photosynthetic rates of leaves grown and measured at their respective growth  $[\text{CO}_2]$  more often than not indicate an increase in photosynthesis with rising  $[\text{CO}_2]$  (Fig. 1A). Based on our analysis, this  $\text{CO}_2$ -induced stimulation averaged 44% across all observations. Although such an increase is expected, it was surprising to note that these observations ranged from instances where photosynthetic rates for plants grown at elevated  $[\text{CO}_2]$  were 40% less than those of their ambient-grown counterparts, to those where rates at elevated  $[\text{CO}_2]$  were three-fold greater than the ambient-grown controls. Of the 39 species represented in this analysis, 9 of them exhibited almost complete acclimation to  $\text{CO}_2$  exposure, with rates of photosynthesis for plants grown and measured at elevated  $[\text{CO}_2]$  being similar to or lower than those of the ambient-grown seedlings (Table 1). Examples of this acclimation response include *Cecropia obtusifolia* (Reekie and Bazzaz 1989), *Cedrus atlantica* (Kaushal et al. 1989), *Ochroma lagopus* and *Pentaclethra macroloba* (Oberbauer et al. 1985), *Pinus ponderosa* (Grulke et al. 1993), *Quercus robur* (Bunce 1992), and *Trichospermum mexicanum* (Reekie and Bazzaz 1989). In contrast, other studies indicated a marked capacity of photosynthesis to increase with atmospheric  $\text{CO}_2$  enrichment, including the species *Citrus aurantium* (Idso et al. 1991), *Alnus rubra* (Arnone and Gordon 1990), *Quercus prinus* (Bunce 1992), and *Salix × dasyclados* (Silvola and Ahlholm 1992), all of which exhibited photosynthetic rates over twice those of the ambient-grown controls (Table 1).

A smaller number of studies have assessed photosynthetic acclimation by measuring rates of net photosynthesis after plants grown at ambient and elevated  $[\text{CO}_2]$  were transferred to a common ambient  $[\text{CO}_2]$ . Application of this tech-

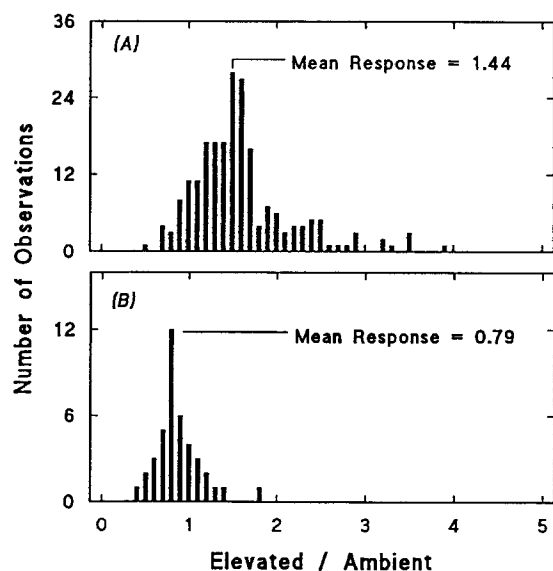


Fig. 1. Frequency distribution for the relative photosynthetic response of trees grown at elevated  $[\text{CO}_2]$  compared to those grown at ambient  $[\text{CO}_2]$ . Number of observations at each ratio interval for (A) trees measured at their respective growth  $\text{CO}_2$  concentration (compare to Table 1) (mean = 1.44) and (B) after being switched to an ambient  $[\text{CO}_2]$  (compare to Table 2) (mean = 0.79). Ratios of elevated/ambient were transformed to common logarithms to calculate the mean.

Table 1. Single-leaf photosynthetic rates for woody perennials grown at an elevated CO<sub>2</sub> concentration, relative to rates of their ambient-grown counterparts. Photosynthesis rates were measured at the growth CO<sub>2</sub> concentration, as listed

Species	Growth CO <sub>2</sub> concentrations (μL L <sup>-1</sup> )	Relative photosynthetic enhancement (elevated/ambient)	Reference
<i>Abies fraseri</i>	374, 713	1.54	Samuelson and Seiler (1992)
<i>Acacia mangium</i>	350, 713	1.35	Ziska et al. (1991)
<i>Acer saccharum</i>	400, 800	1.90	Nobel et al. (1992)
<i>Alnus rubra</i>	350, 650	2.42	Arnone and Gordon (1990)
<i>Betula pendula</i>	350, 700	1.28	Pettersson and McDonald (1992)
<i>Castanea sativa</i>	350, 700	1.30	Mousseau (1993)
<i>Castanea sativa</i>	350, 700	1.87	El Kohen et al. (in press)
<i>Cecropia obtusifolia</i>	350, 525	1.11	Reekie and Bazzaz (1989)
"	350, 700	0.59	"
<i>Cedrus atlantica</i>	350, 800	0.97	Kaushal et al. (1989)
<i>Citrus aurantium</i>	350, 680	2.22	Idso et al. (1991)
<i>Fagus sylvatica</i>	350, 700	2.12	Bestebroer (personal communication)
<i>Fagus sylvatica</i>	350, 700	1.55	El Kohen et al. (in press)
<i>Liquidambar styraciflua</i>	350, 675	1.44	Tolley and Strain (1985)
"	350, 500	0.92	Fetcher et al. (1988)
"	340, 520	1.16	Rogers et al. (1983)
"	340, 718	1.26	Rogers et al. (1983)
"	340, 910	1.72	Rogers et al. (1983)
<i>Liriodendron tulipifera</i>	371, 493	1.21	Norby and O'Neill (1991)
"	371, 787	1.59	"
"	355, 503	1.46	Norby et al. (1992)
"	355, 656	1.66	"
"	376, 540	1.58	Wullschleger, et al. (1992)
"	376, 676	1.63	"
"	354, 503	1.39	Gunderson et al. (1993)
"	354, 656	1.54	"
<i>Malus domestica</i>	350, 700	1.60	Bunce (1992)
<i>Myriocarpa longipes</i>	350, 525	1.18	Reekie and Bazzaz (1989)
"	350, 700	1.06	"
<i>Nothofagus fusca</i>	340, 640	1.45	Hollinger (1987)
<i>Ochroma lagopus</i>	350, 675	0.67	Oberbauer, et al. (1985)
<i>Pentaclethra macroloba</i>	350, 675	0.87	Oberbauer, et al. (1985)
<i>Picea abies</i>	350, 700	1.56	Ceulemans (personal communication)
<i>Picea rubens</i>	374, 713	2.52	Samuelson and Seiler (1994)
<i>Pinus contorta</i>	350, 1000	1.60	Higginbotham et al. (1985)
<i>Pinus contorta</i>	350, 2000	1.40	Higginbotham et al. (1985)
<i>Pinus nigra</i>	350, 800	1.30	Kaushal et al. (1989)
<i>Pinus pinaster</i>	350, 700	1.26	Picon (1992)
<i>Pinus ponderosa</i>	350, 700	1.18	Grulke et al. (1993)
<i>Pinus radiata</i>	340, 660	1.37	Conroy et al. (1990b)
<i>Pinus radiata</i>	340, 660	1.86	Conroy et al. (1990a)
"	330, 660	1.89	Conroy et al. (1988)
"	340, 640	1.55	Hollinger (1987)
"	330, 660	1.65	Conroy et al. (1986)
<i>Pinus taeda</i>	350, 675	0.94	Tolley and Strain (1985)
"	350, 500	1.13	Fetcher et al. (1988)
<i>Pinus taeda</i>	350, 650	1.46	Tissue et al. (1993)
<i>Piper auritum</i>	350, 525	1.56	Reekie and Bazzaz (1989)
"	350, 700	1.62	"
<i>Populus grandidentata</i>	361, 707	1.65	Curtis and Teeri (1992)
<i>Populus euramericana</i>	350, 700	2.16	Ceulemans (personal communication)
<i>Pseudotsuga menziesii</i>	340, 640	1.32	Hollinger (1987)
<i>Quercus alba</i>	389, 496	0.98	Norby and O'Neill (1989)
"	389, 793	1.58	"

Table 1 (Continued)

Species	Growth CO <sub>2</sub> concentrations (μL L <sup>-1</sup> )	Relative photosynthetic enhancement (elevated/ambient)	Reference
<i>Quercus alba</i> (cont.)	376, 540	1.39	Wullschlegel et al. (1992)
"	376, 676	1.51	"
"	354, 503	1.37	Gunderson et al. (1993)
"	354, 656	1.79	"
<i>Quercus ilex</i>	360, 710	1.82	De Angelis et al. (1993)
<i>Quercus petraea</i>	350, 700	1.41	Picon (1992)
<i>Quercus prinus</i>	350, 700	2.21	Bunce (1992)
<i>Quercus robur</i>	350, 700	1.08	Bunce (1992)
<i>Salix x dasyclados</i>	300, 500	1.58	Silvola and Ahlholm (1992)
"	300, 700	2.41	"
<i>Senna multijuga</i>	350, 525	1.59	Reekie and Bazzaz (1989)
"	350, 700	1.26	"
<i>Tabebuia rosea</i>	350, 713	1.64	Ziska et al (1991)
<i>Trichospermum mexicanum</i>	350, 525	1.43	Reekie and Bazzaz (1989)
"	350, 700	0.81	"

Table 2. Single-leaf photosynthetic rates for woody perennials grown at an elevated CO<sub>2</sub> concentration relative to rates of their ambient-grown counterparts, with the exception that rates were measured at a common ambient CO<sub>2</sub> concentration. Growth CO<sub>2</sub> concentrations and the pot size used in the original study are included for the readers convenience (na = not available)

Species	Growth CO <sub>2</sub> concentrations (μL L <sup>-1</sup> )	Pot size (cm <sup>3</sup> )	Photosynthesis at ambient [CO <sub>2</sub> ] (elevated/ambient)	Reference
<i>Abies fraseri</i>	374, 713	175	0.48	Samuelson and Seiler (1992)
<i>Acacia mangium</i>	350, 713	12 500	0.84	Ziska et al. (1991)
<i>Acer saccharinum</i>	350, 700	250	0.80	Bunce (1992)
<i>Cedrus atlantica</i>	350, 800	6000	0.64	Kaushal et al. (1989)
<i>Fagus sylvatica</i>	350, 700	na	1.22	Bestebroer (personal communication)
<i>Liquidambar styraciflua</i>	350, 500	3600	0.73	Fetcher et al. (1988)
<i>Nothofagus fusca</i>	340, 640	4000	0.75	Hollinger (1987)
<i>Ochroma lagopus</i>	350, 675	4580	0.75	Oberbauer et al. (1985)
<i>Pentaclethra macroloba</i>	350, 675	1045	0.69	Oberbauer et al. (1985)
<i>Picea rubens</i>	374, 713	175	0.95	Samuelson and Seiler (1994)
<i>Pinus nigra</i>	350, 800	6000	0.80	Kaushal et al. (1989)
<i>Pinus ponderosa</i>	350, 700	314	0.67	Grulke et al. (1993)
<i>Pinus radiata</i>	340, 640	4000	0.77	Hollinger (1987)
"	330, 660	730	1.28	Conroy et al. (1988)
<i>Pinus taeda</i>	350, 500	3600	0.78	Fetcher et al. (1988)
<i>Populus grandidentata</i>	361, 707	2400	0.98	Curtis and Teeri (1992)
<i>Pseudotsuga menziesii</i>	340, 640	4000	0.94	Hollinger (1987)
<i>Tabebuia rosea</i>	350, 713	12 500	1.17	Ziska et al. (1991)
<i>Salix x dasyclados</i>	300, 500	3535	1.15	Silvola and Ahlholm (1992)
"	300, 700	3535	0.94	"

nique suggests that rates of photosynthesis for leaves previously exposed to elevated [CO<sub>2</sub>] are, in general, slightly lower than those from ambient [CO<sub>2</sub>] treatments (Fig. 1B). Averaged across all observations, there appears to be a

21% reduction or down-regulation in photosynthetic potential for plants grown at elevated CO<sub>2</sub> concentrations compared to the ambient-grown controls. There is, however, tremendous variation in this response, from considerable

down-regulation for the species *Abies fraseri* (Samuelson and Seiler 1992) to an apparent up-regulation of photosynthesis as reported by Conroy et al. (1988) for *Pinus radiata* (Table 2). The length of CO<sub>2</sub> exposure and pot size are included in Table 2, and these factors will be discussed later.

More mechanistic studies on the photosynthetic response of trees grown at elevated

[CO<sub>2</sub>] confirm the occurrence of acclimation, with down-regulation being most commonly observed. Having compiled a series of A/C<sub>i</sub> curves for woody perennials grown either at ambient or elevated [CO<sub>2</sub>], we calculated our own estimates for the maximum rate of carboxylation, V<sub>c<sub>max</sub></sub>, and the maximum rate of electron transport, J<sub>max</sub> (c.f. Harley et al. 1992) from those curves (Table 3). More often than not, V<sub>c<sub>max</sub></sub> was somewhat

Table 3. Estimates for the maximum rate of carboxylation, V<sub>c<sub>max</sub></sub>, and the maximum rate of electron transport, J<sub>max</sub>, as calculated from the A/C<sub>i</sub> curves of woody perennials grown at ambient and elevated CO<sub>2</sub> concentrations. Curves were analyzed according to the biochemical model of Farquhar et al. (1980). Growth CO<sub>2</sub> concentrations and exposure durations used in the original study are also included for the readers convenience (na = not available)

Species	Growth CO <sub>2</sub> concentration (μL L <sup>-1</sup> )	Exposure duration (days)	V <sub>c<sub>max</sub></sub> (μmol m <sup>-2</sup> s <sup>-1</sup> )	Ratio	J <sub>max</sub> (μmol m <sup>-2</sup> s <sup>-1</sup> )	Ratio	Reference
<i>Acacia mangium</i>	350	60–75	60		106		Ziska et al. (1991)
"	700	"	46	0.77	108	1.02	"
<i>Tabebuia rosea</i>	350	60–75	39		67		Ziska et al. (1991)
"	700	"	44	1.13	94	1.40	"
<i>Populus grandidentata</i>	361	45	28		100		Curtis and Teeri (1992)
"	707	"	24	0.86	94	0.94	"
"	361	68	24		70		Curtis and Teeri (1992)
"	707	"	30	1.25	96	1.37	"
<i>Pinus taeda</i>	350	na	18		38		R. Thomas et al. (1994)
"	650	"	16	0.89	36	0.95	"
<i>Pinus taeda</i>	350	"	15		32		R. Thomas et al. (1994)
"	650	"	9	0.60	20	0.62	"
<i>Pinus taeda</i>	350	"	12		25		R. Thomas et al. (1994)
"	650	"	9	0.75	20	0.80	"
<i>Populus euramericana</i>	330	15	53		90		Gaudillère and Mousseau (1989)
"	660	"	45	0.85	80	0.89	"
<i>Liquidambar styraciflua</i>	350	455	61		137		Fetcher et al. (1988)
"	500	"	49	0.80	111	0.81	"
<i>Pinus taeda</i>	350	455	8		21		Fetcher et al. (1988)
"	500	"	7	0.88	20	0.95	"
<i>Picea sitchensis</i>	Ambient	na	23		75		cf. Jarvis (1989)
"	2X	na	21	0.91	70	0.93	"
<i>Betula pendula</i>	350	70	29		91		Pettersson and McDonald (1992)
"	700	"	29	1.00	89	0.98	"
<i>Castanea sativa</i>	350	May 23	16		33		Godard (1989)
"	700	"	28	1.75	47	1.42	"
<i>Castanea sativa</i>	350	June 13	22		43		Godard (1989)
"	700	"	12	0.55	34	0.79	"
<i>Castanea sativa</i>	350	July 17	26		64		Godard (1989)
"	700	"	9	0.35	28	0.44	"
<i>Castanea sativa</i>	350	August 17	15		36		Godard (1989)
"	700	"	8	0.53	25	0.69	"
<i>Liriodendron tulipifera</i>	354	(2 years)	68		128		Gunderson et al. (1993)
"	656	"	61	0.90	126	0.98	"
<i>Quercus alba</i>	354	(2 years)	51		125		Gunderson et al. (1993)
"	656	"	55	1.08	133	1.06	"



lower for plants grown at elevated  $[\text{CO}_2]$  regardless of species, indicating a decrease in either the amount, activity, or kinetic properties of Rubisco. There were also indications among species for up-regulation, as was observed for *Tabebuia rosea* (Ziska et al. 1991) and most notably for *Castanea sativa* early in the growing season (Godard 1989). Changes in  $J_{\text{max}}$  tended to mirror those associated with changes in  $V_{\text{c}_{\text{max}}}$  (Table 3). Other studies report unchanged  $A/C_i$  curves, for example, the  $A/C_i$  curves generated from 3 to 6 saplings of *Liriodendron tulipifera* and *Quercus alba* at each  $[\text{CO}_2]$  were not significantly different at the end of the second season in the field (Gunderson et al. 1993). Other researchers have reported either no change in the  $A/C_i$  curve of trees grown under  $\text{CO}_2$  enrichment (Kull et al. 1993, Teskey 1993) or up-regulation (early in the season) and down-regulation (late in the season) of  $A/C_i$  curves (Ceulemans et al. 1993), but these studies were presented as abstracts and were not available for digitizing and analysis for Table 3.

Based on our analyses, there appears to be sufficient evidence of some degree of photosynthetic acclimation, and in woody perennials, down-regulation in particular. This observation echoes that of Bowes (1991) who argued that although acclimation responses are not reported in all atmospheric  $\text{CO}_2$  enrichment studies, it seems likely that some sort of acclimation is the rule. It is clear, however, that while acclimation does occur in many cases, our ability to judge when it will occur and why it occurs is far from a precise science. The argument has been made that photosynthetic acclimation occurs in response to a reallocation of resources, particularly nitrogen, away from photosynthesis towards some other undefined process or processes, thereby reflecting an optimal use of resources. Potentially more important, from an experimental perspective, is the explanation that sink/source relationships are somehow involved in the acclimation response. We now turn our attention to questions surrounding these issues and to an examination of whether photosynthetic studies using trees can be used to gain additional insights into why acclimation occurs when plants are exposed to elevated  $[\text{CO}_2]$ .

### 3. Possible mechanisms of photosynthetic acclimation

#### *Biochemical considerations in the acclimation response*

Biochemical explanations for why plants may or may not exhibit photosynthetic acclimation when grown at elevated  $\text{CO}_2$  are many, although the majority of these are based either on speculation or derived from observations on herbaceous annuals (cf. Bowes 1991, Stitt 1991). One explanation that is routinely invoked to explain the acclimation response focuses on Rubisco as the prime carboxylation enzyme in the photosynthetic process. As was *inferred* from our earlier analysis of  $A/C_i$  curves, trees grown at elevated  $\text{CO}_2$  often exhibit a decrease in  $V_{\text{c}_{\text{max}}}$  (Table 3), a result that implies a decrease in the amount, activity, or kinetic properties of Rubisco. Can this observation be substantiated, however, for long-lived woody perennials exposed to atmospheric  $\text{CO}_2$  enrichment and subjected to a more thorough biochemical analysis?

Only recently have studies been initiated with trees to examine the acclimation response at a biochemical level of resolution. Spruce (*Picea abies*) needles collected from 4-year-old trees exposed to enriched  $\text{CO}_2$  atmospheres for two seasons showed a 20 to 34% decline in total soluble protein and a 27 to 40% decrease in Rubisco activity (Van Oosten et al. 1992). Total Rubisco content and activity in loblolly pine (*Pinus taeda*) seedlings decreased similarly with atmospheric  $\text{CO}_2$  enrichment, and there was also an increase in activation state of the enzyme (Tissue et al. 1993). An increase in the activation state of Rubisco suggests that plants exposed to  $\text{CO}_2$  enriched atmospheres may compensate for lower Rubisco activity without sacrificing photosynthetic performance. Tissue et al. (1993) also observed that plants grown at elevated  $\text{CO}_2$  had less nitrogen invested in Rubisco and more in the light-harvesting and electron transport components (i.e., chlorophyll), a response that suggests an allocation of resources away from Rubisco and towards other limiting processes of photosynthesis. This observation is not, however, supported by Wilkens et al. (1993) who reported

that a decline in Rubisco protein levels for wild cherry (*Prunus avium*) grown at twice-ambient  $[\text{CO}_2]$  was accompanied by a similar reduction in chlorophyll content and in levels of several major thylakoid membrane components. Therefore, components involved in light harvesting and electron transport declined with elevated  $[\text{CO}_2]$  as did the potential for carboxylation. Contrary to the observation that Rubisco declines with rising  $\text{CO}_2$  concentrations, Koch et al. (1986) reported an increase in Rubisco activity for citrus seedlings.

Biochemical studies more or less support the belief that changes in Rubisco content or activity may be involved in the acclimation response, but it has not been resolved how closely changes in photosynthetic rates are linked to changes in Rubisco. By this we mean, to what extent should decreases in Rubisco content/activity be reflected in decreased photosynthesis? For example, Quick et al. (1991) noted that the strength of Rubisco control of photosynthesis in tobacco was altered by changes in environmental conditions and nitrogen supply during growth, and that in some cases a 49% reduction in Rubisco activity resulted in only a 14% reduction in photosynthesis. An important question in this regard, therefore, is how much down-regulation can occur at the level of Rubisco before an effect is observed at the level of the photosynthetic process? To our knowledge, there have been no attempts to correlate changes in Rubisco quantity or activity with evidence for photosynthetic acclimation in trees. This causal link must be established before a clear picture can emerge on the acclimation response. It should also be remembered that in dealing with a long-lived woody perennial, other long-term acclimation responses, particularly those operating at the leaf or canopy level, must be considered.

#### *Changes at the whole leaf and canopy level*

Many factors, beyond biochemistry, influence leaf-level photosynthetic rates, and if these factors change as a plant adjusts to elevated  $[\text{CO}_2]$  then they may contribute to photosynthetic acclimation and to total carbon assimilation by the tree. Some of the leaf-level changes that have been observed in response to growth at elevated

$[\text{CO}_2]$  that might impact  $\text{CO}_2$  assimilation include changes in leaf size, number, and anatomy, and stomatal features, both physical and functional. Observed changes in leaf longevity and canopy architecture could likewise impact whole canopy assimilation over the season. Observations on these changes will be discussed briefly here insofar as they may impact photosynthesis.

#### *Stomatal changes – functional and physical*

Decreases in stomatal conductance are often, though not always, reported for plants grown at elevated  $[\text{CO}_2]$ , with decreases of 10 to 60% not uncommon. In other cases, however, decreases are either small or not statistically significant (see review in Eamus and Jarvis 1989). The reasons for the variability in stomatal response may relate to whole-plant adjustments in water relations, environmental conditions, measurement techniques, time of day (e.g., Surano et al. 1986), and/or species differences. The contribution of the latter factor is supported by the work of Radoglou and Jarvis (1990b) wherein four hybrid poplar clones differed markedly in the magnitude of their stomatal response to growth at elevated  $[\text{CO}_2]$ , all other factors being comparable.

Although a decrease in conductance could theoretically contribute to a decrease in photosynthetic  $\text{CO}_2$  assimilation, the stomatal limitation of photosynthesis is usually small (Farquhar and Sharkey 1982), and does not necessarily increase upon growth in elevated  $\text{CO}_2$ . In most cases any decrease in internal  $[\text{CO}_2]$  caused by stomatal closure is not sufficient to account for the reported decreases in leaf-level photosynthesis (Oberbauer et al. 1985, Samuelson and Seiler 1992).

In addition to whatever changes in stomatal aperture occur to influence stomatal conductance and thereby photosynthesis, physical changes in leaf anatomy that impact gas exchange have also been reported. The impact of growth at elevated  $[\text{CO}_2]$  on stomatal frequency (assessed as density and/or index), however, is not certain. Historical analyses of herbarium and fossil evidence suggest a decrease in stomatal frequency in trees with increasing atmospheric  $[\text{CO}_2]$  (Woodward 1987, Paoletti and Gellini 1993, Van der Burgh et al.

1993). Manipulative experiments with trees have given more conflicting results. Stomatal density has increased (Gaudillère and Mousseau 1989), decreased (Oberbauer et al. 1985, Paoletti et al. 1993), or not changed (Thomas and Harvey 1983, Oberbauer et al. 1985, Mousseau and Enoch 1989, Radoglou and Jarvis 1990b) in leaves developed in elevated  $[\text{CO}_2]$ . In the case of *Quercus ilex*, Paoletti et al. (1993) inferred that the effect on stomatal conductance of the decrease in stomatal density was less than the effect of decreased aperture, based on the observation that the decline in conductance was greater in older leaves developed before the  $\text{CO}_2$  exposure began (and therefore with unchanged stomatal density).

#### *Changes in leaf internal anatomy*

The most commonly reported anatomical change in response to growth in elevated  $[\text{CO}_2]$  is an increase in leaf thickness (Thomas and Harvey 1983, Conroy et al. 1986, Mousseau and Enoch 1989, Radoglou and Jarvis 1990a), usually correlated with an increase in the dry mass to area ratio (although an increase in this ratio may also reflect accumulation of non-structural carbohydrates). The relationship of leaf thickness to photosynthetic acclimation depends on the nature of the change. If an extra layer of palisade cells develops (Mousseau and Enoch 1989), or if the overall thickness of the mesophyll layer increases, e.g., with larger cells and larger air spaces (Thomas and Harvey 1983, Conroy et al. 1986, Radoglou and Jarvis 1990a), then potential photosynthesis per unit leaf area might increase as internal surface area for  $\text{CO}_2$  absorption increases (Radoglou and Jarvis 1990a), unrelated to any change in leaf biochemistry. For anatomical changes to contribute to a downward trend in photosynthesis, however, they would have to be in the opposite direction from those reported. The changes that do occur might be interpreted as compensating for (or as partially obscuring) any biochemical down-regulation, or simply as increased growth in response to luxury carbon gain. This could also explain the increase in area per leaf reported in newly expanding leaves of *Populus euramericana* (Gaudillère and Mousseau 1989), nodulated *Alnus rubra* (Ar-

none and Gordon 1990) and two citrus rootstock hybrids (Koch et al. 1986), but not reported in most studies.

#### *Changes in the canopy*

At the canopy level, reported increases in total plant leaf area may be a consequence of accelerated ontogeny of the plant rather than a specific response to  $\text{CO}_2$  (Tolley and Strain 1984, Conroy et al. 1986, Radoglou and Jarvis 1990a, Berryman et al. 1993). This accrual of leaf number earlier in the season (or in an earlier season), without a downward acclimation at the leaf level, would increase net carbon assimilation for the plant, at least until maximum leaf area is attained. Canopy leaf area, however, may not change or may decrease with an increase in  $[\text{CO}_2]$ , as in *Castanea sativa* (Mousseau and Enoch 1989) and *Liriodendron tulipifera*, in both growth chamber and field experiments (Norby and O'Neill 1991, Norby et al. 1992). It is important here to emphasize the distinction between a more rapid completion of ontogeny and a  $\text{CO}_2$ -specific effect on the leaf area associated with a given amount of plant tissue. Norby et al. (1992) evaluated this as a change in leaf area ratio (leaf area divided by whole plant mass), and found that this parameter declined significantly and linearly with increasing  $[\text{CO}_2]$  in *Liriodendron tulipifera*. Leaf area ratio also decreased in a tropical Australian tree, *Maranthos corymbosa*, grown in elevated  $[\text{CO}_2]$  (Berryman et al. 1993). This decrease in leaf area ratio might be interpreted as a canopy-level adjustment in carbon assimilation *in lieu of* a photosynthetic acclimation at the biochemical level.

Changes in branching patterns, such as have sometimes been reported in crops (reviewed in Allen 1990, Lawlor and Mitchell 1991) are not easily studied in trees because of their relatively slower growth rate. In general such changes have been found lacking in trees (Mousseau and Enoch 1989, Norby et al. 1992, Pettersson and McDonald 1992), although a suppression of apical dominance has sometimes been reported (Conroy et al. 1990a and references therein). As with leaf area increases, an increase in branch mass does not indicate a  $[\text{CO}_2]$ -specific structural

acclimation if the mass of other plant parts increased proportionately, but may only indicated a more rapid growth rate.

Changes in timing of bud-break or senescence could influence canopy development and whole-season canopy assimilation, but experimental data on these parameters in trees is limited. Mousseau and Enoch (1989) reported early senescence, indicated by premature yellowing and loss of chlorophyll, in *Castanea sativa* seedlings, but Gunderson et al. (1993) found no difference in senescence of either *Liriodendron tulipifera* or *Quercus alba* seedlings grown in the field. Murray et al. (1993) reported a reduced growing season in Sitka spruce exposed to CO<sub>2</sub> enrichment, with earlier bud-set in autumn, and later flushing in the spring. It is not clear at this point what the overall effect of these changes might be on season-long carbon assimilation.

#### 4. Factors controlling the acclimation response

Examination of the data base in section 2, compiled by pooling all the reported responses of trees, has shown that in most cases photosynthesis remains higher in plants growing in elevated [CO<sub>2</sub>]. Despite this enhancement, photosynthesis measured at a common ambient [CO<sub>2</sub>] indicates a trend toward downward acclimation. The trend is not universal, however, and the mixed results from A/C<sub>i</sub> curves suggest that an examination of the environmental or biological variables influencing the photosynthetic responses might reveal some patterns within the overall trend toward acclimation.

##### *Length of CO<sub>2</sub> exposure*

Since acclimation implies a process that occurs over time, many have suggested that for plants grown at elevated [CO<sub>2</sub>] the acclimation response should become increasingly evident as the length of CO<sub>2</sub> exposure increases. This time-dependent pattern of photosynthetic acclimation is often observed as an inability of CO<sub>2</sub>-induced increases in photosynthesis to be sustained throughout a CO<sub>2</sub> enrichment study (Godard 1989, Samuelson and Seiler 1992, Grulke et al. 1993, Mousseau 1993). Grulke et al. (1993)

reported, for example, that photosynthetic rates measured 1 and 6 days after elevated [CO<sub>2</sub>] was supplied to *Pinus ponderosa* seedlings were greater than rates of the ambient-grown controls, but that after 39 and 112 days these differences were lost. Samuelson and Seiler (1992) similarly observed an almost two-fold increase in net photosynthesis for *Abies fraseri* after 5 months of atmospheric CO<sub>2</sub> enrichment, but after 12 months these rates were similar between the two [CO<sub>2</sub>] treatments. Solitary measurements of photosynthesis for two tropical tree species indicated that plants grown at elevated [CO<sub>2</sub>] had rates 13 to 33% lower than those of the ambient-grown controls (Oberbauer et al. 1985). Observations such as these are often interpreted to mean that initial increases in photosynthesis will not be maintained during long-term exposure to elevated [CO<sub>2</sub>].

While a relationship between acclimation and the length of CO<sub>2</sub> exposure has been indicated in a few studies, far more suggest that the stimulatory effects of elevated [CO<sub>2</sub>] on photosynthesis can be sustained throughout months or years of CO<sub>2</sub> exposure (Norby and O'Neill 1989, 1991, Conroy et al. 1990a, Idso et al. 1991, Bunce 1992, Curtis and Teeri 1992, Noble et al. 1992, Pettersson and McDonald 1992, Wullschlegel et al. 1992, Gunderson et al. 1993). Notable among these are the studies of Idso et al. (1991) and Gunderson et al. (1993), both of which report sustained photosynthetic enhancement after three years of atmospheric CO<sub>2</sub> enrichment for plants growing directly in the ground. In the study of Idso et al. (1991), photosynthesis for *Citrus aurantium* more than doubled after being exposed to an approximate two-fold increase in [CO<sub>2</sub>], a response that led these authors to question whether down-regulation of photosynthesis and growth in elevated [CO<sub>2</sub>] occurs in the natural environment. Multiple observations by Gunderson et al. (1993) over a three year period also indicated that enhancement of leaf-level photosynthesis was not lost in either of the forest tree species *Liriodendron tulipifera* or *Quercus alba* as a result of CO<sub>2</sub> enrichment (Fig. 2).

There are a number of possible explanations for the conflicting results from these temporal analyses of photosynthetic acclimation. Photo-

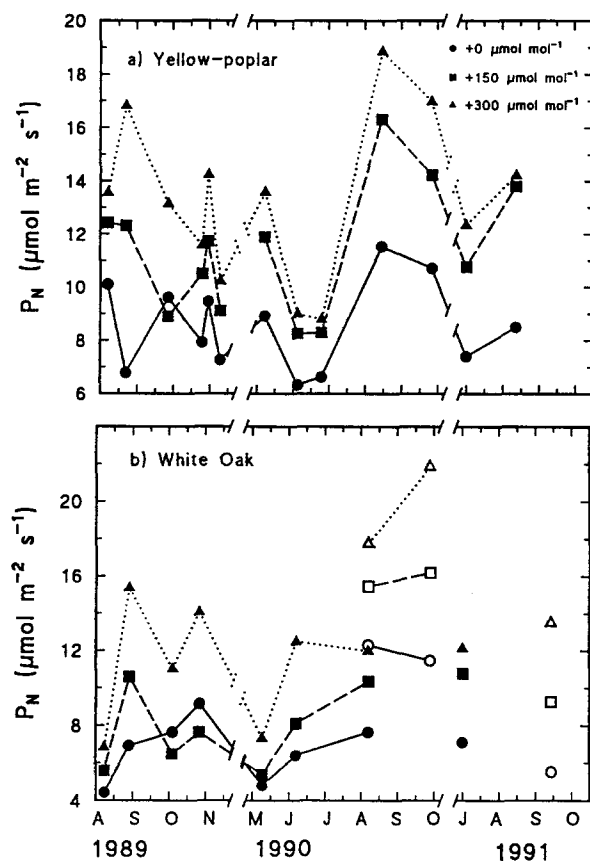


Fig. 2. Mean net photosynthesis ( $P_N$ ) of (a) *Liriodendron tulipifera* and (b) *Quercus alba* grown under three levels of  $\text{CO}_2$  enrichment for three years in the field. Symbols represent the means of periodic measurements taken with an open-flow gas exchange system with a single leaf cuvette. Open symbols in (b) denote response of second flush foliage. Figure reprinted with permission from Gunderson et al. (1993).

synthetic rates are measured only periodically during the course of an experiment. If photosynthesis responds dynamically to changing biochemical status within the leaf, then acclimation based on changes in Rubisco activity, starch concentration, nutrient limitation, etc., may be affected by seasonal shifts in sink strength coinciding with patterns of above and below ground growth. Other environmental limitations, whether those of nutrient availability, restricted rooting volume, light, or temperature, may also vary in relative importance over time, and these factors will be considered further as potential contributors to the acclimation response.

### Sink strength

Considerable evidence has accumulated from studies with herbaceous plants that photosynthetic acclimation is somehow linked to a lack of adequate sinks for the additional carbon being fixed, and feedbacks via starch accumulation and biochemical down-regulation of Rubisco are postulated (see reviews by Arp 1991, Bowes 1991, Stitt 1991). The experimental evidence presented indicates that field-grown crops do not show the same degree of downward acclimation as those raised in pots in growth chambers (see also Lawlor and Mitchell 1991), and that developmental sinks such as fruit set affect acclimation. At least one study used two pot sizes to show root restriction as a factor in photosynthetic acclimation (Thomas and Strain 1991), although it has been suggested that greater nutrient supplies in larger pots may confound the effect of pot volume (McConnaughay et al. 1993).

Again, the evidence in trees supporting the linkage between acclimation and sink strength is much more limited. The two previously mentioned long-term studies of trees planted directly in the ground did not find any evidence for photosynthetic acclimation (Idso et al. 1991, Gunderson et al. 1993), whereas in many of the potted, growth chamber studies acclimation has occurred. A single study with *Abies fraseri* grown in two pot sizes (Samuelson and Seiler 1992) showed a stronger acclimation in the smaller pots, in that photosynthesis at elevated  $[\text{CO}_2]$  was depressed in the small pots; switching them both to ambient  $[\text{CO}_2]$ , however, showed a similar degree of acclimation in the two pot sizes. Unfortunately, because of differences in experimental conditions and seedling size and age, comparisons of pot size between experiments (Table 2) yields little information to either support or refute the root restriction argument, although the most pronounced acclimation responses all occurred at the smallest pot sizes. Table 2 also highlights how few studies of acclimation in trees have been conducted in pots larger than  $10\,000\text{ cm}^3$ , so that if pot size is important, estimates of acclimation in larger seedlings or longer-term studies could have been affected by root restriction.

Since sink strength (other than pot volume) is

difficult to manipulate in trees, e.g. a tree must be several years old to fruit and set seed, and in many cases other sinks may be more important, few studies of this nature have been done in trees. Research at our field site compared photosynthetic enhancement in 3-year-old intact *Liriodendron tulipifera* planted in the ground with that in shoots resprouting from the stumps of trees the same age. In the coppicing shoots, with no restriction of their large root systems and their rapidly growing shoots presumably providing another strong sink, photosynthetic stimulation by elevated  $[\text{CO}_2]$  was no greater than that in intact plants. Measuring photosynthesis of the coppicing shoots at a common ambient  $[\text{CO}_2]$  also indicated a lack of photosynthetic acclimation (Table 4). It could be that trees, at least those growing with adequate nutrition, light and rooting volume, already have an adequate carbon sink in their woody tissues (Mousseau and Saugier 1992), but that does not explain the many instances when acclimation does occur.

#### Nutritional limitations

It has been suggested that the potential of elevated  $[\text{CO}_2]$  to increase photosynthesis will not be met in natural forest stands because nutrients and water may be limiting (Kramer 1981, Eamus and Jarvis 1989), and nutrient limitations could, in fact, be one factor controlling the extent of photosynthetic acclimation to elevated  $[\text{CO}_2]$ . In light of the frequently observed decline in foliar nitrogen concentrations (e.g., Norby et al. 1992), and the link between nitrogen and Rubisco protein, this hypothesis is fairly attractive. In very few of the studies of photosynthetic responses of trees to elevated  $[\text{CO}_2]$ , however, has this hypothesis been tested.

None of the published studies where nutrient supply has been varied have measured acclimation *per se*, that is by measuring both sets of trees at a common  $\text{CO}_2$  concentration, or by constructing  $A/C_i$  curves, but have measured photosynthetic enhancement at the growth  $[\text{CO}_2]$ .

Silvola and Ahlholm (1992) grew *Salix*  $\times$  *dasyclados* at four nutrient and  $\text{CO}_2$  levels, and found that in general, the relative photosynthetic response to  $[\text{CO}_2]$  was greater at higher nutrient levels, although the relative increase with  $[\text{CO}_2]$  was higher than expected in seedlings where no nutrients were added. Norby and O'Neill (1991) grew *Liriodendron tulipifera* in native soil with or without additional nutrients, and found a greater  $\text{CO}_2$  response in the fertilized seedlings on only two of three dates. El Kohen and Mousseau (1993) found that photosynthetic acclimation to  $\text{CO}_2$  enrichment in *Castanea sativa* occurred much earlier in the growing season in plants grown without fertilizers. Tissue et al. (1993) also found that photosynthetic response was correlated with N availability in *Pinus taeda*; photosynthetic rates in low N seedlings were similar in ambient and elevated  $[\text{CO}_2]$ . Low P seedlings in that study, however, showed relative  $\text{CO}_2$ -stimulations of photosynthesis greater than those in high P, high N seedlings, although the absolute rates at both  $\text{CO}_2$  concentrations were lower in the low P seedlings. In contrast, Conroy et al. (1986) found that photosynthesis in P-deficient *Pinus radiata* seedlings did not respond to elevated  $[\text{CO}_2]$ , because of a structural change in the thylakoid membranes and a general dysfunction of Photosystem II as measured with chlorophyll fluorescence techniques. Further experiments found that growth at elevated  $[\text{CO}_2]$  increased the critical concentration of P required

Table 4. Photosynthetic rates, and % change relative to rates in ambient-grown trees, of intact and coppice yellow-poplar trees grown at each of three  $\text{CO}_2$  concentrations. C.A. Gunderson, unpublished data

$\text{CO}_2$ treatment	Photosynthesis ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )		
	Measured at growth $[\text{CO}_2]$ (June 1992)		Measured at ambient $[\text{CO}_2]$ (June 1993)
	Intact	Coppice	Coppice
Ambient	8.4 (–)	9.4 (–)	10.3 (–)
+150 $\mu\text{L L}^{-1}$	11.0 (+31%)	11.6 (+23%)	11.1 (+8%)
+300 $\mu\text{L L}^{-1}$	13.5 (+61%)	15.2 (+62%)	9.6 (–7%)

for photosynthesis, but not by as large a degree as the concentration critical for growth (Conroy et al. 1990b). In fact, in a longer exposure, Conroy et al. (1990a) reported an apparent adjustment to low P; at 110 weeks the low P seedlings had regained the ability to respond to elevated  $[\text{CO}_2]$  that had apparently been lost at 22 weeks.

In another nutrient-related experiment, Pettersson and McDonald (1992) provided small *Betula pendula* with 'optimal nutrition' by raising them in cuvettes where their roots were continuously sprayed with a nutrient solution. The A/C<sub>i</sub> curves generated were no different for plants raised at either ambient or elevated  $[\text{CO}_2]$ . The authors suggest from this that photosynthetic acclimation might be linked to nutrient limitation. In short, while there appears to be a link between inadequate P or N nutrition and photosynthetic acclimation to elevated  $[\text{CO}_2]$ , it is not clear whether this limitation to  $\text{CO}_2$  response is responsible for the general photosynthetic acclimation observed experimentally. A further obstacle is that, even in nutrient-related studies, tissue nutrient levels are seldom measured, making it difficult to establish relationships between nutrient limitations and photosynthetic responses. As a result, the contribution of nutrient limitation to photosynthetic acclimation on a global scale is still difficult to predict.

#### *Other limitations to response*

Other factors, including the availability of water and light, may limit photosynthetic response to elevated  $[\text{CO}_2]$ . Photosynthetic response to elevated  $[\text{CO}_2]$  during drought stress could be particularly important, both in water limited environments and in the event of the regional decreases in rainfall postulated in some global climate change scenarios. If low irradiance limits photosynthetic response to elevated  $[\text{CO}_2]$ , then the acclimation seen in many growth chamber studies could be related to low light levels present during growth. On the other hand, lower light levels may be appropriate for understory species, or for estimating photosynthetic responses in lower leaves after canopy closure. In fact, studies that have compared the light response curves of trees grown at a *single* ir-

radiance level have generally found that quantum yield at low irradiance is higher in trees grown and measured at elevated  $[\text{CO}_2]$ , suggesting enhanced rates of photosynthesis in low light environments (Eamus and Jarvis 1989, Wullschlegel et al. 1992). Unfortunately, few studies on photosynthetic responses of trees to elevated  $[\text{CO}_2]$  have been conducted in multiple light regimens, or under both well-watered and water-stressed growing conditions, and the effect of water-stress or irradiance on acclimation is not resolved. Tolley and Strain (1985) grew two tree species under ambient and elevated  $[\text{CO}_2]$  and both high and low irradiance for 56 d and then subjected half of the seedlings to a 14 d water stress cycle. They concluded that, in *Pinus taeda*,  $[\text{CO}_2]$  had little effect on photosynthesis (at the growth  $[\text{CO}_2]$ ) in either water treatment, e.g., that photosynthesis largely acclimated to the higher  $[\text{CO}_2]$  under all conditions, and that elevated  $[\text{CO}_2]$  delayed the effects of water stress on *Liquidambar styraciflua*, especially at high irradiance. Looking only at the 675 vs. 350  $\mu\text{L L}^{-1} \text{CO}_2$  treatments, at the end of the drying cycle, the only *P. taeda* seedlings that had *not* completely acclimated were those that were water stressed and growing at high irradiance, and the only *L. styraciflua* seedlings that *did* completely acclimate were those in the water-stress, high irradiance treatment. On the other hand, the data of Reekie and Bazzaz (1989) showed that four of five tropical trees exhibited some degree of photosynthetic acclimation, but that there was very little difference in the patterns of response to  $[\text{CO}_2]$  between those grown at high and low light. It is clear that, if water or irradiance do limit the photosynthetic response to elevated  $[\text{CO}_2]$  in some species, that more research is needed to describe the bounds of those effects.

#### *Inherent ability to respond*

Are some species inherently more able to respond to an increase in  $[\text{CO}_2]$  and less likely to exhibit photosynthetic acclimation? It has been suggested that species with a higher relative growth rate will have a stronger sink strength and thus be more responsive to  $[\text{CO}_2]$  and less likely to adjust photosynthesis downward (Oech-

el and Strain 1985, Mousseau and Saugier 1992). The evidence for this in trees is weak, since a species comparison of response across all studies is confounded by differences in growing conditions (e.g., pot size, length of exposure, nutrient regime, light). Such analysis also requires ranking species by potential growth rate at optimal conditions – an estimate at best. Nevertheless, some comparisons can be made within studies of more than one species. For example, Grulke et al. (1993) compared photosynthetic and growth responses of two full-sib families of *Pinus ponderosa* with known differences in growth rates. Both families demonstrated almost complete acclimation to elevated  $[\text{CO}_2]$  within 39 days, and the authors concluded that high growth rate was not correlated with a greater response to elevated  $[\text{CO}_2]$ . Similarly, Norby and O'Neill (1989, 1991) and Gunderson et al. (1993) compared photosynthesis in a 'freely-flushing' species, *Liriodendron tulipifera*, with that in a 'fixed growth' pattern species, *Quercus alba*, in growth chamber experiments and in the field. They found no differences in the photosynthetic responses of the two species, and no evidence of photosynthetic acclimation. Oberbauer et al. (1985) compared a fast-growing pioneer species, *Ochroma lagopus*, with a slower-growing climax species, *Pentaclethra macroloba*. Photosynthesis was lower in seedlings of both species grown in elevated  $[\text{CO}_2]$  relative to photosynthesis in seedlings grown in ambient  $[\text{CO}_2]$ , measured either at the growth  $[\text{CO}_2]$  or at a common  $[\text{CO}_2]$ . Apparent acclimation was greatest in the fast-growing species as measured at the growth  $[\text{CO}_2]$  and similar when measured at a common ambient  $[\text{CO}_2]$ . Kaushal et al. (1989) compared *Cedrus atlantica*, with a long monocyclic aerial growth pattern, and *Pinus nigra*, with a short monocyclic or bicyclic aerial growth pattern, and found a more pronounced down-regulation of photosynthesis in *Cedrus* seedlings, although the *Cedrus* seedlings exhibited a greater growth response to  $[\text{CO}_2]$ . In another study, Ziska et al. (1991) examined the  $\text{A}/\text{C}_i$  curves of nine tropical species grown at ambient and enriched  $[\text{CO}_2]$ , among them a fast-growing introduced tree, *Acacia mangium*, and a native canopy tree, *Tabebuia rosea*. Neither species showed a downward acclimation of

photosynthesis, but the faster-growing *Acacia* showed more evidence of up-regulation. It may be significant that in most studies, photosynthesis was measured only once or twice, usually near the end of the study. While this may be indicative of the response of an 'acclimated' seedling (in the sense of Eamus and Jarvis 1989), the timing of the measurement may not coincide with the active growth phase of the seedling. As a result, even if relative  $\text{CO}_2$  response were correlated with the potential growth rate of the species, this relationship might not be detected. The evidence currently available from these studies, if anything, argues against the inherent ability of some species to sustain higher photosynthetic rates in elevated  $[\text{CO}_2]$  based on their growth rates or patterns, but it cannot be considered conclusive because of the small number of studies carried out in comparable conditions, and the timing of the photosynthetic measurements.

## 5. Summary

In order to place the literature reviewed here into a broader context, several generalizations may be made. First, it is very likely that some degree of leaf-level photosynthetic acclimation will occur in trees as  $\text{CO}_2$  continues to rise into the next century. The practical impact of this phenomenon is that very short-term measurements of photosynthetic response to  $[\text{CO}_2]$  (i.e.,  $\text{A}/\text{C}_i$  curves) cannot be used to predict long-term responses. Second, it is unlikely that this acclimation will be complete. On the contrary, photosynthesis, on a leaf area basis, will probably be noticeably higher in most species growing in elevated  $[\text{CO}_2]$ , unless other environmental factors limit this response. It is also clear that the current understanding of the magnitude of such possible limitations in forest systems is deficient. Hypotheses that either nutrient or water limitations would limit photosynthetic responses in natural environments have not been adequately supported by the currently available data. Further research is needed to evaluate these hypotheses.

Elucidating the biochemical mechanisms involved in photosynthetic acclimation will continue to be an active area for research, and



describing the interactions between these mechanisms and potentially limiting environmental factors will be important in trying to generalize to responses under natural conditions. Other areas of research are needed, however, to improve our ability to predict future global carbon fluxes. In fact, the experiments needed for mechanistic studies tend to be those with carefully controlled seedling growth, non-limiting resources, and plants that can be easily moved to the gas exchange cuvette, i.e., plants in relatively small pots. By contrast, the experiments needed to scale up toward estimating the contributions of trees to the global carbon cycle are those with more natural growth conditions provided by field settings with perhaps uncontrolled, or at least representative levels of water, nutrients, and irradiance, and probably with more mature trees grown under CO<sub>2</sub> enrichment for longer time spans. Unfortunately, by their very nature, these trees will be difficult to evaluate for response mechanisms, and that will be an ongoing challenge.

It should also be pointed out that although photosynthetic acclimation to elevated [CO<sub>2</sub>] at the biochemical or leaf level is an interesting phenomenon, and provides the basis for higher level responses, it does not provide all the information needed for predicting forest responses to a changing environment. There needs to be a new focus on canopy-level, whole tree, and even forest acclimation to rising [CO<sub>2</sub>]. Regardless of the photosynthetic enhancement in trees growing in higher [CO<sub>2</sub>], or regardless of the acclimation, changes in leaf-level photosynthesis may not translate to increased growth or even to greater canopy-level CO<sub>2</sub> assimilation. Future research must include the combined effects of physiological and morphological adjustments to estimate the effects of rising [CO<sub>2</sub>] on growth and carbon flux. Will CO<sub>2</sub>-induced increases in growth cease after canopy closure, or will the higher quantum yields associated with elevated [CO<sub>2</sub>] increase productivity of leaves in lower light environments, resulting in changes in canopy architecture, or perhaps a competitive advantage for shade tolerant or understory species? Will decreases in leaf area ratio result in homeostasis at the whole-tree scale even before canopy closure? Such questions will be appro-

priate as research scales up to address ecosystem and global issues, and may provide new directions for future research.

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