

Carbon Isotopes in Photosynthesis

Fractionation techniques may reveal new aspects of carbon dynamics in plants

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The efficiency of photosynthesis continues to interest biochemists, biologists, and plant physiologists. Scientists interested in CO₂ uptake are concerned about the extent to which the uptake rate is limited by such factors as stomatal diffusion and the chemistry of the CO₂ absorption process. The fractionation of carbon isotopes that occurs during photosynthesis is one of the most useful techniques for investigating the efficiency of CO₂ uptake.

Atmospheric carbon dioxide contains approximately 1.1% of the non-radioactive isotope carbon-13 and 98.9% of carbon-12. During photosynthesis, plants discriminate against ¹³C because of small differences in chemical and physical properties imparted by the difference in mass. This discrimination can be used to assign plants to various photosynthetic groups. The isotope fractionation also reflects limitations on photosynthetic efficiency imposed by the various diffusional and chemical components of CO₂ uptake. When analyzed in detail, this fractionation provides information about water use efficiency and indicates that different strategies are needed for improving water-use efficiency in different kinds of plants.

Isotope fractionation in simple physical and chemical processes is well understood and is commonly

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Current studies include finely tuned, carefully controlled isotope fractionations under defined environmental conditions

used to study mechanisms of chemical (Melander and Saunders 1980) and biochemical (Cleland 1982) processes. Isotopes are used in ecology to establish food chains and biological pathways (Fritz and Fontes 1980, 1986, Rounick and Winterbourn 1986), and isotope studies of tree rings are used to recreate past climates (Hughes et al. 1982). Isotope studies of plants are related to all these areas, because their basis is in fundamental chemical processes, and many of their applications are in the area of ecology (O'Leary 1981, Troughton 1979, Vogel 1980). Recently developed methods are allowing biologists to examine in greater detail the carbon flow in plants.

Measurement of carbon isotopes

The ¹³C content of carbon dioxide is usually determined with a mass spectrometer specially designed for high-precision measurement of the ratio *R*, defined by

$$R = {}^{13}\text{CO}_2/{}^{12}\text{CO}_2$$

Other materials must be converted to CO₂ prior to analysis. Plants are ordinarily converted to CO₂ by combustion. Individual compounds isolated from plants are sometimes converted to CO₂ by chemical or enzymatic degradation.

For natural materials (plants, animals, and minerals), *R* is approximately 0.0112, and only the last digit in this ratio varies. For convenience, *R* values are generally converted to values of δ¹³C,

$$\delta^{13}\text{C} = \left[\frac{R(\text{sample})}{R(\text{standard})} - 1 \right] \times 1000$$

The standard is carbon dioxide obtained from a limestone, called PDB, from the Pee Dee formation in South Carolina (Craig 1957). The units of δ¹³C are called "per mil," or ‰. A more negative δ¹³C means more ¹²C, or lighter in mass; a more positive δ¹³C means more ¹³C, or heavier. Most natural materials have negative δ¹³C values because they contain less ¹³C than the standard. The precision of modern mass spectrometers is at least ±0.02 ‰, but sample preparation errors may bring the total reproducibility of measurements on plant materials to ±0.2 ‰. Thus, interpretations based on differences smaller than 1 ‰ should be made with caution.

In the absence of industrial activity, the δ¹³C value of atmospheric CO₂ is -8 ‰. This value for the atmosphere is slowly becoming more negative due to the combustion of fossil fuel (δ¹³C for fossil fuel is approximately -30 ‰) (Hoefs 1980).

Isotope values of plants

In the 1950s, Craig (1953, 1954) and Baertschi (1953) measured $\delta^{13}\text{C}$ values of a variety of natural materials, including plants (reviewed by O'Leary 1981). They found that most plants had $\delta^{13}\text{C}$ values in the range -25 to -35 ‰. They failed to find large species or environmental effects on these values.

The plants in these initial studies were principally C_3 plants, which fix CO_2 by the action of the enzyme ribulose biphosphate carboxylase. The C_4 photosynthetic pathway, in which CO_2 is initially taken up through carboxylation of phosphoenolpyruvate, was discovered in the 1960s. Following this discovery, Bender (1968, 1971; see also Smith and Epstein 1971) discovered that C_4 plants are isotopically distinct from C_3 plants. C_3 plants have $\delta^{13}\text{C}$ values of approximately -28 ‰, whereas C_4 plants are approximately -14 ‰.

In subsequent years, a number of laboratories around the world made similar measurements on thousands of plants species and established a clear distinction between C_3 and C_4 plants (Figure 1), with little overlap between the two distributions. Therefore, ^{13}C analysis has become a standard test for determining the pathway of CO_2 fixation. What is the biochemical source of this difference?

Fractionations in chemical and physical processes

Plants contain less ^{13}C than the atmosphere because the physical and chemical processes involved in CO_2 uptake discriminate against ^{13}C . This discrimination occurs because ^{13}C is heavier than ^{12}C and forms slightly stronger chemical bonds. In addition, diffusion of $^{13}\text{CO}_2$ is slower than that of $^{12}\text{CO}_2$ because of this difference in mass. For the conversion of compound A into compound B, the isotope fractionation is defined by

$$\Delta\delta = \frac{[\delta^{13}\text{C}(\text{A}) - \delta^{13}\text{C}(\text{B})]}{1 + \delta^{13}\text{C}(\text{A})/1000}$$

This fractionation has units of ‰. To avoid confusion with ordinary $\delta^{13}\text{C}$ values (which represent isotopic compositions, rather than fractionations), we call this value $\Delta\delta$. The

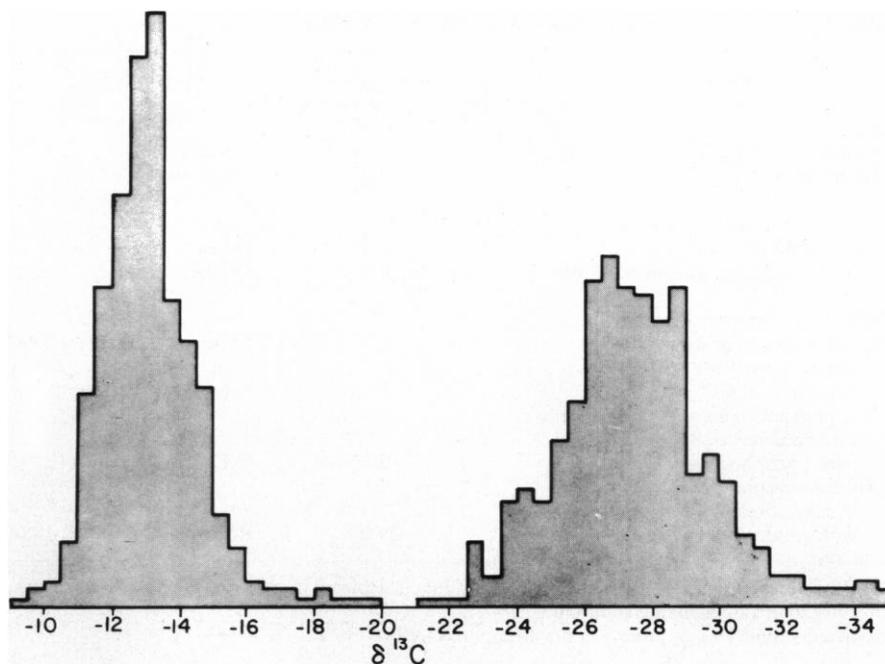


Figure 1. Histogram showing the distribution of $\delta^{13}\text{C}$ values of plant materials. This figure is based on about 1000 analyses performed in five different laboratories.

fractionation has a positive sign when ^{13}C is transformed more slowly than ^{12}C (as is the case in most physical and chemical processes).¹

Many physical, chemical, and biochemical processes have significant isotope fractionations (Cleland 1982, Melander and Saunders 1980). Fractionations can occur both in time-dependent processes (chemical reactions and transport) and in equilibrium processes (chemical equilibria, dissolution, and phase changes), and both are important in plants. Table 1 shows isotope fractionations for processes of importance in photosynthesis.

Theory of isotope fractionation in plants

The principal factor affecting the isotopic compositions of leaves is the isotope fractionation accompanying CO_2 uptake. Following initial suggestions of Craig (1953), Smith and Epstein (1971), and others (O'Leary 1981), models for plant isotope fractionation have focused on the physical and chemical processes accompanying CO_2 uptake, including diffusion, dissolution, and the car-

boxylation step itself. Several mathematical models have been suggested (Deleens et al. 1983, Farquhar et al. 1982, O'Leary 1981, Peisker 1982, 1984, 1985), all of which are based on the component fractionations given in Table 1. The overall fractionation in such a complex system is a combination of these components, but it is not simply the sum of a series of individual fractionations—instead, the fractionation mostly reflects the rate-limiting step or steps (i.e., those with the highest resistivity). As a step becomes more limiting, the observed fractionation approaches the fractionation for that step.

The important steps in CO_2 uptake in C_3 plants are shown in Figure 2. In the first step, external CO_2 is transported through the boundary layer and the stomata into the internal gas space. This process is always to some extent reversible. Internal CO_2 then dissolves in the cell sap and diffuses to the chloroplast, where carboxylation occurs. Because the carboxylation step is irreversible, steps subsequent to carboxylation are not important in determining the isotope fractionation. Both dissolving and diffusion show small isotope fractionations (Table 1), but the largest fractionation is that connected with carboxylation (29 ‰).

It is generally assumed that dissolu-

¹However, note that some workers in the field use the opposite sign convention.

Table 1. Carbon isotope fractions associated with photosynthesis.

Process	$\Delta\delta$, ‰*	Reference
Equilibria		
Solubility of CO ₂ in water	1.1	O'Leary 1984
Hydration of CO ₂	-9.0	Mook et al. 1974
Transport processes		
CO ₂ diffusion in air†	4.4	O'Leary 1981
CO ₂ diffusion in aqueous solution	0.7	O'Leary 1984
Chemical processes		
Spontaneous hydration of CO ₂	6.9	Marlier and O'Leary 1984
Carbonic anhydrase catalyzed hydration of CO ₂	1.1	Paneth and O'Leary 1985
Phosphoenolpyruvate carboxylase-catalyzed reaction of HCO ₃ ⁻ with phosphoenolpyruvate	2.0	O'Leary et al. 1981
Ribulose biphosphate carboxylase-catalyzed reaction of CO ₂ with ribulose biphosphate	29.0	Roeske and O'Leary 1984

*Positive values in this table indicate that the product is depleted in ¹³C compared with the starting state; negative values indicate enrichment.

†Predicted value. This number has not been measured.

tion and liquid-phase diffusion are rapid, but good evidence for this is lacking. If stomatal diffusion is rapid (stomatal resistance is low) and carboxylation is limiting, the predicted isotope fractionation is 28 ‰, and the predicted leaf $\delta^{13}\text{C}$ value is -36 ‰. If diffusion is slow (stomatal resistance is high), the predicted isotope fractionation is 4 ‰ and the predicted leaf $\delta^{13}\text{C}$ value is -12 ‰. To the extent that diffusion and carboxylation jointly limit the rate, the $\delta^{13}\text{C}$ value will be intermediate between these two extremes. Measured $\delta^{13}\text{C}$ values for C₃ plants cluster near -28 ‰, which is nearer to the carboxylation-

limited extreme. More quantitative analysis indicates that the carboxylation resistance is higher than the diffusional resistance by up to a factor of two; diffusion of internal CO₂ back to the outside is faster than carboxylation by up to a factor of two.

Thus, CO₂ uptake in C₃ plants is limited more by the rate of carboxylation of ribulose biphosphate than by diffusion. This finding has important implications for plant breeding. If we could breed plants with a more efficient ribulose biphosphate carboxylase (either because of increased enzyme activity or because of reduced

photorespiration), then we could breed a plant that would take up CO₂ more rapidly without sacrificing water-use efficiency. The alternative possibility, decreasing diffusive resistance, has only a very limited potential for increasing CO₂ uptake, and this increase would come at a substantial cost in water-use efficiency. As we will see below, the situation in C₄ plants is different.

The C₄ pathway involves sequential operation of two carboxylase systems (Figure 3). CO₂ initially enters the leaf through the stomata and is taken up by phosphoenolpyruvate carboxylase in the mesophyll cells. The product of this carboxylation is converted to either malate or aspartate and is transported to the bundle sheath cells, where it is cleaved to CO₂ and some other compound. The CO₂ thus produced is taken up by ribulose biphosphate carboxylase. Although the latter enzyme shows a large isotope fractionation (Table 1), the effects of this fractionation are not seen in C₄ plants because this step is preceded by an irreversible step, the carboxylation of phosphoenolpyruvate.

As in C₃ carboxylation, dissolution and liquid-phase diffusion of CO₂ are assumed to be fast. Carbonic anhydrase is present in C₄ plants (Reed and Graham 1981); thus, CO₂ and HCO₃⁻ are expected to be in equilibrium. The steps that are significant for isotope fractionation are stomatal diffusion and carboxylation of phosphoenolpyruvate. If diffusion is facile and carboxylation is limiting, then the predicted leaf $\delta^{13}\text{C}$ is -1 ‰. On the other hand, if diffusion is limiting and carboxylation is facile, the predicted $\delta^{13}\text{C}$ is -12 ‰.

Observed $\delta^{13}\text{C}$ values for C₄ plants are approximately -14 ‰. Thus, it appears that, unlike the case in C₃ plants, carboxylation capacity in C₄ plants is in excess of that needed for steady-state photosynthesis, and the diffusion is more limiting than carboxylation. Unlike the situation in C₃ plants, further improvements in the efficiency of C₄ plants cannot come about through increases in carboxylation capacity.

The $\delta^{13}\text{C}$ values that are observed in C₄ plants are slightly outside the range allowed by this model, and it is clear that some additional factor is at

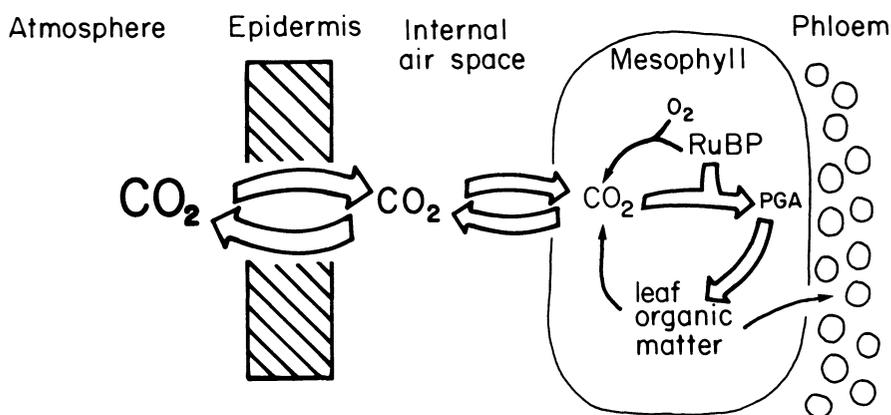


Figure 2. Important steps in CO₂ fixation during C₃ photosynthesis. Sizes of arrows indicate the relative fluxes through the various steps (including the reverse steps) according to the best models available. Sizes of symbols reflect relative concentrations of CO₂ at various stages.

work. The suggestion has often been made that CO_2 is lost from the bundle sheath during CO_2 uptake by ribulose biphosphate carboxylase (Deleens et al. 1983, O'Leary 1981, Peisker 1982). Because of the large isotope discrimination associated with ribulose biphosphate carboxylase, the CO_2 thus lost would be enriched in ^{13}C , leading to a shift in leaf $\delta^{13}\text{C}$ toward more negative values.

In order for this mechanism to work, however, the "lost" CO_2 must escape the leaf completely—it cannot be recaptured by PEP carboxylase in the mesophyll cells. Given the architecture of C_4 leaves and the high efficiency of CO_2 capture by phosphoenolpyruvate carboxylase, it is not clear that this is possible, especially because the CO_2 loss must total 20%–40% of CO_2 fixed. Other important factors may include respiration, translocation, and developmental effects. Evidence in favor of the CO_2 loss hypothesis has been obtained by Hattersley (1982), who showed that $\delta^{13}\text{C}$ values of C_4 plants vary with bundle sheath permeability, with the more negative values being observed for plants in which permeability (and therefore, loss of CO_2) is expected to be highest.

The limiting predictions for C_3 and C_4 plants, along with the observed $\delta^{13}\text{C}$ values, are shown in Table 2. These values remind us that whereas chemical processes are principally limiting in C_3 plants, diffusion is principally limiting in C_4 plants.

CAM plants

Desert plants and other succulents absorb CO_2 by the pathway known as Crassulacean acid metabolism (CAM; Kluge and Ting 1978, Osmond 1978). At night, these plants open their stomates and absorb CO_2 in order to synthesize malic acid by use of phosphoenolpyruvate carboxylase and malate dehydrogenase in a process similar to that seen in C_4 plants. These plants accumulate high levels of malic acid overnight. During the following morning, stomates close and this malic acid is decarboxylated. The CO_2 thus formed is taken up by ribulose biphosphate carboxylase in a process akin to that in the bundle sheath cells of C_4 plants. During the afternoon, many CAM plants open

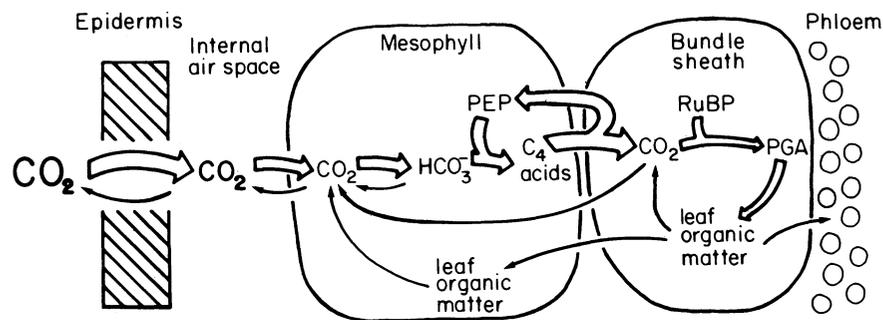


Figure 3. Important steps in CO_2 fixation during C_4 photosynthesis. Sizes of arrows indicate the relative fluxes through the various steps (including the reverse steps) according to the best models available. Sizes of symbols reflect relative concentrations of CO_2 at various stages.

their stomates and engage in direct C_3 photosynthesis using ribulose biphosphate carboxylase (Kluge and Ting 1978, Osmond 1978).

When CAM plants absorb CO_2 only at night, they have $\delta^{13}\text{C}$ values of approximately -11‰ (Nalborczyk et al. 1975, O'Leary 1981). When CAM plants engage in only daytime photosynthesis, they have $\delta^{13}\text{C}$ values of approximately -28‰ , characteristic of C_3 plants (Nalborczyk et al. 1975).

Most often $\delta^{13}\text{C}$ values for CAM plants are in the range -10 to -20‰ . Thus, their $\delta^{13}\text{C}$ values serve to distinguish them from C_3 plants. Distinction from C_4 plants can generally be made on physiological grounds (particularly succulence) and on the basis of diurnal variations in malic acid content.

The balance between night and day CO_2 fixation in CAM plants is re-

flected in $\delta^{13}\text{C}$ values (Figure 4), and one of the common uses of isotopic studies in CAM plants has been to determine the proportions of the two CO_2 fixation pathways and the variation in proportions with changes in environmental conditions (Osmond et al. 1976). Such isotopic data can also be correlated with measurements of titratable acidity and gas exchange. The leaf succulent *Sedum wrightii* grows in a variety of environments in the southwestern United States, and study of herbarium specimens reveals that this species shows a greater variation in leaf thickness than most other species in the family. Kalisz and Teeri (1986) have shown that in various populations of *S. wrightii*, $\delta^{13}\text{C}$ values become more positive, leaves become thicker, and growth rates decrease as an increasing proportion of CO_2 is absorbed at night.

Environmental effects have also

Table 2. Predicted and observed $\delta^{13}\text{C}$ values for C_3 and C_4 plants.

Model	Predicted $\delta^{13}\text{C}$	
	C_3 plants	C_4 plants
diffusion limiting, carboxylation fast ($[\text{CO}_2(i)]$ approaches zero)	-12‰	-12‰
carboxylation limiting, diffusion fast ($[\text{CO}_2(i)]$ approaches $[\text{CO}_2(\text{ext})]$)	-38‰	-1‰
carboxylation and diffusion equally limiting ($[\text{CO}_2(i)] = 1/2 [\text{CO}(\text{ext})]$)	-25‰	-6.5‰
observed $\delta^{13}\text{C}$	-25 to -29‰	-12 to -16‰

been studied in detail for the Mexican perennial succulents *Cremnophila linguifolia* and *Sedum greggii* and their F1 hybrid in an attempt to determine environmental versus genetic determinants of CAM (Teeri and Gurevitch 1984). Large variations in $\delta^{13}\text{C}$ could be seen in all three populations, reflecting variations in the proportion of carbon taken up by the CAM pathway, as expected from Figure 4.

However, it should be noted that the curve shown in Figure 4 is only qualitatively correct. The limiting $\delta^{13}\text{C}$ values for pure C_3 and pure CAM are probably variable with environmental conditions, and this variation has not been taken into account in studies to date.

The combustion studies of $\delta^{13}\text{C}$ values of CAM plants reflect the intrinsic isotope fractionations associated with the two CO_2 fixation pathways, as well as the proportions of carbon fixed by each of the two pathways. The first attempt to measure the two intrinsic fractionations separately was that of Nalborczyk et al. (1975), who exposed one set of CAM plants to CO_2 only at night and another set only during the daylight hours. Detailed studies of the isotope fractionation associated with nocturnal CO_2 fixation have been made by O'Leary and Osmond (1980), who purified malic acid, the initial product of CO_2 fixation, and degraded it to convert carbon-4 of this material (which came from atmospheric CO_2)

to CO_2 for the isotopic analysis.

This isotope fractionation reflects only the CO_2 fixation process, and the resulting isotopic signal is free of complications due to postcarboxylation events, import and export processes, and contributions from daytime CO_2 fixation. The isotopic composition so obtained must be corrected for contributions of respired carbon, randomization of malate by fumarase, and residual malate left over from the previous day. The final $\delta^{13}\text{C}$ value for newly fixed carbon was -4 to -7 ‰ for various species (Deleens et al. 1985, Holtum et al. 1983, O'Leary and Osmond 1980), both for growth-chamber plants and for field-grown plants.

Comparison with models developed in connection with C_4 photosynthesis (Figure 3) reveals that nocturnal CO_2 uptake is controlled jointly by diffusion and carboxylation to provide optimum CO_2 absorption per amount of water lost, and this balance is maintained (by adjustment of stomatal aperture) even in the face of varying CO_2 concentrations (Holtum et al. 1983, O'Leary and Osmond 1980). The partitioning of internal CO_2 between carboxylation and return to the atmosphere is approximately 1:1. This balance is different from that in C_4 plants. Gas-exchange studies confirm this conclusion (Holtum et al. 1983). Temperature effects, which are not visible in combustion studies, can be seen in these studies.

For *Kalanchoe daigremontiana*, the isotope fractionation associated with malate synthesis changes from -4 ‰ at 17°C to 0 ‰ at 27°C because of an increase in carboxylation capacity coupled to a decrease in stomatal aperture (Deleens et al. 1985).

There is an interesting discrepancy between these results and results of combustion studies. As noted above, combustion studies indicate that when CAM plants absorb CO_2 only at night, the leaf $\delta^{13}\text{C}$ value is approximately -11 ‰. However, studies of new carbon incorporated into malate give approximately -7 ‰. This difference may be due to CO_2 loss during the morning; during malate decarboxylation and CO_2 reabsorption by ribulose biphosphate carboxylase, the internal CO_2 concentration becomes quite high (Cockburn et al. 1979), and a small amount of CO_2 escapes from the leaf. Because of the large isotope discrimination associated with ribulose biphosphate carboxylase, this lost CO_2 is very heavy, with a $\delta^{13}\text{C}$ value of approximately $+20$ ‰.² Loss of this heavy CO_2 is a principal cause of the shift of $\delta^{13}\text{C}$ value.

Respiration

The $\delta^{13}\text{C}$ value of a leaf reflects principally the isotope fractionation associated with photosynthetic carbon fixation and thus provides a useful indication of the operation of the C_3 , C_4 , and CAM photosynthetic pathways. However, other effects may also contribute to the overall isotopic picture. In addition to the possible contribution of CO_2 loss from the bundle sheath cells during C_4 photosynthesis and CO_2 loss during deacidification in CAM plants, other losses of carbon from leaves may also contribute.

All plants respire, and in so doing, they may lose significant amounts of CO_2 . If this CO_2 has the same $\delta^{13}\text{C}$ value as the leaf from which it is lost, then this loss is of no consequence for the isotope content of the leaf. However, if respired carbon is depleted in ^{13}C compared to the leaf, then the leaf will become ^{13}C enriched as a result

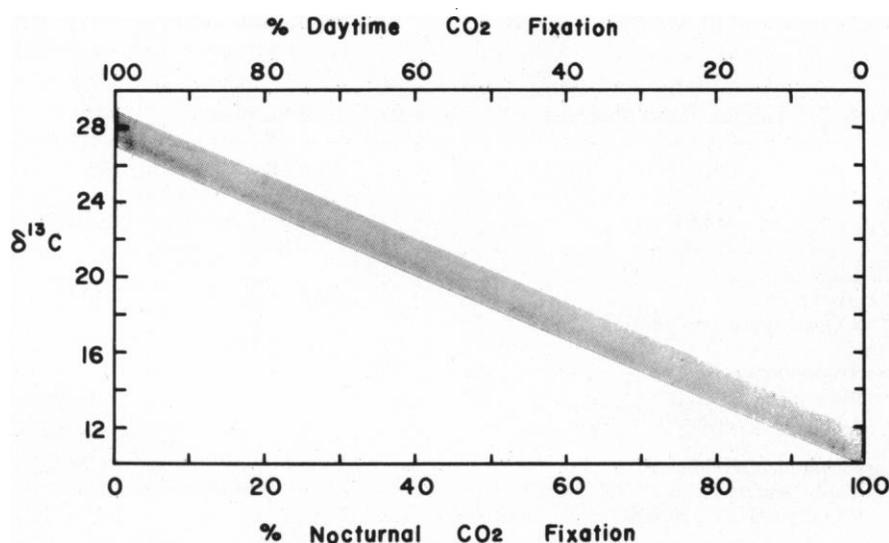


Figure 4. Predicted $\delta^{13}\text{C}$ value for CAM plants as a function of the proportions of CO_2 fixed at night and during the day.

²M. H. O'Leary and I. W. Treichel, 1987. Unpublished data, University of Wisconsin, Madison.

of respiration. Unfortunately, it is not possible to measure the isotopic consequences of respiration during steady-state photosynthesis, because respiration-derived CO₂ cannot be separated from other CO₂. Measurement made under nonphotosynthetic conditions may not give an accurate indication of either the quantity or the isotopic nature of normal respiration, especially for photorespiration, which is associated with CO₂ uptake by ribulose biphosphate carboxylase in C₃ plants.

Experimental data to date do not provide a clear answer to the question of whether respiration fractionates carbon isotopes (O'Leary 1981), much less the question of whether the isotopic consequences of respiration may change with species or environment. The lack of information on this issue becomes particularly troublesome in such quantitative studies as water-use efficiency.

Longer-term effects

According to this picture of CO₂ fixation, the ¹³C content of leaves reflects the isotope fractionation associated with CO₂ uptake, with perhaps small modifications as a result of respiratory processes. This snapshot view of CO₂ fixation is not entirely correct.

The isotopic content of a leaf provides an integrated view of carbon gain and loss over the whole history of the leaf. Although most leaf carbon appears to be introduced directly by photosynthesis within the leaf, total carbon also includes carbon that was imported from elsewhere in the plant during early stages of leaf development and excludes carbon that has been lost from the leaf by respiratory processes and by export to the remainder of the plant. An accurate model of the isotopic composition of a leaf must include all these processes.

Import and export processes are poorly understood and are often slighted in development of quantitative isotopic models. These processes can be ignored only to the extent that the carbon gained and lost is isotopically the same as whole leaf carbon. Studies of the changes in isotopic content during development are inconclusive with regard to the question of whether these effects are significant.

Aquatic plants and algae

Aquatic plant δ¹³C values are more difficult to understand than those of terrestrial plants because of the importance of diffusion in photosynthesis of aquatic plants. Diffusion of CO₂ dissolved in water is orders of magnitude slower than diffusion of CO₂ in air. Not surprisingly, in aquatic plants diffusion is often limiting, so the isotope fractionation is small, even for C₃ plants. Although the δ¹³C value of CO₂ in air is relatively constant, the δ¹³C value of dissolved CO₂ is quite variable, and dissolved CO₂ differs from dissolved HCO₃⁻ by approximately 9 ‰.

Recent studies have attempted to account quantitatively for these factors. Osmond et al. (1981) surveyed a variety of aquatic plants and simultaneously made measurements of the isotopic composition of dissolved inorganic carbon. In rapidly flowing streams in which mixing was good and carbon was readily available, plants often showed isotope fractionations similar to those of terrestrial C₃ plants, indicating that neither mixing nor diffusion was rate limiting. In sluggish water, isotope fractionations were small, because diffusion is limiting in CO₂ uptake. Similar results have been reported by Raven et al. (1982).

Recent identification of the aquatic plants *Isoetes lacustris* L. and *Isoetes howellii* as CAM species is based on criteria other than isotopic composition (Keeley and Busch 1984, Richardson et al. 1984). The isotope fractionation is small, because of limited availability of CO₂. The occurrence of CAM in these cases appears to be a response to limited CO₂ availability.

Likewise, algae, which take up CO₂ by means of ribulose biphosphate carboxylase, show isotope fractionations that vary with environmental CO₂ concentration (Kerby and Raven 1985). In laboratory experiments, small isotope fractionations (sometimes approaching 0 ‰) are observed when CO₂ is limiting, and fractionations of 20 ‰ or more are observed when CO₂ concentration is high. In field studies, isotope fractionations may vary over this entire range, with most of the variation presumably being due to variations in CO₂ availability.

Water-use efficiency

With the realization that isotope fractionation in plants is a reflection of the balance between diffusional and chemical processes, Farquhar et al. (1982) suggested that isotopic compositions of plants should correlate with internal CO₂ concentration, [CO₂(i)], according to the equation

$$\Delta\delta = a + [b - a][\text{CO}_2(i)]/[\text{CO}_2(\text{ext})]$$

where *a* is the isotope fractionation in CO₂ diffusion (4.4 ‰) and *b* is the carboxylation fractionation (29 ‰ for C₃ plants), [CO₂(i)] is the internal gas-phase CO₂ concentration, and [CO₂(ext)] is the external CO₂ concentration. Because gas exchange also provides a value of [CO₂(i)], this equation provides an important bridge between the two methods and helps to validate the theoretical models for isotope fractionation.

Several studies have now shown that the isotopic method and the gas-exchange method give consistent values of [CO₂(i)]. Isotopic analysis of malate and independent gas-exchange measurements both show that in *K. daigremontiana* changing the external CO₂ concentration over the range from 100 to 1000 ppm does not change the ratio [CO₂(i)]/[CO₂(ext)], in spite of a substantial change in CO₂ uptake rate. Instead, stomatal aperture is adjusted to keep this ratio constant and optimize water-use efficiency (Holtum et al. 1983).

Isotopic compositions of halophytes become more positive by up to 10 ‰ with increasing salinity (Guy et al. 1980, Neales et al. 1983). The above equation suggests that this observation might be due to a decrease in [CO₂(i)], and gas-exchange studies are consistent with that suggestion (Guy and Reid 1986). Most of the change in photosynthetic rate with salinity is due to a change in stomatal conductance. In spinach, increasing salinity to 200 mM NaCl shifted δ¹³C to more positive values by 5 ‰, indicating a decrease in [CO₂(i)], consistent with results of gas exchange studies (Downton et al. 1985). Similarly, the flacca mutant of tomato has a high stomatal conductance, a high [CO₂(i)], and an isotope fractionation 1–2 ‰ larger than wild type (Bradford et al. 1983).

Following the correlation of $\delta^{13}\text{C}$ with $[\text{CO}_2(i)]$ came the realization that for C_3 plants, the isotopic composition should correlate with water-use efficiency. More water-efficient plants should have more positive $\delta^{13}\text{C}$ values. Recent studies from Farquhar's group demonstrate that this correlation can be used to screen cultivars for water-use efficiency (Condon et al. 1987, Farquhar and Richards 1984). In wheat, various cultivars differing in water-use efficiency from 2.0 to 3.7 mmol C/mol H_2O varied in isotope fractionation from 22 to 19 ‰.³ This method is faster and cheaper than other screening methods and thus has tremendous economic implications for plant breeding. However, it should be noted that the method is probably practical only when comparing a single species grown in a single environment. It is unlikely that the same method can be used for C_4 plants because of the low $[\text{CO}_2(i)]$ and the smaller range of $\delta^{13}\text{C}$ values that would result from changes in $[\text{CO}_2(i)]$.

Fine tuning the measurements

The use of isotopic methods for distinguishing among C_3 , C_4 , and CAM plants is now well established. This procedure depends on large differences in $\delta^{13}\text{C}$ values, which are easily measured and easily interpreted. However, there is currently considerable interest in the question of whether small variations in $\delta^{13}\text{C}$ values (of the order of 1–4 ‰) may be of use in biology. The water-use efficiency studies cited above are one example of this approach.

Combustion analyses of large collections of C_3 plants grown under a variety of conditions and analyzed in a number of different laboratories give a range of $\delta^{13}\text{C}$ values. Although an adequate statistical analysis of all the data in the literature has not been done, comparison of large data sets from a number of laboratories gives a mean for all C_3 plants of -27.1 ± 2.0 ‰ and for C_4 plants of -13.1 ± 1.2 ‰.⁴ The reproducibility of individu-

al measurements is approximately ± 0.2 ‰. The remainder of the variability must be due to individual differences among species or environmental effects.

The observed variation becomes smaller when measurements are restricted to a single species and a single environment. For soybean (a C_3 plant), combustion analysis of 25 cultivars grown side-by-side showed a standard deviation of ± 0.36 ‰.⁵ A similar experiment with 120 cultivars of *Zea mays* (a C_4 plant) grown side-by-side showed $\delta^{13}\text{C} = -11.6 \pm 0.4$ ‰ in a system in which the experimental error for multiple measurements of a single sample was ± 0.3 ‰.⁶

Interestingly, differences in ploidy level do not seem to translate into differences in $\delta^{13}\text{C}$ in either C_3 or C_4 plants. In *Z. mays*, a tetraploid strain had a $\delta^{13}\text{C}$ value only 0.3 ‰ more negative than the mean value for 120 other strains of the same species. In alfalfa, genetically identical diploid, tetraploid, and octaploid strains differed by less than 0.3 ‰.⁷ Thus, the ratio $[\text{CO}_2(i)]/[\text{CO}_2(\text{ext})]$ seems to be preserved across ploidy levels, in spite of significant biochemical and anatomical differences.

In addition, there are small organ and biochemical differences within a plant: Nonphotosynthetic tissues (e.g., stem and roots) are generally 2–4 ‰ more positive than leaves (O'Leary 1981). Individual chemical components may also differ. Lipids, in particular, are several per mil more negative than other materials, apparently as a result of the isotope fractionation associated with the decarboxylation of pyruvic acid (O'Leary 1981).

Recent studies indicate that there are small environmental effects on $\delta^{13}\text{C}$ values in C_3 plants. When tomato plants were grown in a controlled-environment chamber at varying light levels and temperatures, systematic trends were found in $\delta^{13}\text{C}$ values. As growth temperature was increased from 17° C to 32° C, the $\delta^{13}\text{C}$ value became more negative by 3 ‰. As

light intensity increased from 100 to 500 $\mu\text{Einsteins}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, the observed $\delta^{13}\text{C}$ value became more positive by 2 ‰. No such trend is apparent in similar studies with C_4 plants.⁸ These studies provide an important control for attempts to use $\delta^{13}\text{C}$ of tree rings to construct past climates (Hughes et al. 1982).

Short-term measurements

As noted above, combustion studies integrate over the entire life of a leaf, and it is likely that useful short-term information is lost in this integration. Methods are currently being developed that permit measurement of isotope fractionation during a short period (1–12 hr) in the life of a plant. These methods may provide a new window on environmental and other short-term effects.

The first short-term method (O'Leary and Osmond 1980) made use of malate accumulated overnight in CAM plants. Studies of environmental and species variations have demonstrated that the method gives useful indications of environmental effects on stomatal aperture and carboxylation capacity.

Other short-term methods make use of the change in isotopic composition of atmospheric CO_2 during photosynthesis in a closed system. This approach can be applied either in a flowing system like that used in gas exchange (Evans et al. 1986) or in a closed, nonflowing system (O'Leary et al. 1986). The advantage of the flowing system is that measurements are made at steady-state, and the properties of the plant are likely to be quite reproducible. The disadvantage is that the isotopic change in the CO_2 stream due to photosynthesis is quite small, and thus the calculated isotope fractionation (which may be 5–10 times larger than the observed change) is subject to a large uncertainty. Larger isotopic changes are obtained in the second method, but results are rendered uncertain by changes in CO_2 concentration in the atmosphere (and consequently changes in stomatal aperture) that occur during the experiment. Both methods give results consistent with data from the combustion method.

⁸See footnote 2.

³Note that the numbers given here are isotope fractionations, not isotopic compositions.

⁴M. H. O'Leary, 1987. Unpublished data, University of Wisconsin, Madison.

⁵J. Teeri and J. Gurevich, 1986. Unpublished data, University of Chicago, Chicago.

⁶J. Holtum, D. Weber, and M. H. O'Leary, 1983. Unpublished data, University of Wisconsin, Madison.

⁷See footnote 2.

These methods will be useful for studying a variety of environmental and species effects.

Conclusions

The first phase of studies of carbon isotope fractionation in plants took advantage of the large differences among C₃, C₄, and CAM plants. During this period the isotopic method became a standard method by which new species could be placed in these categories. The current phase involves finely tuned, carefully controlled studies of isotope fractionation under defined environmental conditions. These studies are providing details on carbon flow during photosynthesis and on how various steps contribute to the overall rate of CO₂ uptake in plants. We can anticipate that studies in the future will reveal many new aspects of both long-term and short-term dynamics of carbon movements in living systems.

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