

CARBON ISOTOPE DISCRIMINATION AND PHOTOSYNTHESIS

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INTRODUCTION

There are two naturally occurring stable isotopes of carbon, ^{12}C and ^{13}C . Most of the carbon is ^{12}C (98.9%), with 1.1% being ^{13}C . The isotopes are unevenly distributed among and within different compounds, and this isotopic distribution can reveal information about the physical, chemical, and metabolic processes involved in carbon transformations. The overall abundance of ^{13}C relative to ^{12}C in plant tissue is commonly less than in the carbon of atmospheric carbon dioxide, indicating that carbon isotope discrimination occurs in the incorporation of CO_2 into plant biomass. Because the isotopes are stable, the information inherent in the ratio of abundances of carbon isotopes, presented by convention as $^{13}\text{C}/^{12}\text{C}$, is invariant as long as carbon is not lost. Numerous contributions have been made to our understanding of carbon isotope discrimination in plants since this area was extensively reviewed by O'Leary (97). Here we discuss the physical and enzymatic bases of carbon isotope discrimination during photosynthesis, noting how knowledge of discrimination can be used to provide additional insight into photosynthetic metabolism and the environmental influences on that process.

ISOTOPE EFFECTS

Variation in the $^{13}\text{C}/^{12}\text{C}$ ratio is the consequence of "isotope effects," which are expressed during the formation and destruction of bonds involving a carbon atom, or because of other processes that are affected by mass, such as gaseous diffusion. Isotope effects are often classified as being either kinetic or thermodynamic, the distinction really being between nonequilibrium and equilibrium situations. One example of a kinetic effect is the difference between the binary diffusivity of $^{13}\text{CO}_2$ and that of $^{12}\text{CO}_2$ in air. Another example is the difference between the kinetic constants for the reaction of $^{12}\text{CO}_2$ and $^{13}\text{CO}_2$ with ribulose biphosphate carboxylase-oxygenase (Rubisco). Both these examples are called "normal" kinetic effects in that the process discriminates against the heavier isotope. Thermodynamic effects represent the balance of two kinetic effects at chemical equilibrium and are therefore generally smaller than individual kinetic effects. An example of a thermodynamic effect is the unequal distribution of isotope species among phases in a system (e.g. in CO_2 in air versus in CO_2 in solution). Thermodynamic effects, like some kinetic ones, are temperature dependent.

Isotope effects, denoted by α , are also called fractionation factors because they result in fractionations of isotopes. They are here defined (as by some, but not all chemists) as the ratio of carbon isotope ratios in reactant and product

$$\alpha = \frac{R_r}{R_p}, \quad 1.$$

where R_r is the $^{13}\text{C}/^{12}\text{C}$ molar ratio of reactant and R_p is that of the product. Defined in this way, a kinetic isotope effect can be thought of as the ratio of the rate constants for ^{12}C and ^{13}C containing substrates, k^{12} and k^{13} , respectively. Thus

$$\alpha_{\text{kinetic}} = \frac{k^{12}}{k^{13}}. \quad 2.$$

A simple equilibrium isotope effect would be the ratio of the equilibrium constants for ^{12}C and ^{13}C containing compounds, K^{12} and K^{13} , respectively:

$$\alpha_{\text{eqbm}} = \frac{K^{12}}{K^{13}}. \quad 3.$$

Diffusional effects belong to the category of kinetic effects, and the isotope effect is the ratio of the diffusivity of the ^{12}C compound to that of the ^{13}C compound. The above effects are discussed more fully in Part I of the Appendix. Isotope effects may occur at every reaction of a sequence, but the overall isotope effect will reflect only the isotope effects at steps where the reaction is partially reversible or where there are alternative possible fates for atoms, until an irreversible step is reached (97). Kinetic isotope effects of successive individual reactions are usually not additive, but the thermodynamic ones are. If all reactants are consumed and converted to product in an irreversible reaction, there is no fractionation. For example, plants grown in a closed system, where all CO_2 was fixed, showed no isotope effect (6).

ISOTOPIC COMPOSITION AND DISCRIMINATION

Definitions

Farquhar & Richards (39) proposed that whole plant processes should be analyzed in the same terms as chemical processes. From Equation 1 it is evident that this requires measurements of isotopic abundance of both source and product. For plants this means measuring R_a (isotopic abundance in the air) and R_p (isotopic abundance in the plant, where the plant can be considered the product referred to in Equation 1). For numerical convenience, instead of using the isotope effect ($\alpha = R_a/R_p$), Farquhar & Richards (39) proposed the use of Δ , the deviation of α from unity, as the measure of the carbon isotope discrimination by the plant:

$$\Delta = \alpha - 1 = \frac{R_a}{R_p} - 1. \quad 4.$$

The absolute isotopic composition of a sample is not easy to measure directly. Rather, the mass spectrometer measures the deviation of the isotopic composition of the material from a standard,

$$\delta_p = \frac{R_p - R_s}{R_s} = \frac{R_p}{R_s} - 1, \quad 5.$$

where R_s is the molar abundance ratio, $^{13}\text{C}/^{12}\text{C}$, of the standard. The reference material in determinations of carbon isotopic ratios has not normally been CO_2 in air but traditionally has been carbon in carbon dioxide generated from a fossil belemnite from the Pee Dee Formation, denoted PDB [for which $R = 0.01124$, (17)]. In this review all compositions that are denoted δ are with respect to PDB.

In contrast to δ , the discrimination, Δ , is independent of the isotopic composition of the standard used for measurement of R_p and R_a , and is also independent of R_a . Plants show a positive discrimination (Δ) against ^{13}C . Typically C_3 plants have a discrimination of $\sim 20 \times 10^{-3}$, which is normally presented in the literature as 20‰ (“per mil”). Consistent with this notation, we will use ‰ as equivalent to 10^{-3} . Note that “per mil” is not a unit, and is analogous to per cent; discrimination is therefore dimensionless. Equations involving the δ notation have been made unnecessarily complex by including the factor 1000 in the definition (i.e. $\delta_p = (R_p/R_s - 1) \cdot 1000$). We have opted for simplicity, but the reader should note that factors of 1000 in other treatments (including our own) should be deleted when comparing to the equations presented here. Other possible definitions of discrimination are discussed in Part III of the Appendix.

The value of Δ as defined above is obtained from δ_a and δ_p , where a and p refer to air and plant, respectively, using Equation 4, and the definitions of δ_a and δ_p ($R_a/R_s - 1$; $R_p/R_s - 1$, respectively):

$$\Delta = \frac{\delta_a - \delta_p}{1 + \delta_p}. \quad 6.$$

On the PDB scale, free atmospheric CO_2 ($R_a \sim 0.01115$ in 1988) currently has a deviation, δ_a , of approximately -8‰ , and typical C_3 material ($R_p \sim 0.01093$) a deviation, δ_p , of -27.6‰ , which yields $\Delta = (-0.008 + 0.0276)/(1 - 0.0276) = 20.1\text{‰}$. O’Leary (97) pointed out that the simultaneous use of discrimination and δ is confusing for work with plants, since the discrimination values (Δ) are usually positive while those of δ are usually

negative when PDB is the reference. Where possible, it is preferable to use molar abundance ratios (R) and compositional deviations (δ) only as intermediates in the calculation of final isotope effects (97).

Isotopic Composition of Source Air

The advantage of reporting Δ is that it directly expresses the consequences of biological processes, whereas composition, δ_p , is the result of both source isotopic composition and carbon isotope discrimination. This distinction is particularly important in the interpretation of some growth cabinet work where the isotopic composition of CO_2 can be affected by mixing of CO_2 derived from fossil fuel combustion with normal atmospheric CO_2 . Of course, it is relevant for vegetation grown near vents outgassing the CO_2 produced from burning underground coal (for which $\delta_a = -32.5\text{‰}$) (46). Of wider relevance, the distinction between δ and Δ is important when interpreting results from canopies, if turbulent transfer is poor. In these conditions, there is a gradient, with height, in isotopic composition of CO_2 in the air, δ_a . This gradient occurs because of both canopy photosynthetic activity and soil respiration and litter decomposition. On the one hand, since photosynthetic processes discriminate against ^{13}C , the remaining CO_2 in air should be enriched in ^{13}C when CO_2 concentration is drawn down (32, 35). On the other hand, decomposition processes, which release CO_2 with an isotopic composition similar to that of the decaying vegetation, result in a much lower ^{13}C content of the soil CO_2 (1, 68, 116, 122, 123, 148). Francey et al (42) reported a CO_2 concentration of 20 ppm lower, 1 m above the ground, than outside the canopy in the daylight period in a dense (14 m) canopy of huon pine in Tasmania. The difference in δ_a between the top and bottom of the canopy was 0.8‰. In warm and dense tropical rainforests, the CO_2 concentration, c_a , is large near the forest floor, and δ_a is small [$c_a = 389$ ppm, $\delta_a = -11.4\text{‰}$ at 0.5 m (133); see also (88)]. The isotopic composition, δ_a , and CO_2 concentration, c_a , should be negatively related within a canopy (as in the above reports) so that for those field-grown plants where the gradients of c_a are found to be small, the gradient of δ_a is also likely to be small.

The isotopic composition of the free atmosphere also changes, slowly becoming depleted in ^{13}C (41, 45, 70, 92, 108). The progressive decrease in δ_a is caused by the anthropogenic burning of fossil fuels ($\delta \sim -26\text{‰}$). From 1956 to 1982, δ_a has decreased from -6.7‰ (at 314 ppm) to -7.9‰ (at 342 ppm) (70, 92).

There is also an annual cycle of 10 ppm in c_a , and 0.2‰ in δ_a , in the northern hemisphere, associated with seasonal changes in standing biomass; the amplitudes of changes in c_a and δ_a are much smaller in the southern hemisphere (92). In major metropolitan areas, δ_a may vary by as much as 2‰ both daily and annually, because of human activities (64, 65). Throughout

this review when discussing studies where isotopic composition of plant material is presented without corresponding measurements of δ_a ; we also provide an estimate of discrimination (Δ) using the assumption (for field-grown plants) of an atmospheric composition (δ_a) of -8‰ .

"On-line" Measurement of Carbon Isotope Discrimination

In most studies, composition of CO_2 from combustion of plant material (δ_p) has been compared to that of the atmosphere in which the material was grown (δ_a) to yield an average discrimination over the period in which the carbon was fixed. A more direct and nondestructive means of measuring short-term carbon isotope discrimination is to measure the changes in the $^{13}\text{C}/^{12}\text{C}$ ratio of the CO_2 in air as it passes a leaf within a stirred cuvette, such as those commonly used for whole-leaf gas-exchange measurements (32, 36, 62, 125). If the reactions associated with photosynthetic CO_2 fixation discriminate against ^{13}C , the remaining CO_2 should be enriched in ^{13}C . Discrimination can be calculated from measurements of the concentration (c) and the isotopic composition (δ) of the CO_2 of the air entering (c_e and δ_e) and leaving (c_o and δ_o) the cuvette according to an equation derived by Evans et al (32),

$$\Delta = \frac{\xi(\delta_o - \delta_e)}{1 + \delta_o - \xi(\delta_o - \delta_e)} \quad 7.$$

where $\xi = c_e/(c_e - c_o)$. Note that Evans et al (32) used the constant 1000 in the denominator rather than 1, because their values of δ had also been multiplied by 1000.

O'Leary et al (102) used a different "on-line" technique, where the plant was enclosed in a bell jar and allowed to deplete the CO_2 . The continuing isotopic enrichment of the remaining CO_2 was monitored and discrimination calculated from a set of differential equations.

Estimates from these "on-line" methods are usually comparable to those from tissue combustion analyses (32, 62, 125). The clear advantage over tissue combustion of the "on-line" approaches is that they are nondestructive and rapid (~ 30 min), permitting studies of isotope discrimination as a function of time or of physiological and environmental conditions. The measurement of tissue is of course invaluable for longer-term integration, and for the ease with which small amounts of material can be collected, stored, and subsequently analyzed.

THEORY OF CARBON ISOTOPE DISCRIMINATION DURING PHOTOSYNTHESIS

Carbon isotope composition of plants was first used to indicate photosynthetic pathways in plants (2, 3, 89, 93, 106, 120, 127, 128, 130, 144, 145, 150, 151, 156, 159, 160, 163). This is because phosphoenolpyruvate (PEP)

carboxylase, the primary carboxylating enzyme in species having a C_4 metabolism, exhibits a different intrinsic kinetic isotope effect and utilizes a different species of inorganic carbon that has an isotopic composition at equilibrium different from that of Rubisco. Isotopic screening was a simple test for determining the photosynthetic pathway when it was unknown for a species. Over the past 15–20 years, the results of such surveys have provided a broad base of the distribution of photosynthetic pathways among different phylogenetic groups and ecological zones (97, 99, 106). Although major photosynthetic groups could clearly be distinguished by their isotopic composition, the results of these early studies also indicated that there was substantial variation in isotopic values at both the interspecific and intraspecific levels, as well as variation associated with different environmental growth conditions and with variation in dry-matter composition. Substantial theoretical and experimental progress has been made over the past ten years in understanding the biochemical, metabolic, and environmental factors contributing to the different isotopic compositions among plants. The major isotope effects of interest are listed in Table 1 and include kinetic discrimination factors associated with diffusion (denoted by a) and enzyme fractionation (denoted by b), as well as equilibrium discrimination factors (denoted by e). We refer to this table as we review the theory and supporting evidence.

C_3 Photosynthesis

HIGHER PLANTS Several models have been developed to describe the fractionation of carbon isotopes during C_3 photosynthesis (38, 69, 97, 109, 122, 149). The models are similar in structure, each assuming that the major components contributing to the overall fractionation are the differential diffusivities of CO_2 containing ^{12}C and ^{13}C across the stomatal pathway and the fractionation by Rubisco. Each of the models suggests additivity of fractionation factors weighted by the relative “limitation” or CO_2 partial pressure difference imposed by the step involved. Of the models, that of Farquhar et al (38) has been the most extensively developed and tested. Their expression for discrimination in leaves of C_3 plants in its simplest form is,

$$\Delta = a \frac{p_a - p_i}{p_a} + b \frac{p_i}{p_a} = a + (b - a) \frac{p_i}{p_a}, \quad 8.$$

where a is the fractionation occurring due to diffusion in air (4.4‰, a theoretical value that has not been confirmed experimentally), b is the net fractionation caused by carboxylation (mainly b_3 , discrimination by Rubisco; see Table 1 and also Part IV of the Appendix) and p_a and p_i are the ambient and intercellular partial pressures of CO_2 , respectively. Equation 8 is derived in Part II of the Appendix; see also reference 5.

Table 1 Isotope effects of steps leading to CO₂ fixation in plants.

Process	Isotope effect (α)	Discrimination (‰)	Symbol	Reference
Diffusion of CO ₂ in air through the stomatal pore ^a	1.0044	4.4	a	Craig (16)
Diffusion of CO ₂ in air through the boundary layer to the stomata ^a	1.0029	2.9	a_b	Farquhar (33)
Diffusion of dissolved CO ₂ through water	1.0007	0.7	a_l	O'Leary (98)
Net C ₃ fixation with respect to p_i	1.027	27	b	Farquhar & Richards (39)
Fixation of gaseous CO ₂ by Rubisco from higher plants	1.030 (pH = 8)	30	b_3	Roeske & O'Leary (119) ^b
Fixation of HCO ₃ ⁻ by PEP carboxylase	1.029 (pH = 8.5)	20	b_3	Guy et al (50)
	1.0020	2.0	b_4^*	O'Leary et al (101)
	1.0020	2.0		Reibach & Benedict (117)
Fixation of gaseous CO ₂ (in equilibrium with HCO ₃ ⁻ at 25°C) by PEP carboxylase	0.9943	-5.7	b_4	Farquhar (33)
Equilibrium hydration of CO ₂ at 25°C	0.991	-9.0	e_b	Emrich et al (31)
	0.991	-9.0		Mook et al (91)
Equilibrium dissolution of CO ₂ into water	1.0011	1.1	e_s	Mook et al (91)
	1.0011	1.1		O'Leary (98)

^aTheoretical value^bData corrected for dissolution of CO₂

The significance of Equation 8 is that when stomatal conductance is small in relation to the capacity for CO₂ fixation, p_i is small and Δ tends towards 4.4‰ (see Figure 1). Conversely, when conductance is comparatively large, p_i approaches p_a and Δ approaches b (perhaps 27–30‰; see Appendix Part IV). Nevertheless, it is a little dangerous to take the argument further and say that when p_i and Δ are small, stomata are necessarily limiting photosynthesis. That conclusion would only follow if the relationship between assimilation rate, A , and p_i remained linear beyond the operational point (40).

There are several cases where measurements of both Δ and p_i/p_a have been made in controlled conditions. Farquhar et al (35) found a significant correlation between Δ in dry matter and discrete measurements of p_i/p_a among different species over the range of p_i/p_a 0.3–0.85. The best fit, taking a as 4.4‰, was observed with a value for b of 27‰. The leaf with the lowest p_i/p_a was from an *Avicennia marina* plant, which showed discrimination of 11.8‰. Such low values of Δ had previously been considered to be in the range of C₄ plants. Downton et al (using spinach; 21) and Seemann & Critchley (using beans; 124) also observed significant correlations between Δ in dry matter and p_i/p_a , the best fit being obtained by setting b equal to 28.5‰ and 26.4‰,

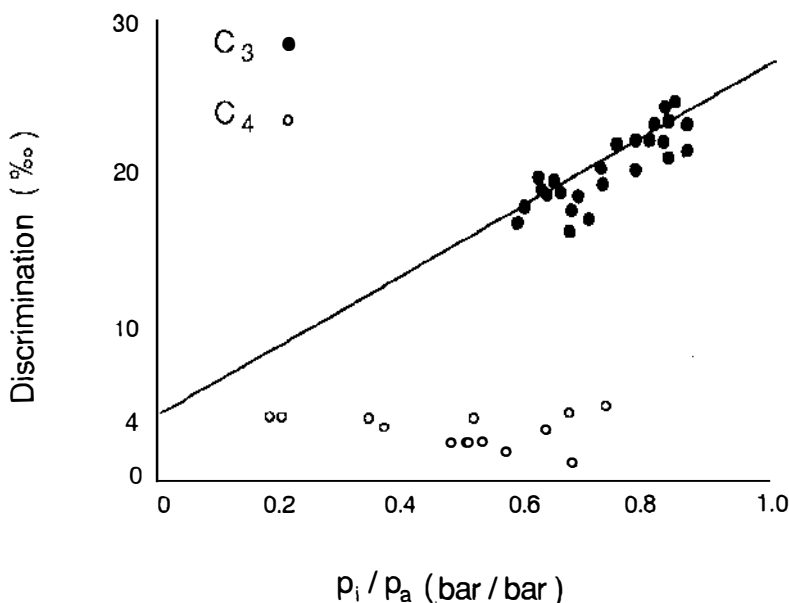


Figure 1 Carbon isotope discrimination, Δ , versus the ratio of intercellular and ambient partial pressures of CO_2 , p_i/p_a , when all are measured simultaneously in a gas exchange system (36). The line drawn is Equation 8 with $a = 4.4\text{‰}$ and $b = 27\text{‰}$.

respectively. However, it should be noted that in none of the above studies was δ_a directly measured. Winter (155) showed that both Δ and p_i/p_a of leaves became smaller as *Cicer arietinum* plants were water stressed. Conversely, Bradford et al (9) showed that both were greater in a tomato mutant lacking abscisic acid (ABA) than in its isogenic parent. Phenotypic reversion of Δ and p_i/p_a occurred when the mutant was sprayed with ABA during its growth. Measurements of mistletoes and their hosts (25, 30) showed interspecific variation in both Δ and p_i/p_a . Guy et al (52) found that increased salinity decreased Δ in *Puccinellia* and p_i/p_a as expected from theory. Over the short term, Brugnoli et al (11) showed that the assimilation-weighted value of p_i/p_a and Δ of sugar produced by a leaf in a single day followed the predicted theoretical relationship (Equation 8) with a fitted value for a of 4.1‰ and for b of $24\text{--}25.5\text{‰}$. The overall discrimination to starch appeared to be slightly smaller. In all of the above cases, Δ , inferred from the carbon composition of leaf material, and p_i/p_a were positively correlated. The values of b that gave the best fit showed variation, which could have many causes (see Part IV of the Appendix for further elaboration).

NONVASCULAR PLANTS Surveys of isotopic composition have been made on species of mosses, liverworts, and lichens. Isotope ratio variation in the

range of -21.3‰ to -37.5‰ ($\Delta = 13.6\text{--}30.4\text{‰}$) has been reported (121, 128, 135, 136).

For mosses, and some liverworts, the gametophytes are morphologically similar to higher plants but are restricted in size by their lack of vascular tissue. Their leaflike photosynthetic structures tend to be just one cell layer thick and do not have the specialized anatomy of higher plants. They do not consistently have an epidermis with impermeable cuticle and stomata, so we might not expect to observe variation in isotope discrimination arising from short-term variation in permeability to gases as with higher plants. It is possible, however, that permeability changes with water content. Even if this resistance remains constant, the gradient in partial pressure across it will change if the flux changes. For example, assimilation rate may change because of differing light levels, and this should increase the gradient and decrease Δ (32). For other liverworts with a thicker thallus and an epidermis, there may be pores that lead to air chambers, like stomata in higher plants, and we would expect to see variation in discrimination similar to that in higher plants.

In contrast to our explanations for variation in Δ in mosses and liverworts, Rundel et al (121) attributed the very negative values of δ_p in mosses in humid conditions to a large content of lipid in the tissue of those species [as lipids are depleted in ^{13}C compared to other plant compounds (97)]. Teeri (135) suggested that these differences may have arisen because of differences in the amount of carbon fixed by PEP carboxylase, but this is unlikely to differ from that in higher plants.

Among lichens, there are differences in carbon isotope discrimination that depend on the phycobionts in the symbiotic association (74, 76). Green algae as phycobionts are able to maintain positive photosynthetic rates when only misted, whereas when cyanobacteria are the phycobionts, surface liquid water is required for photosynthetic activity (75). This difference suggests that the CO_2 diffusion rate may be limiting when cyanobacteria are the phycobionts; correspondingly, the carbon isotope discrimination by lichens with cyanobacteria is 2–4‰ less than that of lichens with green algae. Another possibility is that liquid water is needed for a bicarbonate transport system, which also has a characteristically smaller discrimination (see the section below on algae). A further complication is that discrimination by Rubisco, b_3 , is 21‰ in the only cyanobacterium measured compared to 29‰ in higher plants (50). A great deal more work is required before an equation like Equation 8 could be applied with confidence to lichens.

C₄ Photosynthesis

Variation in isotopic composition among plants with the C_4 photosynthetic pathway is less than in C_3 plants, because the term b from Equation 8 (largely reflecting b_3 , the discrimination by Rubisco, $\sim 30\text{‰}$) is replaced by ($b_4 +$

$b_3\phi$) which is numerically much smaller than b_3 . This is because b_4 (the effective discrimination by PEP carboxylase) is $\sim -5.7\text{‰}$ (see Table 1) and ϕ [the proportion of the carbon fixed by PEP carboxylation that subsequently leaks out of the bundle sheath, thereby allowing limited expression of Rubisco discrimination (b_3)] is necessarily less than unity (33). The bases for this model of discrimination are as follows: CO_2 diffuses through stomata to the mesophyll cells, where it dissolves (e_s) and is converted to HCO_3^- (e_b). At equilibrium, the heavier isotope concentrates in HCO_3^- compared to gaseous CO_2 —i.e. the combined terms $e_s + e_b$ are negative (Table 1). In turn, PEP carboxylase discriminates against $\text{H}^{13}\text{CO}_3^-$ —i.e. b_4^* is positive and normal for a kinetic effect. Thus if the gaseous intercellular CO_2 is in equilibrium with HCO_3^- , then the net discrimination from CO_2 to OAA is

$$b_4 = e_s + e_b + b_4^* \quad 9.$$

which is negative because of the magnitude of e_b . Various transformations then occur, depending on C_4 subtype, but the net result in all cases is that CO_2 is released within the bundle sheath cells and refixed by Rubisco. There is little opportunity for discrimination in the release of CO_2 in the bundle sheath cells because of the lack of significant biochemical branches. No further discrimination would occur if the bundle sheath were gas tight (153). However, some quantities of CO_2 and HCO_3^- are likely to leak out of these cells and into the mesophyll cells, especially through the apoplastic portions of the bundle sheath cells, where they can then mix with other CO_2 that has diffused in through the stomata. The leak is a branch from the main path of carbon and allows some discrimination by Rubisco in the bundle sheath cells (b_3).

Various models (18, 33, 56, 110, 117) have addressed aspects of the ^{13}C discrimination during C_4 photosynthetic metabolism. Intrinsic to all of these models is the notion that variation in isotopic composition in C_4 plants is associated with leakage of CO_2 and/or HCO_3^- . The “leakiness” (ϕ) may also be regarded as a measure of the “overcycling” by PEP carboxylase that occurs in mesophyll cells, raising the partial pressure of CO_2 within the bundle sheath cells (33).

Farquhar (33) developed an expression for the discrimination occurring in C_4 photosynthesis, in which

$$\Delta = a \frac{p_a - p_i}{p_a} + (b_4 + b_3\phi) \frac{p_i}{p_a} \quad \frac{p^*}{p_a} \quad 10.$$

Depending whether $(b_4 + b_3\phi - a)$ is positive, zero, or negative, the dependence of Δ on p_i/p_a will be positive, zero, or negative. Experimental evidence suggests that the factor is often close to zero, with short-term

discrimination responding little to variation in p_i/p_a (32, 36, and see Figure 1). From Table 1 it may be seen that the zero value is obtained with $\phi = 0.34$.

Farquhar (33) and Hattersley (56), using the Farquhar model, predicted that bundle sheath "leakiness" above $\phi = 0.37$ (the value differs from 0.34 because a smaller value was assumed for b_3) should result in a positive response of Δ to increasing p_i/p_a . Anatomical variations between C_4 types (55) may be associated with variations in ϕ . For example, Ehleringer & Pearcy (29) observed that quantum yields for CO_2 uptake are lower for all C_4 dicots and NAD-ME (malic enzyme) C_4 type grasses than for NADP-ME or PCK (phosphoenolpyruvate carboxykinase) types, which have bundle sheaths with suberized lamellae (12, 57). Diminished quantum yields are to be expected as a result of increased leakiness—i.e. increased overcycling within the mesophyll cells. The measured differences in carbon isotope discrimination by NAD-ME, NADP-ME, and PCK type C_4 grasses as deduced from isotopic composition (10, 56, 150, 163) and from "on-line" measurements (32, 36) are consistent with the expectation that leakage is greatest in the first type. Because ϕ is a measure of the overcycling as a proportion of the rate of PEP carboxylation, it is likely that Δ will increase whenever Rubisco activity is diminished more than PEP carboxylase activity by some treatment. Thus ϕ and Δ depend as much on coordination of mesophyll and bundle sheath activity as on anatomical features.

C₃-C₄ Intermediacy

Monson et al (90) measured isotopic composition of 6 C_3 - C_4 intermediate species in *Flaveria* and reported Δ values of 9.6–22.6‰. They suggested that the isotopic variation resulted from differences in bundle sheath leakage (according to Equation 10). While this probably accounts for some of the variation, another biochemical factor may also be important. The C_3 - C_4 "intermediate" species appear to have glycine decarboxylase confined to the bundle sheath cells (63, 115). The effect is that CO_2 released by photorespiration is released and partially refixed in the bundle sheath, so that discrimination by Rubisco can occur twice (S. von Caemmerer, unpublished). The modification to b , the C_3 carboxylation parameter from Equation 8, is thus the product of the proportion, A_s/A , of carbon fixed twice (where A_s is the rate of assimilation in the bundle sheath, and A that by the whole leaf), and ϕ , the proportion of the carbon supplied to the bundle sheath that leaks out. In the simplest form the equation becomes (G. D. Farquhar, unpublished)

$$\Delta = a \frac{p_a - p_i}{p_a} + b \left(1 + \frac{\phi A_s}{A} \right) \frac{p_i}{p_a} = a + \left[b \left(1 + \frac{\phi A_s}{A} \right) - a \right] \frac{p_i}{p_a}. \quad 11.$$

This theoretical prediction awaits experimental testing.

Crassulacean Acid Metabolism

The details of Crassulacean acid metabolism (CAM) that affect Δ have been recently reviewed by O'Leary (99). In this section, we present equations for Δ analogous to those discussed earlier for C_3 and C_4 carbon assimilation pathways. Plants in the full CAM mode take up CO_2 and synthesize oxaloacetate using PEP carboxylase, and the oxaloacetate (OAA) is then reduced and stored as malate (103). At dawn the plants close their stomata and decarboxylate the malate, refixing the released CO_2 using Rubisco. The malate that is stored at night will show the same discrimination as for C_4 species with zero leakage (33), i.e.

$$\Delta = a + (b_4 - a) \frac{p_i}{p_a} \quad 12.$$

Winter (154) reported that nocturnal values of p_i/p_a in *Kalanchoe pinnata* started at a C_4 -like value (~ 0.4) and increased with time to a C_3 -like value (~ 0.7) before dawn. On this basis, we could expect instantaneous Δ of the carbon being fixed to have decreased from ~ 0.4 to -2.7‰ as the night progressed. This is consistent with observations that Δ of crystalline oxalate and of carbon-4 of malic acid were near to zero (58, 100).

If the stomata closed completely at dawn, the photosynthetic tissue would form a closed system and there would be no fractionation of the carbon between malate and the sugar products. However, consider the case where the stomata were not closed in the light while a CAM plant was enclosed in a cuvette with no external CO_2 . In this case, there should be a discrimination in going from malate to the new C_3 carbon because we no longer have a closed system. The discrimination is given by $\phi(b_3 - a)$, where ϕ is the proportion of decarboxylated carbon that leaks out of the leaf. Nalborczyk (94) allowed CAM plants to fix CO_2 only at night and found that the overall discrimination was $\sim 3\text{‰}$. This result implies that ϕ was about 0.05–0.15. However, when CAM plants are growing in normal air, evolution of CO_2 in the light is usually negligible.

Toward the end of the light period, after decarboxylation of all the stored malate, there is sometimes CO_2 uptake [denoted phase IV by Osmond (103)] via Rubisco, and possibly involving PEP carboxylase as well. Nalborczyk et al (95) allowed plants to fix carbon only in the light and observed a discrimination of 21‰, which is what one would expect with a typical C_3 value of p_i/p_a in Equation 8.

Therefore in the simplest case of C_4 fixation in the dark and C_3 fixation in the light, the average discrimination over a 24 hr period is

$$\Delta = a + \frac{\int^D A(b_4 - a) \frac{p_i}{p_a} dt + \int^L A(b_3 - a) \frac{p_i}{p_a} dt}{\int^D A dt + \int^L A dt}, \quad 13.$$

where A is the assimilation rate, $\int^D dt$ denotes the time integral in the dark, and $\int^L dt$ that in the light, and b for the light period is the average of b_3 and b_4 weighted by the rates of RuBP and PEP carboxylation (if the latter occurs), respectively.

Aquatic Plants and Algae

Carbon isotope combinations measured in aquatic plants range between -11‰ and -39‰ , potentially leading to the mistaken impression that both C_3 and C_4 photosynthetic pathways are present in aquatic plants (4, 22, 105, 113, 132). However, with limited exceptions (86, 147), C_4 plants are not known from aquatic habitats. When CO_2 fixation is via the normal C_3 pathway, Equation 8 applies, but with the parameter a modified to reflect diffusion in the aqueous phase ($e_s + a_l$) so that

$$\Delta = (e_s + a_l) \frac{p_a - p_c}{p_a} + b \frac{p_c}{p_a} = e_s + a_l + (b - e_s - a_l) \frac{p_c}{p_a}, \quad 14.$$

where the equivalent partial pressure of CO_2 at the site of carboxylation is denoted as p_c . Note that the discrimination during diffusion of CO_2 in water (a_l) is 0.7‰ (98) and not 11‰ as some authors have written. Much of the diffusion of inorganic carbon in the aqueous environment will be as bicarbonate rather than CO_2 , but the discrimination here should also be small (38). Note further that the discrimination is with respect to gaseous CO_2 in equilibrium with the aqueous environment.

However, there is a widespread mechanism(s) among marine and freshwater organisms for raising the concentration of CO_2 at the site of carboxylation above that of the environment (5, 80). Farquhar (33) suggested that the equation for C_4 discrimination could be adapted to describe discrimination if the active species transported is bicarbonate as

$$\begin{aligned} \Delta &= (e_s + a_l) \frac{p_a - p_c}{p_a} + (e_s + e_b + b_m + b_3\phi) \frac{p_c}{p_a} \\ &= e_s + a_l + (e_b + b_m + b_3\phi - a_l) \frac{p_c}{p_a}, \end{aligned} \quad 15.$$

where b_m is the fractionation during membrane transport. The value of b_m is unknown, but it has been cautiously assumed to be small, making $(e_s + e_b +$

b_m), which is the analog for b_4 from the C_4 model, close to -7.9% (33). Note that in both Equations 14 and 15 the discrimination is again expressed in relation to a gaseous source. As with Equation 14, the discrimination in relation to dissolved CO_2 as the source, provided it were in equilibrium with the gas phase, would be found by subtracting e_s and with reference to bicarbonate (again, if in equilibrium) would be found by subtracting $e_s + e_b$. However, it is convenient to retain the same convention for source carbon as used for aerial plants (i.e. gaseous CO_2), especially when we have chosen gaseous CO_2 as our substrate for carboxylation by Rubisco (see definition of b_3 in Table 1). The latter choice is also reasonable in a mechanistic sense because the Rubisco site, with RuBP bound, probably reacts with gaseous substrates only.

The effects on Δ of induction of active carbon accumulation were elegantly demonstrated by Sharkey & Berry (125). The green alga *Chlamydomonas reinhardtii* was grown at 5% CO_2 and then transferred to normal air levels of CO_2 . Before transfer, Δ was 27–29‰, and after 4 hr of induction Δ was 4‰. Sharkey & Berry (125) discussed their results in terms of slightly simplified versions of Equations 14 and 15. Berry (5) noted that measurements of Δ alone are insufficient to distinguish between a CO_2 concentrating mechanism (Equation 15) and a normal C_3 mechanism (Equation 14) with a large resistance to diffusion. In both cases, Δ is small because most of the CO_2 reaching Rubisco is fixed.

ENVIRONMENTAL EFFECTS ON CARBON ISOTOPE DISCRIMINATION

Goudriaan & van Laar (47), Körner et al (72), and Wong et al (161) were among the first to note a strong correlation between the photosynthetic rate and leaf conductance. This correlation was maintained over a wide variety of plant species and under a diversity of environmental treatments, implying some level of regulation between CO_2 demand by the chloroplasts and CO_2 supply by stomatal control. If in fact there were no deviations from the slope of the photosynthesis-versus-conductance relationship and if the intercept were zero (as was the case in the original papers), then the intercellular CO_2 pressure (p_i) of all plants would have been constant, dependent only on photosynthetic pathway. This constancy was mistakenly suggested in at least one early review (126). Although a number of studies that followed showed a significant tendency for photosynthesis and conductance to be correlated (161), many of these data sets exhibited some deviation from a linear relationship or a nonzero intercept (112, 152). It is unfortunate that in the search for general patterns the variance in p_i was, for a time, ignored. When it was recognized that there was a fundamental relationship between Δ or δ_p and p_i ,

more effort was put into documenting and understanding the isotopic variation at both the environmental and genetic (intra- and interspecific) levels. In the next sections, we describe what is known about the relationship between p_i (as measured by isotope discrimination) and environmental parameters.

Light

While some of the first experiments reported no consistent pattern between leaf isotopic composition, δ_p , and irradiance (129), later studies have indicated that δ_p increased with an increase in growth irradiance. Interpretation of carbon isotope composition of leaves experiencing different light levels has been somewhat controversial. The controversy lies in separating the effects of light on discrimination from correlated effects on δ_a (source air), both of which affect leaf carbon isotopic composition. In field studies, Vogel (148) was among the first to describe a consistent pattern of isotopic variation in leaves under canopy conditions where light levels varied substantially. He noted that δ_p within a canopy decreased by 3‰ between the top (19 m) and bottom (1 m) of the canopy. He further noted that the isotopic composition of soil CO₂ was approximately -19‰, while that of the atmosphere was only -7‰. He attributed all of the decrease in δ_p of leaves at lower layers to a recycling of soil CO₂ (a lighter source CO₂), although the isotopic composition of CO₂ within the canopy, δ_a , was not measured. He calculated that recycled CO₂ accounted for 15% of the carbon incorporated in lower leaf layers—assuming that the physiological discrimination was constant. Medina & Minchin (87) pursued these observations, reporting $\delta^{13}\text{C}$ gradients of 4.7 and 5.6‰ between upper and lower canopy leaves for two different tropical-forest types. Again the decrease in $\delta^{13}\text{C}$ of leaves at lower levels was attributed to a lighter source CO₂, with the implication that as much as 20% of the carbon fixed in lower leaf layers was derived from soil respiration. A third study by Schleser & Jayasekera (122) reports a similar pattern for forest beech and isolated lime trees. Again, they attributed this result to recycled soil CO₂.

Some recent studies have examined both δ_a and δ_p . In their study in a huon pine forest but without δ_a decreasing in a corresponding manner, which indicates a physiological effect. They found that leaves from lower in the canopy had greater p_i values than those from the upper canopy, suggesting, according to Equation 8, a greater discrimination in lower leaves. Ehleringer et al (27, 28) observed a similar pattern with ten shrub and tree species from a subtropical monsoon forest. Leaf δ_p decreased (i.e. became more negative) and p_i increased as observations were made deeper in the canopy. Furthermore, when only outer canopy leaves were measured on plants with differing degrees of canopy closure, δ_p was decreased with decreasing irradiance, consistent with the model of increasing p_i at lower light levels. These measurements were

confirmed with gas exchange observations of the dependence of p_i on irradiance. While it is undoubtedly true that a fraction of the soil CO_2 is incorporated within leaves at the lower canopy level, much of the decrease in leaf isotopic composition is likely to be associated with stomatal and photosynthetic effects. Higher p_i values in understory leaves are likely to benefit plant performance when leaves are exposed to higher irradiances during sunflecks and when leaves are allowed to operate at higher quantum yields (71, 107). In the field, effects of irradiance on p_i are difficult to separate from those of leaf-to-air vapor pressure difference (vpd). The smaller vpd at the bottom of the canopy could also cause greater p_i , and greater Δ (another complication is discussed after Equation A13 in the Appendix).

Water

PHYSIOLOGICAL RESPONSE TO DROUGHT When soil moisture levels are decreased, a common response is simultaneous decreases in photosynthesis, transpiration, and leaf conductance (40). If the "supply function" of photosynthesis (leaf conductance) decreases at a faster rate under stress than the "demand function" [photosynthetic dependence on p_i , *sensu* Farquhar & Sharkey (40)], then p_i will decrease. This effect should be measurable as either an increase in δ_p or correspondingly as a decrease in Δ . Over the short term when new growth has not occurred, the impact of stress can be detected in carbohydrate fractions within leaves (11, 163a, 81). Alternatively, the reduction in p_i/p_a can be measured using the "on-line" approach (62). In longer-term observations under both growth-chamber and field conditions, plants under water stress induced by low soil moisture availability produced leaves with lower p_i values as estimated by carbon isotopic composition (19, 23, 26, 39, 59–62, 131, 140, 155). Increasing the soil strength (physical resistance to root penetration), such as might occur in drier soils, induces a reduction of Δ , as observed with reduced soil moisture levels (84).

An increase in the leaf-to-air vapor pressure difference will also cause diminution of p_i and Δ in the short term (11) and long term (35, 39, 157).

PHOTOSYNTHETIC PATHWAY SWITCHING In response to changes in leaf water status, a number of species show dramatic shifts in carbon isotope composition (up to 10–15‰) associated with changes in photosynthetic metabolism. Thus upon exposure to increased drought, some species can shift from C_3 to CAM photosynthesis (8, 54, 67, 78, 137–140, 146, 158). Correspondingly, there is an increase in δ_p (decrease in Δ). This shift in metabolism is reversible, dependent primarily on plant water status, and can occur in both annual and perennial leaf succulents of arid habitats. Other plants, notably "stranglers" of tropical habitats, exhibit CAM metabolism as epiphytic juveniles, but later switch to C_3 metabolism when roots reach the soil surface (111, 134, 143).

PHOTOSYNTHETIC TWIGS AND STEMS In an interesting twist on the photosynthetic-shift theme, at least two stem succulents native to southern Africa exhibit C_3 metabolism in the leaves (which are shed early in the drought period) and CAM in the stems (77, 142). In recent studies on green-twig plants from arid lands of North America, high rates of photosynthesis have been observed in twig tissues that are comparable to those observed in leaves (13, 24, 104, 131). Unlike the previous example, the twigs of these species all have C_3 photosynthesis. In all such species examined to date, p_i values as measured by gas exchange techniques are lower in twig than leaf tissues, leading to a significant difference in carbon isotopic composition of the two tissue types. Thus, in these cases, the decrease in Δ of the twigs is associated with increased diffusional constraints rather than with a change in metabolic pathway as described in the previous section.

Salinity

In nonhalophytic species, increased salinity has numerous metabolic effects (48). Stomatal closure is typically associated with increased salinity (20, 21, 79, 124). Thus it should not be surprising to note that in those species Δ decreased with increasing salinity, indicating a decrease in p_i with increasing stress (124). What is perhaps more intriguing is that halophytic species also exhibit a similar pattern whether in field or laboratory conditions (35, 51–53, 96, 163a).

Air Pollution

A long-term consequence of exposure to air pollutants (e.g. ozone, sulfur dioxide) at the leaf level is normally a decrease in both leaf conductance and photosynthesis (118). It is not clear, however, whether this decrease in gas exchange represents overall decline in metabolic activity or an increased diffusion limitation imposed by stomata. In each of the limited number of studies available that examine carbon isotope discrimination by leaves of plants exposed to pollutants, exposed plants exhibited lower Δ values, suggesting lower p_i (43, 49, 81). Changes in isotopic composition of leaf tissues from these studies of 1‰ or greater were common even under modest exposures to air pollution. Under long-term, chronic exposure to air pollutants, clear differences exist in the carbon isotope ratios of the wood of annual growth rings that are consistent with short-term, leaf-level observations (43, 44, 81).

WATER-USE EFFICIENCY OF C_3 SPECIES

Transpiration Efficiency and Carbon Isotope Discrimination

Measurements of Δ in C_3 species may usefully contribute to the selection for transpiration efficiency—i.e. the amount of carbon biomass produced per unit water transpired by the crop.

The instantaneous ratio of CO₂ assimilation rate of a leaf, A , to its transpiration rate, E , is given approximately by

$$\frac{A}{E} = \frac{p_a - p_i}{1.6\nu}, \quad 16.$$

where ν is the water vapor pressure difference between the intercellular spaces and the atmosphere. The factor 1.6 arises because the binary diffusivity of water vapor and air is 1.6-fold greater than that of CO₂ and air. Equation 16 may be rewritten as

$$\frac{A}{E} = \frac{p_a \left(1 - \frac{p_i}{p_a}\right)}{1.6\nu}, \quad 17.$$

to emphasize that a smaller value of p_i/p_a is equivalent to an increase in A/E , for a constant water vapor pressure difference, ν . Thus selecting for lower p_i/p_a should be, to a first approximation, a screen for greater A/E , which, in turn, is a component of transpiration efficiency. From Equation 8, Δ may be used as a surrogate measure of p_i/p_a in C₃ plants.

In all of the experiments relating gas exchange properties and short- and long-term discrimination (see the section above on C₃ photosynthesis) and where vapor pressure difference, ν , was maintained constant, the ratio of assimilation and transpiration rates, A/E , was negatively related to Δ , as expected from Equation 17. However, during whole-plant growth, losses of carbon and water occur that are not included in Equation 17. A proportion, ϕ_c , of the carbon fixed via the stomata during the day is lost from the shoot at night or from nonphotosynthetic organs such as the roots, during both the day and night. Further, some water is lost from the plant independently of CO₂ uptake. The stomata may not be completely closed at night, cuticular water loss occurs, and there is unavoidable evaporative loss from the pots in whole-plant experiments. If this "unproductive" water loss is a proportion, ϕ_w , of "productive" water loss, Equation 17 may be modified to describe the molar ratio, W , of carbon gain by a plant to water loss

$$W = \frac{p_a \left(1 - \frac{p_i}{p_a}\right) (1 - \phi_c)}{1.6\nu(1 + \phi_w)}, \quad 18.$$

which, when combined with Equation 8, predicts a negative linear dependence of W on Δ (38, 60). By substitution, Equation 18 can be rewritten as

$$W = \frac{p_a \left(\frac{b - d - \Delta}{b - a} \right) (1 - \phi_c)}{1.6\nu(1 + \phi_w)}, \quad 19.$$

where d is a correction related to assimilation rate (see Part III of the Appendix). The data from pot experiments using a combination of watering treatments and genotypes fit the theory reasonably well for a number of species—wheat (39, 84), peanuts (61, 62, 162), cotton (59), tomato (83), and barley (60). We suggest that future studies will provide better understanding of the relationships between W and Δ when account is taken of environmental and genetic effects on ϕ_c and ϕ_w .

Scaling from the Plant to the Canopy

Water-use efficiency is difficult to measure in the field. There have, however, been a few attempts to relate it to Δ , or at least to relate yield under water-limited conditions to Δ . Wright et al (162) measured total above-ground biomass yield and water use of eight peanut genotypes receiving adequate water (under a rain-excluding shelter). They obtained a negative relationship between W and leaf Δ .

There are several reasons why the negative relationship between W and Δ , given by Equation 19, might work well for individual plants in pots, or even for small plots in the field, but become inconsistent over larger areas. The uncontrolled loss of water is not an independent, fixed proportion (ϕ_w) of transpiration because, for example, soil evaporation tends to be negatively related to leaf area development. If ν fluctuates, then those genotypes that might grow more when ν is small will obtain a greater W for the same Δ .

Equation 19 also contains a simplification that becomes more problematic with increase of scale. The equation is written as if the vapor pressure difference, ν , were an independent variable. To some extent, however, it must vary as stomatal conductance, g_s , changes (as is the case for a single leaf). A reduction in g_s , and therefore in E , means more heat has to be lost by sensible heat transfer. The presence of a leaf boundary layer resistance to the transfer of heat translates this into an increase of leaf temperature and of ν and so the effect of decreased g_s on E is moderated. This moderating effect increases as the ratio of boundary layer resistance to stomatal resistance increases. With a sufficiently high ratio, the proportional reduction in E caused by partial stomatal closure is no greater than the associated proportional reduction in A . Farquhar et al (36) discussed the above problems and defined the conditions that would be necessary for A/E to become independent of stomatal conductance, p_i/p_a and Δ .

The problem is exacerbated in the field, where the aerodynamic resistance of the crop has to be taken into account. If the canopy and leaf boundary layer

resistances to heat are very large, there is the possibility that a genotype with a greater stomatal conductance than another otherwise identical genotype will have a greater value of W (15), despite also having a greater Δ (36). This is more likely to occur at high temperatures. On the other hand, it is less likely to occur when crops have very small leaf area indexes, as would normally be the case under conditions where stress occurs early, and in crops sown in areas prone to severe, early water stress, because under these conditions the crop is more closely "coupled" to the atmosphere, like an isolated plant (15, 66). If the source of variation in Δ is the capacity for photosynthesis, the effects of boundary layers are unimportant (15). This appears to be the case for peanuts (62). Therefore at the crop level, identification of the causes underlying differences in Δ may become important—differences in conductance having different micrometeorological consequences from differences in photosynthetic capacity.

Carbon Isotope Discrimination and Plant Growth Characteristics

Hubick et al (62) found a negative relationship between dry matter production and Δ of peanut cultivars grown in field trials. On the other hand Condon et al (14) saw a positive relationship between yield and Δ for wheat cultivars in two years that included periods of greater than usual rainfall. The sign of the relationship under well-watered conditions is difficult to predict. It is clear that any associations between Δ and patterns of carbon partitioning will be important. The relative growth rate, r (sec^{-1}), of a plant depends on the assimilation rate per unit leaf area, A ($\text{mol C m}^{-2} \text{sec}^{-1}$), and the ratio of total plant carbon to leaf area, ρ (mol C m^{-2}), according to the following identity (84)

$$r = \frac{lA(1 - \phi_c)}{\rho}, \quad 20.$$

where l is the photoperiod as a proportion of a day. Masle & Passioura (85) observed that wheat seedlings grew more slowly in soil of increased strength than in controls. Masle & Farquhar (84) showed that ρ increased with increasing soil strength. They also found that Δ decreased with increasing soil strength. Changing soil strength thus induced a negative relationship between ρ and Δ . They noted that a similar, negative, but genetic association between ρ and Δ would tend to cause a positive relationship between growth rate and Δ . A negative association between ρ and Δ has been observed among wheat and sunflower genotypes during early growth (J. Virgona, personal communication). If ν is low early in the life of a crop, then a positive association between Δ and relative growth rate among genotypes will confound the relationship between final W and Δ .

Genetic Control of Discrimination

Genetic studies of W , p_i/p_a , and Δ are in their infancy. These traits are most likely to be polygenic, since any gene that affects either assimilation rate per unit leaf area or stomatal conductance can have an effect. Despite the considerable genetic and environmental (nutrition, light intensity, etc) effects on the individual components A and g , separately, it is likely that the variation in the ratio A/g , and hence in p_i/p_a and Δ , is smaller, because of coordination between A and g (37). The coordination can lead to predictable genotypic differences in p_i/p_a and Δ as assessed from gas exchange (62), as well as in Δ assessed from δ_p .

The genetic control of Δ appears to be strong in wheat. Condon et al (14) showed that genotypic ranking was maintained at different sites and between plants grown in pots and in the field. The broad sense heritabilities [proportion of total variance of Δ that can be ascribed to genotype, rather than to environment or to interactions between the two ($G \times E$)] ranged between 60 and 90%. From analyses of Δ in 16 peanut genotypes grown at 10 sites in Queensland, Hubick et al (62) calculated an overall broad sense heritability of 81%. With *Phaseolus vulgaris* in Colombia, it was 71% (23). Hubick et al (62; and see earlier discussion in reference 36) examined the progeny of a cross between Tifton 8, a peanut genotype having a small value of Δ , and Chico, which has a large value of Δ . Statistical analyses of measurements of Δ and W in the F_2 generation gave estimates for the heritability of 53% for Δ and 34% for W . The phenotypic correlation between W and Δ was -0.78 . As expected, the Δ values of F_2 plants were highly variable and there were several transgressive segregants with values of Δ lower than those of Tifton 8.

The Δ values of the F_1 generation of the Tifton 8 and Chico cross, while somewhat intermediate between the two parents, were very close to those of Tifton 8 in Δ and W . Martin & Thorstenson (83) examined the F_1 plants from a cross between *Lycopersicon pennellii*, a drought-tolerant species related to tomato, with tomato itself, *Lycopersicon esculentum*. *L. pennellii* had a lower Δ than *L. esculentum*, and again Δ of the F_1 was intermediate, but closer to the low- Δ parent. Both sets of data suggest some dominance of the low- Δ attribute.

Genetic analysis of a polygenic trait like Δ is obviously difficult, yet considerable progress has recently been made using modern techniques. Martin et al (82) reported that 70% of the variance for Δ in a variable tomato population derived from further generations of the above cross was associated with three restriction fragment length polymorphisms (RFLPs)—i.e. genetic markers identifying discrete DNA sequences within the genome. Additive gene action was observed in the three cases, and in one of them, there was also a significant nonadditive component. This kind of work may enable breeders to follow the results of backcrossing material with desirable Δ into

commercial cultivars. However, in parallel with pursuing research on the genetic control of carbon isotope discrimination by the plant, it is important to establish what values of Δ are appropriate in a particular environment and for a particular species. This requires extensive physiological work at different scales, from the organelle to the canopy, and a much better understanding of the interactions among plants, canopies, and their microclimates.

CONCLUDING REMARKS

Carbon isotope discrimination has become a tool to help us understand photosynthesis and its coordination with water use in ecological and physiological studies of C_3 species. Future work will relate these more to growth characteristics and will differentiate between effects of photosynthetic capacity and stomatal conductance. The latter may perhaps be studied using observations of isotopic composition of organic oxygen and hydrogen (36). These compositions are affected by the ratio of ambient and intercellular humidities and should therefore reflect changes in the energy budgets of leaves, which are themselves influenced by stomatal conductance.

It is possible that measurements of Δ in C_4 species may aid in seeking changes in coordination between mesophyll and bundle sheath tissue during photosynthesis, perhaps revealing differences in quantum requirements.

Technological advances in combining gas chromatography and isotope ratio mass spectrometry should facilitate measurements of carbon isotope discrimination between and within organic compounds, thereby increasing our ability to identify origins of materials and to study the nature of the control of metabolic pathways following photosynthesis.

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APPENDIX

Part I. Definitions Isotope effects (α) are here defined as the ratio of carbon isotope ratios in reactant and product (39)

$$\alpha = \frac{R_r}{R_p}, \quad A1.$$

where R_r is the $^{13}\text{C}/^{12}\text{C}$ molar ratio of reactant and R_p is that of the product.

In a first order kinetic reaction, the definition is obvious, i.e.

$$\alpha = \frac{k^{12}}{k^{13}}, \quad \text{A2.}$$

where k^{12} and k^{13} are the rate constants for reactions of the respective isotopic substances. Higher-order kinetic reactions including Michaelis-Menten ones (38) can be treated similarly (102), and k^{12} and k^{13} become pseudo-first-order rate constants. The isotope effect associated with diffusion is the ratio of the ^{12}C and ^{13}C diffusivities. The analogy with Equation A1 is the diffusion from a source (reactant) to a sink where the "product" is kept at a vanishingly small concentration. In an equilibrium, the "product" is the carbon-containing compound of interest on the right-hand side of an equilibrium reaction. So if the reaction of interest is



where A and B might be CO_2 and HCO_3^- , for example, then application of this rule yields

$$\alpha = \frac{\frac{A^{13}}{A^{12}}}{\frac{B^{13}}{B^{12}}} = \frac{K^{12}}{K^{13}}, \quad \text{A4.}$$

where K^{12} is the equilibrium constant,

$$K^{12} = \frac{k_1^{12}}{k_{-1}^{12}}, \quad \text{A5.}$$

for the ^{12}C compounds and K^{13} is the analogous constant for ^{13}C compounds. Note that the equilibrium isotope effect, α , is the kinetic isotope effect for the forward reaction (α_1) divided by that of the reverse reaction (α_{-1})—i.e.

$$\alpha = \frac{K^{12}}{K^{13}} = \frac{\left[\frac{k_1^{12}}{k_{-1}^{12}} \right]}{\left[\frac{k_1^{13}}{k_{-1}^{13}} \right]} = \frac{\left[\frac{k_1^{12}}{k_{-1}^{13}} \right]}{\left[\frac{k_{-1}^{12}}{k_{-1}^{13}} \right]} = \frac{\alpha_1}{\alpha_{-1}}. \quad \text{A6.}$$

It is pleasing then that the forms of the isotope effect (α) for kinetic effects (k^{12}/k^{13}) and equilibrium effects (K^{12}/K^{13}) are superficially similar. We denote the discrimination for either effect as α minus one (39). In most cases

discrimination associated with a kinetic effect will be positive, but there is no a priori reason why a thermodynamic discrimination should be positive.

Part II. Discrimination in a simple two stage model—diffusion followed by carboxylation The carbon isotope ratio of CO_2 in air is R_a , and in the plant product is R_p . In turn R_p must be the same as the ratio of $^{13}\text{CO}_2$ assimilation rate, A^{13} , and $^{12}\text{CO}_2$ assimilation rate, A [no superscript is given here for a variable relating to the major isotope ^{12}C —i.e.

$$R_p = \frac{A^{13}}{A}. \quad \text{A7.}$$

Further, if the isotope effect associated with carboxylation is $1 + b$, then we must have

$$\frac{R_i}{R_p} = 1 + b, \quad \text{A8.}$$

where R_i is the carbon isotope ratio of the intercellular CO_2 .

In turn, R_i is simply found by relating A to g (conductance) and P (total pressure). Thus,

$$A = \frac{g(p_a - p_i)}{P}. \quad \text{A9.}$$

The kinetic isotope effect for diffusion is the ratio of the diffusivities of $^{12}\text{CO}_2$ and $^{13}\text{CO}_2$ in air. Thus,

$$1 + a = \frac{g}{g^{13}}, \quad \text{A10.}$$

and so

$$A^{13} = \frac{g(R_a p_a - R_i p_i)}{(1 + a)P}. \quad \text{A11.}$$

Substituting Equations A9 and A11 in A7,

$$R_p = \frac{R_a p_a - R_i p_i}{(1 + a)(p_a - p_i)}.$$

Rearranging,

$$\frac{R_a}{R_p} = (1 + a) \frac{p_a - p_i}{p_a} + \frac{R_i}{R_p} \frac{p_i}{p_a}.$$

Thus, using the definition of discrimination and Equation A8

$$\alpha = 1 + \Delta = \frac{R_a}{R_p} = (1 + a) \frac{p_a - p_i}{p_a} + (1 + b) \frac{p_i}{p_a}.$$

Thus

$$\Delta = a \frac{p_a - p_i}{p_a} + b \frac{p_i}{p_a},$$

which is Equation 8 from the main text. Note that no assumption of linearity is made about the response of A to p_i in the derivation of this equation.

Part III. Alternative definitions of discrimination There are other possible definitions of discrimination. For example one could write

$$\text{Discrimination}^* = 1 - \frac{R_p}{R_a}.$$

This would correspond to $(1 - k^{13}/k^{12})$ for kinetic effects and to $(1 - K^{13}/K^{12})$ for equilibrium effects. The asterisk superscript is added to emphasize that the numerical values obtained differ from those made using Equation 4. On this basis

$$\Delta^* = \frac{\delta_r - \delta_p}{1 + \delta_r}.$$

The numerical differences between this and our chosen definition of discrimination are usually less than 0.5‰. In the case of discrimination by ribulose biphosphate carboxylase (Rubisco), the two definitions differ by ~0.9‰, which is significant. However, formulation of discrimination as Δ^* rather than as $(R_a/R_p - 1)$, would make derivation of the theory much more complicated. This may be seen by repeating the derivation in Part II using $a^* = 1 - g^{13}/g$ and $b^* = 1 - R_p/R_i$.

Although it may seem odd to have the abundance ratio of the source, R_a , in the numerator of our chosen definition (Equation A1), we note that R_a/R_p may equally be thought of as S_p/S_a , where S is the molar ratio $^{12}\text{C}/^{13}\text{C}$.

Yet another notation is to use $R_p/R_a - 1$, $k^{13}/k^{12} - 1$, and $K^{13}/K^{12} - 1$, which leads to negative values of discrimination.

Part IV. Complications to the use of $\Delta = a + (b - a)p_i/p_a$ Farquhar (34) showed that the appropriate value of p_i in Equation 8 is the assimilation-rate-weighted value of p_i , whereas normal gas exchange gives a conductance-weighted value of p_i . These two estimates will differ if there is heterogeneity of stomatal opening (73, 141) and restricted lateral diffusion within the leaf. Greater degrees of heterogeneity will therefore cause smaller best fit values for b . The simplest value of b would be the isotope discrimination factor of Rubisco carboxylation, taking gaseous CO_2 as the substrate (b_3). Roeske & O'Leary (119) measured the isotope effect as 1.029, but with respect to dissolved CO_2 , so that the result must be multiplied by the isotope effect of the dissolution of CO_2 in water (1.0011) making b_3 approximately 30‰ (36). Guy et al (50) measured the effect directly with respect to the gas by monitoring continuing isotopic enrichment of CO_2 in a reaction vessel and calculated b_3 to be $\sim 29\text{‰}$ using an equation analogous to that for Rayleigh distillation (7, 97). However, Farquhar & Richards (39) suggested that the net discrimination in C_3 photosynthesis should be less than that in the Rubisco carboxylation, because even in C_3 species a portion, β , of CO_2 fixation is via PEP carboxylase. With b_4 being the net fractionation by PEP carboxylase with respect to gaseous CO_2 in equilibrium with HCO_3^- , (-5.7‰ ; see Table 1) they suggested a net discrimination value of

$$b = (1 - \beta)b_3 + \beta b_4 = b_3 - \beta(b_3 - b_4).$$

The difference ($b_3 - b_4$) is $\sim 36\text{‰}$, so that b is sensitive to the proportion of β -carboxylation. The latter depends on the amount of aspartate to be formed—unlikely to vary much between plants—and the amount of HCO_3^- formed for pH balance. This latter factor may contribute to the greater discrimination shown by *Ricinus* plants grown with NH_4^+ as N source than when NO_3^- was the sole source, although the phenomenon was interpreted in terms of changed stomatal behavior (114). In an unpublished study by Melzer & O'Leary (personal communication), C_4 fixation was found to reduce carbon discrimination by no more than 1‰ in C_3 species. Assuming p_i/p_a was ~ 0.7 , this means that b could be reduced from b_3 by 1.9‰.

Other effects are ignored in the simple model represented by Equation 8. These include the presence of resistance between the intercellular spaces and the sites of carboxylation, and effects of respiratory losses and translocation. Many of these effects are taken into account in a more detailed equation (32) for which Equation 8 is a simplification:

$$\Delta = a_b \frac{p_a - p_s}{p_a} + a \frac{p_s - p_i}{p_a} + (e_s + a_l) \frac{p_i - p_c}{p_a} + b \frac{p_c}{p_a} - \frac{\frac{eR_d}{k} + f\Gamma^*}{p_a}, \text{ A12.}$$

where p_s is the $p(\text{CO}_2)$ at the leaf surface, p_c is the equivalent $p(\text{CO}_2)$ at the sites of carboxylation, a_b is the fractionation occurring during diffusion in the boundary layer (2.9‰), e_s is the fractionation occurring as CO_2 enters solution [1.1‰ at 25°C; (149)] a_l is the fractionation due to diffusion in water [0.7‰; (98)], e and f are fractionations with respect to average carbon composition associated with "dark" respiration (R_d) and photorespiration, respectively, k is the carboxylation efficiency, and Γ^* is the CO_2 compensation point in the absence of R_d (32).

Equation 8 overestimates discrimination compared to Equation A12 by

$$d = [r_b(a - a_b) + r_w(b - e_s - a_l)] \frac{AP}{p_a} + \frac{\frac{eR_d}{k} + f\Gamma^*}{p_a}, \text{ A13.}$$

The resistances r_b and r_w ($\text{m}^2 \text{ sec mol}^{-1}$) are those of the boundary layer, and between the intercellular spaces and the sites of carboxylation, respectively, and P is the atmospheric pressure. Thus Equation 8 should overestimate discrimination at a fixed p_i/p_a by an amount (d) that increases with increasing assimilation rate, as may tend to occur naturally with increasing light intensity (32).

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