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On the Relationship between Carbon Isotope Discrimination and the Intercellular Carbon Dioxide Concentration in Leaves

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Abstract

Theory is developed to explain the carbon isotopic composition of plants. It is shown how diffusion of gaseous CO_2 can significantly affect carbon isotopic discrimination. The effects on discrimination by diffusion and carboxylation are integrated, yielding a simple relationship between discrimination and the ratio of the intercellular and atmospheric partial pressures of CO_2. The effects of dark respiration and photorespiration are also considered, and it is suggested that they have relatively little effect on discrimination other than *via* their effects on intercellular $p(CO_2)$. It is also suggested that various environmental factors such as light, temperature, salinity and drought will also have effects *via* changes in intercellular $p(CO_2)$. A simple method is suggested for assessing water use efficiencies in the field.

Introduction

Higher plants with the conventional (C_3) pathway of carbon assimilation have a $^{13}C/^{12}C$ ratio about 20 per mille less than that in the atmosphere while plants with the dicarboxylic acid (C_4) pathway have a ratio which is lower than the atmosphere by about 10 per mille (Bender 1968). Plants exhibiting crassulacean acid metabolism (CAM) exhibit intermediate values (Bender 1971) which appear to be related to the relative proportions of C_3 and C_4 fixation by these species (Osmond 1978). Within the C_3 and C_4 groupings there is still variation. The purpose of this communication is to provide some understanding of possible causes of this variation.

The basis of the biochemical discrimination against ^{13}C in C_3 plants lies with the primary carboxylating enzyme, ribulose-1,5-bisphosphate (RuP_2) carboxylase (Park and Epstein 1960) which discriminates against ^{13}C because of the intrinsically lower reactivity of ^{13}C (Melander and Saunders 1979). Isotopic discrimination by enzymes may vary with pH, temperature, and metal ion concentrations (O'Leary 1978). For RuP_2 carboxylase, Whelan *et al.* (1973) reported increasing discrimination with temperature of 1·2‰ per °C. However Christeller *et al.* (1976) found that the discrimination by soybean RuP_2 carboxylase was independent of temperature. These and other recent estimates of the discrimination by the enzymes are in the range 27–38‰ (Whelan *et al.* 1973; Estep *et al.* 1978*a*, 1978*b*; Wong *et al.* 1979*b*). It is likely that much of the variation presently evident in the literature reflects experimental uncertainties rather than intrinsic variations in the capacity of the enzyme to fractionate carbon isotopes.

With the fractionation of the enzymatic reaction being in this range, and the isotopic composition of the air being about $-8‰$ with respect to the standard Pee Dee belemnite (PDB) (Keeling *et al.* 1979; Goodman 1980), plants might be expected to have a $\delta^{13}C$ in the range -46 to $-35‰$. More than 90% of the measurements made in C_3 species fall between -30 and $-22‰$ (Troughton *et al.* 1974): thus the isotope ratio predicted on the basis of the enzymatic fractionation does not agree with that during photosynthetic CO_2 assimilation *in vivo*.

This lack of agreement is not altogether surprising since the enzymatic reaction *in vivo* occurs under conditions which differ from those used to measure the fractionation *in vitro*. The fundamental difference is that, because of the resistances to diffusion from the atmosphere to the chloroplast, the CO_2 at the site of the carboxylation reaction is not in complete equilibrium with the atmosphere. *In vitro*, special precautions are taken to ensure equilibrium between the source and the CO_2 fixed. Although stromal CO_2 concentrations are lower than those ordinarily used *in vitro*, concentration variations do not ordinarily affect isotope fractionations by enzymes (see Appendix 1).

The physical principles of gaseous diffusive transport are reasonably well understood, and in this paper we develop a quantitative treatment of the influence of these processes on isotopic fractionation. While we acknowledge that other factors in addition to diffusion complicate isotopic fractionation during photosynthesis, our approach reconciles most of the differences between the observed and expected fractionations, and it permits a link to be made between measurements of gas exchange physiology and isotopic fractionation. On the basis of arguments developed here, we predict that factors which contribute to changes in water use efficiency in photosynthesis will generally result in predictable changes in carbon isotope fractionation. These arguments also permit the CO_2 concentration at the site of the RuP_2 carboxylase reaction to be estimated from the measured isotopic fractionation. These measurements suggest that there is only a small gradient in CO_2 concentration from the intercellular air spaces to the sites of carboxylation in chloroplasts of C_3 species.

Theory

Isotopic compositions are specified as $\delta^{13}C$ values:

$$\delta(‰) = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1000,$$

where R_{sample} and R_{standard} are the abundance ratios, $^{13}C/^{12}C$, of the sample and the standard, PDB, respectively. Isotopic discrimination, d, is defined by

$$d = 1000 \times (1 - R_{\text{product}}/R_{\text{source}}).$$

Using the above definition of δ, this is equivalent to

$$d = \frac{\delta_{\text{source}} - \delta_{\text{product}}}{1 + \delta_{\text{source}}/1000}.$$

We note that, as defined, isotopic compositions of plants are negative, whereas the processes of diffusion and RuP_2 carboxylation have positive discriminations (i.e. against $^{13}CO_2$).

Park and Epstein (1960) noted that the isotopic fractionation associated with enzymatic fixation and with diffusion would not be additive *in vivo*. If photosynthesis were exclusively limited by diffusion the fractionation would reflect only the diffusive processes, while if diffusion placed no limitation the fractionation would be equivalent to that of the enzymatic step.

For the purpose of this paper we will assume that the real value for discrimination in the carboxylation reaction, b, is 30‰. This value could be in error or might vary with genotype or condition by a few parts per mille without substantially affecting the arguments developed below.

A second source of discrimination is the gaseous diffusion through the boundary layer and stomata of the leaf. The diffusivity, D_{12}, of one gas in another depends on such factors as molecular shape and interaction potentials and is also inversely proportional to the square root of the reduced masses (Mason and Marrero 1970). The former effects are unchanged by isotopic substitution leaving the effects of mass given by

$$D_{12} \propto \left(\frac{m_1 m_2}{m_1+m_2}\right)^{-\frac{1}{2}}.$$

Thus the diffusivity of $^{13}CO_2$ in air (mol. wt 28·8) is 4·4‰ less than that of $^{12}CO_2$ in air (Craig 1953): the discrimination, a, associated with diffusion in air is 4·4‰.

It may be seen that when stomata place no limitation on diffusion of CO_2, C_3 leaves should discriminate against $^{13}CO_2$ by 30‰ with respect to the atmosphere. When stomata are sufficiently closed that they become the sole source of limitation, the discrimination should be 4‰. Intermediate cases give intermediate discriminations. We now consider a simple model which can be used in such cases. We later modify the resulting equation to take into account various complications.

The molar flux, A, of $^{12}CO_2$ into the plant from the atmosphere is given by

$$A = g(c_a - c_i)/P, \tag{1}$$

where g is the conductance of the boundary layer and stomatal pores to the diffusion of CO_2, c_a and c_i are the partial pressures of CO_2 in the external atmosphere and in the intercellular spaces, respectively, and P is the atmospheric pressure. Diffusion from the intercellular spaces to the site of carboxylation is through liquid or solid phases, and little fractionation should take place, as we discuss later. We initially assume that the gradient of concentration of CO_2 from the intercellular spaces to the chloroplasts is negligible. We also initially ignore dark respiration and photorespiration. The rate of carboxylation is then given by (Farquhar *et al.* 1980)

$$A = kc_i^{\dagger}. \tag{2}$$

As shown in Appendix 2, k is a complicated parameter and depends on $c_i^{\dagger}$, the total concentration of $^{12}CO_2$ and $^{13}CO_2$ in the intercellular spaces, but this does not affect our result.

Eliminating c_i from (1) and (2) we obtain

$$A = \frac{kg/P}{k+g/P}\, c_a. \tag{3}$$

Similarly, the rate of assimilation of $^{13}CO_2$, A', is given by

$$A' = g'(c'_a - c'_i)/P \tag{1'}$$

$$A' = k' c'_i \tag{2'}$$

$$A' = \frac{k' g'/P}{k' + g'/P} c'_a, \tag{3'}$$

where the prime refers to $^{13}CO_2$.

$$g' = g(1 - a/1000) \tag{4}$$

$$k' = k(1 - b/1000). \tag{5}$$

From our earlier discussion, $a = 4{\cdot}4$ and $b = 30$, but ti will be convenient later if we now retain some generality. Dividing (3') by (3) and substituting using (4) and (5) we obtain

$$\frac{A'/A}{c'_a/c_a} = \frac{k'(1 - a/1000) + g'(1 - b/1000)/P}{k' + g'/P} \tag{6}$$

and the discrimination (d, ‰) against $^{13}CO_2$ is given by

$$d = \left(1 - \frac{A'/A}{c'_a/c_a}\right) \times 1000 \tag{7}$$

$$= \frac{k' a + g' b/P}{k' + g'/P}. \tag{8}$$

From (1') we can replace g' by $A' P/(c'_a - c'_i)$ and from (2') this becomes

$$g' = \frac{k' c'_i P}{c'_a - c'_i}. \tag{9}$$

Substituting this into (8) we obtain the discrimination

$$d = a + (b - a)c'_i/c'_a. \tag{10}$$

The atmosphere has about 7·8‰ less $^{13}CO_2$ than does the standard PDB (Keeling *et al.* 1979; Goodman 1980). This value changes with latitude and time as the total atmospheric CO_2 concentration changes (Keeling *et al.* 1979). For generality, call this δ_{atm}. The isotopic composition of the plant with respect to PDB is given by

$$\delta = \delta_{atm} - a - (b - a)c'_i/c'_a. \tag{11}$$

To within 2%, c'_i/c'_a is equal to c^+_i/c^+_a and so equation (11) may be rewritten as

$$\delta = \delta_{atm} - a - (b - a)c^+_i/c^+_a. \tag{12}$$

Related expressions, derived by alternative approaches, are given by O'Leary and Osmond (1980) and Vogel (1980).

Substituting $\delta_{atm} = -7{\cdot}8$, $a = 4{\cdot}4$, and $b = 30$ into equation (12) yields

$$\delta = -12{\cdot}2 - 25{\cdot}6c^+_i/c^+_a. \tag{13}$$

Troughton *et al.* (1974) found a bimodal distribution of δ values in land plants; the second group of species (which included the C_3 species) had a range of −36 to −21‰ and a mean of −27·8‰. Using equation (13) [adjusted to a 1973 δ_{atm}

of $-7{\cdot}4$ (Keeling *et al.* 1979)], this corresponds to a range for c_i^+/c_a^+ of 0·95–0·36 with a mean of 0·63. Reliable values of c_i^+/c_a^+ are not available for a large number of species. However, Wong *et al.* (1979*b*) observed a mean value for c_i^+/c_a^+ in eight C_3 species of 0·7. Constable and Rawson (1980) observed variation between crop species in the range 0·48–0·76, and Sharkey and Raschke (1981) observed the range 0·87–0·98.

Gradient of Concentration from the Intercellular Spaces to the Site of Carboxylation

In the following sections we delete the superscript (+) denoting total partial pressure of CO_2. If the partial pressure of CO_2 at the site of carboxylation, c_c, is significantly less than c_i, then equation (12) is replaced by

$$\delta = \delta_{atm} - a - (b-a)c_i/c_a + br, \tag{14}$$

where r is this drop in concentration (as a proportion of the ambient CO_2 concentration), i.e.

$$r = (c_i - c_c)/c_a. \tag{15}$$

In a particular leaf, r increases with increasing CO_2 assimilation rate, but leaves with large photosynthetic capacities tend to have large internal surface areas, thus ensuring that r does not become too large (Raven and Glidewell 1981).

Some authors have considered r to be large, and some have even assumed c_c equals the compensation point. The latter assumption is obviously incorrect, since net assimilation would then have to be zero. It arose from mistakenly interpreting the almost linear response of CO_2 assimilation rate to changes in c_i near the compensation point as being due to the presence of a physical resistance; we now believe that this near linearity is due mainly to the kinetic characteristics of RuP_2 carboxylase–oxygenase (Farquhar *et al.* 1980; Farquhar and von Caemmerer 1982).

Nevertheless, r must be finite. From a consideration of the (uncertain) resistances involved, Raven and Glidewell (1981) estimated r at approximately 0·15. Farquhar and von Caemmerer (1982) considered it to be small. We may, in the future, be able to estimate r more precisely from measurements of c_i/c_a and δ. We rewrite equation (14) with r as the subject:

$$r = [\delta - \delta_{atm} + a + (b-a)c_i/c_a]/b. \tag{16}$$

At present we can only place an upper bound on r in limited cases. Since, for a leaf assimilating CO_2, $c_i/c_a < 1$,

$$r < (\delta - \delta_{atm} + b)/b.$$

The limiting case will be most nearly met in situations where δ is most negative, although there is a possibility that r is unusually low in such situations, or that δ_{atm} was less than $-7{\cdot}8$. From the survey of Troughton *et al.* (1974), this corresponds to $\delta = -36‰$. In other words, even in cases when c_i/c_a is greatest ($\delta = -36$), c_i/c_a must still be less than one and, with δ_{atm} taken as $-7{\cdot}4$ in 1973,

$$r < (-36 + 7{\cdot}4 + b)/b.$$

The estimate of the upper bound depends on the estimate of the fractionation by the enzyme. With b taken variously as 27, 32 or 38 the upper bound is $-0{\cdot}06$, 0·11 or 0·25, respectively.

On the Effects of 'Dark' Respiration and Photorespiration

In Appendix 2 it is shown that if 'dark' respiration in the light and photorespiration discriminate against ^{13}C by e and f‰, respectively, equation (12) is replaced by

$$\delta = \delta_{atm} - a - (b-a)\frac{c_i}{c_a} + \frac{f\Gamma_* + eR_d/k}{c_a}, \quad (17)$$

where Γ_* is the CO_2 compensation point which would occur in the absence of R_d, and R_d is the rate of 'dark' respiration. In this context R_d includes all non-photorespiratory release of CO_2, such as respiration by non-chlorophyllous cells and continued tricarboxylic acid cycling in the light (Azcon-Bieto *et al.* 1981).

It is also shown in Appendix 2 that, although the leaf will be depleted in ^{13}C compared with the atmosphere, the proportion of CO_2 present as $^{13}CO_2$ in the intercellular spaces will be higher than in the atmosphere by

$$\delta_i - \delta_{atm} = \frac{c_a - c_i}{c_a}\left(b - a - \frac{f\Gamma_* + eR_d/k}{c_i}\right)‰.$$

Park and Epstein (1960) found that respired CO_2 from tomato was up to 8‰ richer in ^{13}C than the plant. Troughton *et al.* (1974), however, found no consistent differences. In *Triticum aestivum*, the respired CO_2 was 5‰ richer, in *Pinus radiata* 4‰ depleted, and in three C_4 species, less than 2‰ richer. Thus e is probably not large. In Appendix 3, estimation of f is also discussed. In the model of Farquhar *et al.* (1980), Γ_* and R_d/k are 31 and 11 μbar, respectively, at 25°C and 21% O_2 and their sum is a close approximation to the CO_2 compensation point occurring in the presence of dark respiration. Because Γ_* and R_d/k are small compared to c_a, and because f and e are probably not large, equation (12) should be a good approximation of equation (17).

If there is no *intrinsic* discrimination by dark respiration and photorespiration, i.e. $e = f = 0$, then these processes only contribute to δ via their effects on c_i/c_a, and equation (12) remains valid. This is because the isotopic composition of the CO_2 released is the same as that of the leaf, which in turn must be the same as the average composition of that entering the stomata during photosynthesis.

Other Factors Affecting δ

δ_{atm} is not constant and varies spatially and temporally in natural conditions (Keeling *et al.* 1979) and in growth chambers (Smith *et al.* 1976). To the extent that the supply of CO_2 is restricted in a closed volume, fractionation is reduced (Berry and Troughton 1974). In closed canopies, respired CO_2 will be reassimilated (Grinstead 1977; Medina and Minchin 1980). However the finding that trees lower in the canopy have more negative values of δ may reflect the high value of c_i/c_a which occurs at low irradiances. Obviously c_i/c_a equals unity at the light compensation point. Farquhar (1980) suggested this explanation for observations of more negative values of δ in innermost rings of trees.

Secondary isotope fractionations also occur in the synthesis of certain compounds (Park and Epstein 1960; O'Leary 1981). CO_2 fixation into the tricarboxylic acid cycle (Walker 1962) may also affect δ, if the cells have 'access' to CO_2 from outside the plant.

Aquatic and C_4 Species

In plants growing under water, the parameter '*a*' is probably close to zero. The diffusion coefficients of substances in water decline as the inverse square root of the molecular weight over a range of weights including CO_2 and HCO_3^- (Stein 1962) and this led Raven (1970) and others, subsequently, to suggest that a fractionation of 11‰ should occur in diffusion of CO_2 through water. However, the above relationship reflects changes in the molecular radius of the solute (Stein 1962) and addition of a neutron to the nucleus is unlikely to affect this. Mills and Harris (1976) have reviewed the effect of isotopic substitution on diffusion in liquids. They conclude that diffusion rates of tracers are determined primarily by bulk solvent properties and not by changes in tracer mass. They comment on 'the special case of liquid water where there is a large isotopic effect', but refer here to a comparison of the diffusivities of HDO and HTO in H_2O (D = deuterium, T = tritium), and not to such processes as the diffusion of CO_2 or HCO_3^- in water. Mills and Harris argue that the differences in diffusivities of HDO and HTO in H_2O are not due to mass effects *per se*. For the present we must conclude that isotopic effects on the diffusion of inorganic carbon species in aqueous solutions are negligible. Nevertheless, careful measurements of these effects are needed.

Seagrasses are known to have large values of δ (typically -13‰) and this is thought to be due to an effectively low c_i/c_a caused by a large liquid boundary layer resistance (Andrews and Abel 1979; Benedict *et al.* 1980; Smith and Walker 1980). Similar considerations may apply to other aquatic plants (Raven 1970; Smith and Walker 1980; Osmond *et al.* 1981; Raven 1981). In some algae the situation is complicated by a CO_2-concentrating mechanism (Badger *et al.* 1980). The discrimination is also more complex in C_4 species because of the interposition of phospho*enol*pyruvate carboxylase and will be discussed in a separate publication.

Discrimination against ^{14}C

The previous equations may be adapted for use with $^{14}CO_2$ by doubling '*a*' and multiplying *b* by 1·9, since the latter relates to a chemical process (Stern and Vogel 1971).

Use of $\delta^{13}C$ for Measurements of Comparative Water Use Efficiency

Measurements of δ may provide a simple means for estimating the average c_i during the growth of a leaf in the field, weighted according to the instantaneous rate of net photosynthesis, $A(t)$, i.e. average c_i (from δ),

$$\bar{c}_i = \frac{\int c_i(t)\, A(t)\, dt}{\int A(t)\, dt}.$$

If dark respiration and photorespiration both occur but do not discriminate significantly, *e* and *f* are close to zero and the above integral is over only the portion of the growth period in which the leaf is illuminated, which we can denote as $\int^{(l)}$. Thus if *r* is zero

$$\bar{c}_i = \frac{\int^{(l)} c_i(t)\, A(t)\, dt}{\int^{(l)} A(t)\, dt} = (\delta_{atm} - a - \delta)\frac{c_a}{b-a}.$$

Such a measure could be useful to ecophysiologists, agronomists and foresters as a comparative measure of the water use efficiency during growth. This is because an equation for the rate of transpiration of water per unit area of leaf, E, may be written, analogous to equation (1) as

$$E = 1{\cdot}6g(e_i - e_a)/P = 1{\cdot}6g\,\Delta e/P,$$

where e_i and e_a are the vapour pressures in the intercellular spaces and in the external atmosphere, respectively. The factor $1{\cdot}6$ arises because water vapour diffuses through air more rapidly than does CO_2 (Andrussow 1969) and differs from the ratio of the reduced masses of H_2O and air and of CO_2 and air because of such factors as molecular shape and interaction potentials.

Assuming that the stomata are closed at night the total water used per unit area of leaf is

$$\int^{(t)} E\,dt = 1{\cdot}6\overline{\Delta e}\int^{(t)}(g/P)\,dt,$$

where

$$\overline{\Delta e} = \int^{(t)}(g\,\Delta e/P)\,dt/\int^{(t)}(g/P)\,dt.$$

The total accumulation of carbon is

$$\int A\,dt = (1-\phi)\int^{(t)} A\,dt,$$

where ϕ is the proportion of carbon fixed which is respired by the leaf at night and by other parts of the plant over the whole period. Using equation (1),

$$\begin{aligned}\int^{(t)} A\,dt &= \int^{(t)}[g(c_a - c_i)/P]dt \\ &= c_a\int^{(t)}(g/P)dt - \bar{c}_i^*\int^{(t)}(g/P)\,dt \\ &= (c_a - \bar{c}_i^*)\int^{(t)}(g/P)\,dt,\end{aligned}$$

where

$$\bar{c}_i^* = \int^{(t)}(c_i g/P)\,dt/\int^{(t)}(g/P)\,dt.$$

Thus the water use efficiency is

$$\begin{aligned}\frac{\int A\,dt}{\int E\,dt} &= \frac{(1-\phi)(c_a - \bar{c}_i^*)\int^{(t)}(g/P)\,dt}{1{\cdot}6\overline{\Delta e}\int^{(t)}(g/P)\,dt} \\ &= \frac{(1-\phi)(c_a - \bar{c}_i^*)}{1{\cdot}6\overline{\Delta e}}.\end{aligned}$$

Wong *et al.* (1979*a*) have shown that, in the light, A and g are often correlated, despite changes in age, nutrition, irradiance, stress etc. Thus c_i averaged with respect to conductance, $\bar{c}_i^*$, should approximate c_i averaged with respect to assimilation rate in the light, $\bar{c}_i$, and

$$\begin{aligned}\frac{\int A dt}{\int E dt} &= \frac{(1-\phi)c_a}{1{\cdot}6\overline{\Delta e}}\left(1 - \frac{(\delta_{atm} - a - \delta)}{(b-a)}\right) \\ &= \frac{(1-\phi)c_a}{1{\cdot}6\overline{\Delta e}}\,\frac{b - \delta_{atm} + \delta}{b-a}.\end{aligned}$$

It is already established that C_4 and CAM species, which have a greater water use efficiency, are more enriched in ^{13}C than are C_3 species. This is in part due to

the different discrimination properties of the primary carboxylating enzymes involved. We are now suggesting that, within C_3 species, plants which are more enriched in ^{13}C will show greater water use efficiency. The vapour pressure difference $(e_i - e_a)$ between the inside of the leaf and the external atmosphere is largely determined by micrometeorological conditions, and to some extent by the leaf itself (Cowan and Farquhar 1977). At a particular site, however, a ranking of leaves of C_3 species according to their δ values should give a ranking of their efficiencies of water use at the leaf level.

For example, consider the case of wheat. Troughton *et al.* (1974) measured $\delta^{13}C$ in wheat as $-30{\cdot}4‰$. For an average crop plant growing under good conditions, $\phi \approx 0{\cdot}3$ (McCree 1976). If $\overline{\Delta e} = 12 \times 10^{-3}$ bar, (a weighted mean difference in vapour pressure of 12 mbar), $c_a = 330 \times 10^{-6}$ bar, δ_{atm} (1973) $= -7{\cdot}4$, $a = 4{\cdot}4$ and $b = 30$, then the crop should have a water use efficiency of $3{\cdot}3$ mmol C per mol H_2O. Assuming that carbon makes up 42% of dry matter in wheat (Fischer and Turner 1978), this corresponds to $5{\cdot}2$ mg dry matter per g H_2O. Long-term measurements of water use efficiency of a wheat crop have been made in New South Wales and, after allowing for soil evaporation, the value was $5{\cdot}1$ mg dry matter per g H_2O (Fischer and Turner 1978). The close agreement is fortuitous, but indicates that the measurement of δ may be useful for comparative purposes in this context.

Environmental Effects on δ via Changes in c_i/c_a

Wong *et al.* (1979*a*) have shown that c_i/c_a tends to be a somewhat conservative parameter for particular species, though it can vary with temperature and humidity. Farquhar (1980) has discussed these and other possible influences on c_i/c_a and their likely effects on δ in the context of examining tree rings for evidence of climatic change. He concluded that, when CO_2 assimilation rate is reduced by factors directly affecting leaf metabolism, c_i should increase and δ decrease. In contrast, to the extent that CO_2 assimilation rate is indirectly reduced by reduction of stomatal conductance, c_i should decrease and δ increase. This may explain the increase in δ with salinity observed in two halophytes by Guy *et al.* (1980). De Jong (1978) showed that water use efficiency increased with salinity in three beach species, which is equivalent to a decrease in c_i.

Consistent with the above, Winter (1981) has found that leaves of *Cicer arietinum* plants exposed to drying cycles had lower c_i and greater (i.e. less negative) δ values than those of control plants. Further, Winter *et al.* (1981) found that specimens of two C_3 halophytes from Western Australia had less negative δ values after 3 months of dry conditions.

Farquhar *et al.* (1982) correlated measurements of δ with spot measurements of c_i/c_a in leaves of plants grown under varying conditions. Wide ranges of values for both parameters were obtained, which were reasonably consistent with equation (12). It was concluded that, *in vivo*, RuP_2 carboxylation discriminates against $^{13}CO_2$, relative to $^{12}CO_2$, by about 27‰ (i.e. $b \approx 27$).

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Appendix 1.

On the competition between $^{12}CO_2$ and $^{13}CO_2$ during Carboxylation

$^{12}CO_2$ and $^{13}CO_2$ may be considered as competitors for the same enzymatic site. Denoting the partial pressure of $^{12}CO_2$ by C and that of $^{13}CO_2$ by C', the velocities of the competitive reactions involved are written, respectively, as

$$V = \frac{V_{max} C}{C + K(1 + C'/K')} \tag{A1}$$

and

$$V' = \frac{V'_{max} C'}{C' + K'(1 + C/K)}. \tag{A1'}$$

It is convenient to define the parameters ε_1 and ε_2 which interrelate the Michaelis–Menten constants and the maximum velocities, respectively, by the following expressions

$$K' = K/(1 - \varepsilon_1) \tag{A2}$$

and

$$V'_{max} = V_{max}(1 - \varepsilon_2), \tag{A3}$$

where

$$\varepsilon_1, \varepsilon_2 \ll 1.$$

We note that to the order of terms like C', ε_1 and ε_2,

$$\begin{aligned} C + K(1 + C'/K') &= C + K + C'(1 - \varepsilon_1) \\ &= C^+ + K - \varepsilon_1 C' \text{ (where } C^+ = C + C') \\ &= C^+ + K, \end{aligned}$$

since the third term is the product of two small terms. Thus equation (A1) may be rewritten as

$$V = \frac{V_{max} C}{C^+ + K} \tag{A4}$$

and (A1′) as

$$V' = \frac{V'_{max} C' K/K'}{C^+ + K} \tag{A5}$$

$$= \frac{V_{max} C'}{(1+\varepsilon_1+\varepsilon_2)(C^+ + K)}, \tag{A6}$$

since ε_1, ε_2 are small.

Thus in the form of equations (A4) and (A5), the Michaelis constant is effectively the same for both species with the same total concentration, C^+, in the denominator. In equation (A6) the properties of discrimination are then effectively absorbed into the maximum velocity in a manner which is convenient for treatments such as that in Appendix 2.

Note that $(V'/V)/(C'/C)$ (and hence discrimination) is independent of concentration. O'Leary (1977) shows the same result from a consideration of individual rate constants.

Appendix 2.

The Relationship between Carbon Isotope Discrimination and Intercellular CO_2 Concentration in Leaves of C_3 Species, taking into account Photorespiration and Dark Respiration

The rate of assimilation of $^{12}CO_2$ is given by

$$A = V_c - F - R_d, \tag{B1}$$

where F is the rate of $^{12}CO_2$ release due to photorespiration, and R_d is the rate of $^{12}CO_2$ released due to 'dark' respiration in the light.

Similarly, for $^{13}CO_2$,

$$A' = V'_c - F' - R'_d. \tag{B1′}$$

The rate of photorespiration is given by (Farquhar *et al.* 1980)

$$0{\cdot}5V_o = 0{\cdot}5\frac{V_{o(max)} O}{O + K_o(1 + c_i^+/K_c)} \frac{R}{R + K_r}, \tag{B2}$$

where V_o is the rate of oxygenation, K_o and K_c are the Michaelis constants for oxygen and carbon dioxide, O is the oxygen concentration, $V_{o(max)}$ is the maximum rate of oxygenation, and c_i^+ is the total intercellular concentration of $^{12}CO_2$ and of $^{13}CO_2$. R is the concentration of free ribulose bisphosphate (RuP_2), and K_r is the effective Michaelis constant for RuP_2.

We assume that the proportion of ^{12}C in the tissue depends on the history of net assimilation of $^{12}CO_2$, A, and of $^{13}CO_2$, A', and is given by $A/(A+A')$. The rate of photorespiratory release of $^{12}CO_2$, F, is then given by

$$F = 0{\cdot}5\,\frac{V_{o(max)}\,O}{O+K_o(1+c_i^+/K_c)}\,\frac{R}{R+K_r}\,\frac{A}{A+A'}. \tag{B3}$$

Similarly,

$$F' = 0{\cdot}5\,\frac{V'_{o(max)}\,O}{O+K_o(1+c_i^+/K_c)}\,\frac{R}{R+K_r}\,\frac{A'}{A+A'}. \tag{B3'}$$

As discussed in Appendix 1, the properties of discrimination by an enzyme are effectively absorbed into the V_{max}. Equation (B3') assumes that a particular 'photorespiratory enzyme' is acting on a substrate with the same isotopic composition as the rest of the leaf. Such would be the case if RuP_2 oxygenase discriminated against RuP_2 containing ^{13}C, and if there were no further discrimination up to and including the decarboxylation of glycine. In fact, there are branch points along the way and so equation (B3') is merely a convenient fiction that summarizes the discrimination over a complex series of steps, in favour of the release of $^{12}CO_2$. It describes the relationship, which may be variable, between the isotope composition of photorespired CO_2 and that of the whole leaf.

Dividing (B3') by (B3)

$$F'/F = V'_{o(max)}\,A'/(V_{o(max)}\,A). \tag{B4}$$

We put

$$V'_{o(max)} = V_{o(max)}(1-f/1000), \tag{B5}$$

and since

$$A'/A = (c'_a/c_a)(1-d/1000), \tag{B6}$$

where d(‰) is the fractionation (net discrimination against ^{13}C with respect to the atmosphere), equation (B4) may be rewritten as

$$F'/F = (c'_a/c_a)[1-(d+f)/1000], \tag{B7}$$

since $df/1000$ is an order smaller than the terms in which we are interested.

Similarly we put

$$R'_d/R_d = (1-e/1000)(A'/A), \tag{B8}$$

where e‰ is the intrinsic discrimination against ^{13}C in dark respiration. Using equation (B6),

$$R'_d/R_d = (c'_a/c_a)[1-(d+e)/1000]. \tag{B9}$$

The rate of carboxylation, V_c, of $^{12}CO_2$ is (Farquhar *et al.* 1980)

$$V_c = \frac{V_{c(max)}\,c_i}{c_i^+ + K_c(1+O/K_o)}\,\frac{R}{R+K_r}. \tag{B10}$$

The parameter, k, is then

$$k = \frac{V_{c(max)}}{c_i^+ + K_c(1+O/K_o)}\,\frac{R}{R+K_r}. \tag{B11}$$

Using equations (B1), (B3) and (B11), the rate of net assimilation of $^{12}CO_2$ is given by

$$A = k(c_i-\Gamma_*)-R_d, \tag{B12}$$

where Γ_*, the compensation point in the absence of dark respiration, is given by

$$\Gamma_* = 0{\cdot}5 \frac{V_{o(max)}}{V_{c(max)}} \frac{O}{K_o} K_c \frac{A}{A+A'}. \tag{B13}$$

Similarly,

$$A' = k'(c_i' - \Gamma_*') - R_d', \tag{B12'}$$

where

$$k'/k = V_{c(max)}'/V_{c(max)}, \tag{B14}$$

$$\Gamma_*'/\Gamma_* = V_{o(max)}' V_{c(max)} A'/(V_{o(max)} V_{c(max)}' A). \tag{B15}$$

With

$$k' = k(1-b/1000), \tag{B16}$$

and using equations (B5), (B6) and (B16), and the approximation which we will commonly use from here on, i.e.

$$1/(1-\delta) \simeq 1+\delta,$$

$$\Gamma_*'/\Gamma_* = (c_a'/c_a)[1+(b-d-f)/1000]. \tag{B17}$$

Equations (1) and (1′) are still valid.

$$A = g(c_a - c_i)/P \tag{B18}$$

$$A' = g'(c_a' - c_i')/P. \tag{B18'}$$

Combining these with equations (B12) and (B12′)

$$A = \frac{k(c_a - \Gamma_* - R_d/k)g/P}{g/P+k} \tag{B19}$$

$$A' = \frac{k'(c_a' - \Gamma_*' - R_d'/k')g'/P}{g'/P+k'}. \tag{B19'}$$

The actual compensation point in the model of Farquhar *et al.* (1980) approximates closely to $\Gamma_* + R_d/k$.

Dividing (B19′) by (B19)

$$\frac{A'}{A} = \frac{k'g'/P}{k'+g'/P} \frac{k+g/P}{kg/P} \frac{c_a' - \Gamma_*' - R_d'/k'}{c_a - \Gamma_* - R_d/k}. \tag{B20}$$

From equations (B9), (B16) and (B17) and again approximating $1/(1-\delta)$ by $1+\delta$

$$\frac{c_a' - \Gamma_*' - R_d'/k'}{c_a - \Gamma_* - R_d/k} = \frac{c_a'}{c_a}\left(1 - \frac{(b-d-f)\Gamma_*' + (b-d-e)R_d'/k'}{1000(c_a' - \Gamma_*' - R_d'/k')}\right). \tag{B21}$$

Substituting (B21) into (B20) and using (B16) and

$$g' = g(1-a/1000), \tag{B22}$$

$$\frac{A'/A}{c_a'/c_a} = 1 - \frac{bg'/P + ak'}{1000(g'/P+k')} - \frac{\Gamma_*'(b-d-f) + R_d'/k'(b-d-e)}{1000(c_a' - \Gamma_*' - R_d'/k')}. \tag{B23}$$

Using equations (B6), (B12′) and (B18′),

$$d = a+(b-a)(c'_i/c'_a)-(f\Gamma'_*+eR'_d/k')/c'_a. \tag{B24}$$

To a good approximation, $c'_i/c'_a = c_i^+/c_a^+$ etc., and the discrimination with respect to PDB is given by

$$\delta = \delta_{atm}-a-(b-a)\frac{c_i^+}{c_a^+}+\frac{f\Gamma_*^+ + eR_d^+/k}{c_a^+}. \tag{B25}$$

It is interesting to note that if the definition

$$d_i = \left(1-\frac{A'/A}{c'_i/c_i}\right)\times 1000 \tag{B26}$$

is used, and equations (B6), (B7), (B9) and (B17) redefined accordingly, the previous treatment may be repeated and yields

$$d_i = b-\frac{f\Gamma'_*+eR'_d/k'}{c'_i}, \tag{B27}$$

and since

$$1000\left(1-\frac{c'_i/c_i}{c'_a/c_a}\right) = d-d_i,$$

the $^{13}CO_2$ in the intercellular spaces is enriched with respect to the atmosphere by

$$\frac{c_a^+-c_i^+}{c_a^+}\left(b-a-\frac{f\Gamma_*^+ + eR_d^+/k}{c_i^+}\right).$$

Appendix 3.

Estimation of Carbon Isotope Discrimination due to Photorespiration

Troughton *et al.* (1974) collected CO_2 respired by plants into approximately 100% O_2 and a low CO_2 concentration. We now examine an expression for the discrimination, d_o ‰ against ^{13}C in this respired air, compared to that of the leaf material.

We assume that the leaf contents of ^{12}C and ^{13}C were l and l', respectively. Thus

$$-A'/-A = (1-d_o/1000)(l'/l), \tag{C1}$$

where $-A$ and $-A'$ are the net rates of evolution of $^{12}CO_2$ and $^{13}CO_2$ respectively. Equation (C1) replaces equation (B6) from Appendix 2.

Equations (B1), (B1′) and (B2) remain unchanged. In (B3) and (B3′), $A/(A+A')$ and $A'/(A+A')$ are replaced by $l/(l+l')$ and $l'/(l+l')$, respectively. In equations (B4) and (B8), A'/A is replaced by l'/l. Equation (B5) remains unchanged. Equations (B6), (B7) and (B9) are not used. Equations (B10), (B11), (B12), (B12′), (B14), (B16), (B18), (B18′), (B19), (B19′) and (B20) remain. In equations (B13) and (B15), A is replaced by l, and A' by l'. Equation (B17) becomes

$$\frac{\Gamma'_*}{\Gamma_*} = \frac{l'}{l}\left(1+\frac{b-f}{1000}\right).$$

The first term of equation (B20) is given by

$$\frac{k'g'/P}{k'-g'/P}\,\frac{k+g/P}{kg/P} = 1-\frac{ak'+bg'/P}{1000(k'+g'/P)}.$$

To evaluate the second term of equation (B20), we need an additional equation relating the buildup of CO_2 in the enclosure to the evolution of CO_2. This buildup is proportional to the rate of evolution of CO_2.

$$c_a'/c_a = -A'/-A = (1-d_o/1000)(l'/l).$$

Using this and earlier equations,

$$\frac{c_a'-\Gamma_*'-R_d'/k'}{c_a-\Gamma_*-R_d/k} = \frac{l'}{l}\left(1-\frac{(f-b)\Gamma_*'+(e-b)R_d'/k'+d_o c_a'}{1000(\Gamma_*'+R_d'/k'-c_a')}\right).$$

Finally

$$d_o = \left(1-\frac{(-A')/(-A)}{l'/l}\right)\times 1000 = -\frac{(b-a)c_i^+ + ac_a^+ - f\Gamma_*^+ - eR_d^+/k}{\Gamma_*^+ + R_d^+/k}.$$

If d_o is measured, f may be determined from

$$f = \frac{(b-a)c_i^+ + ac_a^+ + d_o(\Gamma_*^+ + R_d^+/k) - eR_d^+/k}{\Gamma_*^+}.$$

In 100% O_2, Γ_*^+ may be estimated at 148 μbar at 25°C (Farquhar *et al.* 1980). The estimation of c_i^+ and c_a^+ is more problematic. We estimate that, if the CO_2 evolution in 100% O_2 is 10 μmol m^{-2} s^{-1}, the p(CO_2) in the chamber will be 10 μbar. If the stomatal conductance to the diffusion of CO_2, g, is 0·25 mol m^{-2} s^{-1} (corresponding to a conductance to the diffusion of water vapour of 0·4 mol m^{-2} s^{-1}, or 1 cm s^{-1} in the older units), the intercellular p(CO_2), c_i^+, will be 60 μbar. We assume $a = 4·4$, $b = 30$, $e = 0$ and $R_d^+/k = 21·4$ μbar (since k is less in 100% O_2).

Troughton *et al.* (1974) found that the evolved CO_2 was 9·7‰ heavier ($d_o = -9·7$) than the leaf material in an experiment using *Gossypium hirsutum*, and 12·5‰ heavier ($d_o = -12·5$) with *Triticum aestivum*. Using the above estimates of c_i^+, c_a^+, etc., these correspond to values of f of $-0·4$ and $-3·6$, respectively. On the basis of this analysis, the conclusion of Troughton *et al.* is confirmed, namely that $\delta^{13}C$ value was less negative than the tissue because some CO_2 was reassimilated by a reaction (RuP_2 carboxylation) which discriminated against $^{13}CO_2$. The present estimate of f is obviously dependent on the estimate of c_i^+ and c_a^+. Nevertheless, it appears that f is small in magnitude. Certainly, in normal air (340 μbar CO_2, 21% O_2), $f\Gamma_*^+/c_a^+$ is small and equation (12) remains a good approximation to equation (17).

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