\(\delta^{13}C\) Values of \(C_4\) Types in Grasses

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Abstract

\(\delta^{13}C\) values were determined for leaves of \(C_4\) grasses (Poaceae) of different photosynthetic types. For plants grown in the same environment at the same time, mean \(\delta^{13}C\) values for the three \(C_4\) types were: NADP-ME-type, \(-11.35\% \pm 0.13\) s.e. (11 spp.); PCK-type, \(-11.95\% \pm 0.19\) s.e. (11 spp.); and NAD-ME-type, \(-12.7\% \pm 0.21\) s.e. (9 spp.). Although there is some overlap between the values for individual species of the three groups, the difference between any two means is highly significant \([P(t) < 0.01]\) and is not due to taxonomic sampling bias at the subfamily level. The differences in means may suggest that \(C_4\) types differ in rates of leakage of CO\(_2\) and HCO\(_3^-\) from PCR tissue ('photosynthetic carbon reduction' tissue: equivalent to 'Kranz' tissue), and/or, using Farquhar's (Appendix) expression for plant \(\delta\) values, that \(C_4\) types differ in their average intercellular CO\(_2\) concentrations \((c)\). It is also possible that differences between \(C_4\) types exist in some other, unknown, leaf fractionation process. Apoplastic CO\(_2\) leakage from PCR tissue in NAD–ME-type \(C_4\) grasses, which do not possess a PCR 'suberized lamella' as found in NADP–ME-type and PCK-type \(C_4\) grasses, may give these species the most negative \(\delta^{13}C\) values. Expressions for \(C_4\) plant \(\delta^{13}C\) values, and a model for the \(\delta^{13}C\) values of CO\(_2\) and HCO\(_3^-\) in various pools and fluxes in \(C_4\) plant leaves, are given.

Introduction

\(\delta^{13}C\) values of terrestrial plants fall into two discrete ranges which are correlated with photosynthetic pathway \((C_3\ or\ C_4\): Whelan et al. 1970; Bender 1971; Smith and Epstein 1971), both in the Poaceae (Bender 1968; Winter et al. 1976; Brown 1977; Vogel et al. 1978) and other plant families (e.g. Smith and Epstein 1971; Bender et al. 1973; Troughton et al. 1974; Sankhla et al. 1975).

Discrimination against \(^{13}C\) occurs during diffusion of CO\(_2\) into the leaf and during the 'enzymatic conversion of dissolved CO\(_2\) in the cytoplasm to carbohydrates' (Park and Epstein 1960). In \(C_3\) terrestrial angiosperms, the discrimination associated with RuP\(_2\)* carboxylation is recognized as the major fractionation process (Whelan et al. 1973; Christeller et al. 1976; Estep et al. 1978; Schmidt and Winkler 1979; Wong et al. 1979; Vogel 1980; O'Leary 1981; Farquhar et al. 1982b).

If one assumes that the fractionation by RuP\(_2\) carboxylase is constant, whatever the \(\delta^{13}C\) value of the CO\(_2\) pool available to this enzyme, then the difference between

* Abbreviations used: RuP\(_2\), ribulose 1,5-bisphosphate; PEP, phosphoenolpyruvate; PCA, primary carbon assimilation; PCR, photosynthetic carbon reduction; NAD–ME, NAD malic enzyme; NADP–ME, NADP malic enzyme; PCK, phosphoenolpyruvate carboxykinase; PDB, Pee Dee Belemnite.
the δ¹³C value of the atmosphere and that of the plant tissue is largely dependent on the extent to which the isotopic composition of the CO₂ pool available to RuP₂ carboxylase can equilibrate with the isotopic composition of atmospheric CO₂ (Lerman 1975; Schmidt and Winkler 1979; O’Leary 1981; Farquhar et al. 1982b; and see Berry and Troughton 1974). In C₃ plants during steady-state photosynthesis, this extent will vary according to assimilatory, photorespiratory, and dark respiratory rates, and stomatal conductance.

In C₄ plants the CO₂-HCO₃⁻ pool at the site of RuP₂ carboxylation is not in the mesophyll (PCA tissue)* but in PCR or ‘Kranz’ tissue. The isotopic composition of this pool will depend not only upon the same factors listed for C₃ plants (above), but also upon: (i) fractionation associated with the CO₂-HCO₃⁻ equilibrium (Saruhashi 1955; Deuser and Degens 1967; Wendt 1968; Mook et al. 1974); (ii) fractionation associated with PEP carboxylation (Whelan et al. 1973; Reibach and Benedict 1977; O’Leary and Osmond 1980); and (iii) on the conductance of PCR (‘Kranz’) tissue to CO₂ and HCO₃⁻ and, therefore, rates of CO₂-HCO₃⁻ leakage from PCR to PCA (mesophyll) tissue (Hattersley 1976).

One of the principal functions of C₄ plant leaf anatomy is to provide a ‘CO₂-tight’ PCR compartment which facilitates the concentration of CO₂ at the site of RuP₂ carboxylase (e.g. Björkman 1971; Hatch 1976; Hattersley et al. 1977). If PCR tissue were totally ‘CO₂-tight’, its CO₂-HCO₃⁻ pool would be unable to equilibrate with any pool ‘external’ to it, no fractionation by RuP₂ carboxylase would occur (Whelan et al. 1973; O’Leary 1981), and C₄ plant δ¹³C values would be less negative than C₃ plant values. This is because the net discrimination against ¹³C associated with the CO₂-HCO₃⁻ equilibration and with PEP carboxylation is much less than that associated with RuP₂ carboxylation.

Although it is sometimes assumed that the PCR compartment is totally ‘CO₂-tight’ (Deleens 1976; Wong et al. 1979; Benedict et al. 1980), this cannot be true since leakage must at least occur via the symplast. With respect to the PCR apoplast, it has been suggested that leakage is small or negligible when a ‘suberized lamella’ is present in PCR cell walls (Carolin et al. 1973; Hatch and Osmond 1976). However, a ‘suberized lamella’ has yet to be reported in any C₄ dicotyledon, and Hattersley and Browning (1981) have found that NAD-ME-type C₄ grasses also lack such a lamella in PCR cell walls, unlike NADP-ME and PCK-type grasses. Considering PCR cell wall characteristics alone, it seems likely that there will be differences in PCR cell wall conductance between C₄ species. The idea is that if a suberized lamella is present in a PCR cell wall, that wall will be impermeable to water (cf. Soliday et al. 1979 and Kolattukudy 1980) and therefore, I assume, to dissolved CO₂ and HCO₃⁻.

In grasses, the lack of a PCR ‘suberized lamella’ in NAD-ME-type species could mean that a greater proportion of carbon released during C₄ acid decarboxylation (in PCR cells) will leak back to PCA tissue in this type, than in other C₄ types. The difference might be reflected in plant δ¹³C values: the more leaky the PCR tissue, the more negative the δ¹³C value. I have therefore grown NADP-ME, NAD-ME, and PCK-type grass species, in the same environment, and measured leaf δ¹³C values in order to assess if there are differences between C₄ types.

* The PCR and PCA terminology was introduced (Hattersley et al. 1977) to refer to the two chlorenchymatous tissues involved in C₄ photosynthesis, in a functional sense. Anatomically, the terms are usually, but not always, equivalent to bundle sheath and mesophyll tissues respectively.
Materials and Methods

The species sampled, including three dicotyledons, are listed in Table 1. Grass classification follows Watson and Dallwitz (1980). C₄ typing is based on known biochemistry, or on anatomy (Hattersley and Watson 1976).

Plants were grown from seed, except Cynodon dactylon, Paspalurn paspalodes and Panicum decompositurn which were grown from small portions of stolon or rootstock. All sowings and plantings were simultaneous (22–27 Feb. 1980), except for eight species where first attempts at germination were unsuccessful (Table 1). Plants were grown in the same glasshouse, fitted with a ventilator fan and with the door open. The temperature range was 15–30°C (thermostatically controlled upper and lower limits). Plants were well spaced and well watered (bottom irrigated).

Mature, non-senescing, healthy leaves (blades only) were harvested as soon as available in adequate numbers, dried in an oven at 70°C, then ground finely with a soil grinder. Ground stock material of each species consisted of many leaves, from more than one plant. Samples of 80–100 mg from well mixed stock material were combusted in excess O₂ following a method modified from Craig (1957), as outlined in Osmond et al. (1976). Isotope ratios of the collected CO₂ were measured on a Micromass 602D mass spectrometer.

The $^{13}\text{C}/^{12}\text{C}$ ratios are reported as $\delta^{13}\text{C}$ values in $\%_{\text{o}}$, $\delta^{13}\text{C} = \frac{R_{\text{sample}} - R_{\text{standard}}}{R_{\text{standard}}} \times 10^3$, where $R$ is the ratio of mass 45 to mass 44, corrected for $^{12}\text{C}^{16}\text{O}^{18}\text{O}$. An internal Zea mays plant standard was used, calibrated against several standard limestones tied to PDB (from South Carolina). $\delta^{13}\text{C}$ values are expressed relative to PDB standard. Mass spectrometric readings were accurate to within 0.05$\%_{\text{o}}$, and four complete sample replicates (Table 1) indicate an accuracy within 0.1$\%_{\text{o}}$. Analyses were performed in a sequence (Table 1) which was deliberately disordered with regard to C₄ type. Plants were grown to anthesis to check identity.

Results and Discussion

$\delta^{13}\text{C}$ values obtained are given in Table 1. The range for all grass values is narrow ($-10.6$ to $-13.5\%_{\text{o}}$). Fig. 1 gives the $\delta^{13}\text{C}$ value range for each C₄ type, with group means and standard errors. There are overlaps between the values for different types, with PCK species straddling both the NAD–ME and NADP–ME-type ranges. However, only one NAD–ME result (for Panicum stapjanum) lies in the NADP–ME-type range. Indeed $t$-tests show the mean of each group to be significantly different from the means of both other groups (all comparisons with $P(t) < 0.01$; see Fig. 1). Variances for the three C₄ groups are similar ($F$-test). NAD–ME-type grasses generally exhibit the most negative values (mean $-12.7\%_{\text{o}} \pm 0.21$), NADP–ME-type the least negative (mean $-11.35\%_{\text{o}} \pm 0.13$), and PCK-type species are intermediate (mean $-11.95\%_{\text{o}} \pm 0.19$). As the statistical analysis indicates (Fig. 1 caption: 95% confidence intervals for differences between true means), the difference in true means between the PCK-type and either the NADP–ME-type or the NAD–ME-type, though significant, may be very small.

Significant differences in mean $\delta^{13}\text{C}$ values for C₄ types as found in this study are not apparent in three earlier extensive $\delta^{13}\text{C}$ surveys of grasses (Troughton et al. 1974; Winter et al. 1976; Brown 1977), although it is possible from available records to assign only a small proportion of the species examined to type. Other reports, however, do contain information where significant C₄ type differences are detectable (Vogel et al. 1978; Ziegler et al. 1981; also Brown’s 1977 data for U.S. collections; Table 2). Generally, one might expect C₄ type differences to be masked in such surveys, since collection times and sites of the plants measured varied widely, and herbarium material was often employed. Environmental factors can influence $\delta^{13}\text{C}$
Table 1. $\delta^{13}$C values of plants of different $C_4$ type

31 grass species and three dicotyledon species were tested in the order shown in the fourth column. Species marked with an asterisk have been biochemically typed (Andrews et al. 1971; Huber et al. 1973; Gutierrez et al. 1974; Hatch and Kagawa 1974; Hatch et al. 1975; Gutierrez et al. 1976; Rathnam 1978). All species were anatomically typed (Hattersley and Watson 1976). $\delta^{13}$C values in parenthesis are replicates. All sowing dates were in 1980; where no sowing date is given, plants were propagated from small pieces of stolon or rootstock. ‘Age at harvest’ is expressed in days after sowing.

<table>
<thead>
<tr>
<th>$C_4$ type</th>
<th>Subfamily or family</th>
<th>Species</th>
<th>Order</th>
<th>$\delta^{13}$C (‰)</th>
<th>Sowing date</th>
<th>Age at harvest</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grasses</td>
<td>Eu-panicoid</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NADP-ME</td>
<td>* Axonopus compressus (Swartz) Beauv.</td>
<td>28</td>
<td>$-11.0$</td>
<td>19 Mar.</td>
<td>93</td>
<td></td>
</tr>
<tr>
<td></td>
<td>* Digitaria sanguinalis (L.) Scop.</td>
<td>20</td>
<td>$-11.2$</td>
<td>22 Feb.</td>
<td>47</td>
<td></td>
</tr>
<tr>
<td></td>
<td>* Echinochloa frumentacea (Roxb.) Link</td>
<td>29</td>
<td>$-11.2$</td>
<td>18 Mar.</td>
<td>49</td>
<td></td>
</tr>
<tr>
<td></td>
<td>* Panicum bulbosum H.B.K.</td>
<td>19</td>
<td>$-10.6$</td>
<td>22 Feb.</td>
<td>75</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Paspalum paspalodes (Michx.) Lams.-Scribn.</td>
<td>1</td>
<td>$-11.6$</td>
<td>36</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>* Pennisetum typhoides (Burm.) Stapf &amp; Hubb.</td>
<td>7</td>
<td>$-11.4$</td>
<td>22 Feb.</td>
<td>47</td>
<td></td>
</tr>
<tr>
<td></td>
<td>* Setaria italica (L.) Beauv.</td>
<td>27</td>
<td>$-11.1$</td>
<td>16 Apr.</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Andropogonoid</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bothriochloa macra (Steu.) S. T. Blake</td>
<td>33</td>
<td>$-12.0$</td>
<td>19 Mar.</td>
<td>79</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Heteropogon contortus (L.) Beauv. ex R. &amp; S.</td>
<td>3</td>
<td>$-11.4$</td>
<td>16 Apr.</td>
<td>96</td>
<td></td>
</tr>
<tr>
<td></td>
<td>* Sorghum bicolor (L.) Moench</td>
<td>24</td>
<td>$-12.1$</td>
<td>22 Feb.</td>
<td>74</td>
<td></td>
</tr>
<tr>
<td></td>
<td>* Zea mays L.</td>
<td>25</td>
<td>$-11.3$</td>
<td>22 Feb.</td>
<td>74</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PCK</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Eu-panicoid</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>* Brachiaria eruciformis (Smith) Griseb.</td>
<td>22</td>
<td>$-12.1$</td>
<td>16 Apr.</td>
<td>51</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Eriochloa meyeriana (Nees) Pilg.</td>
<td>32</td>
<td>$-12.7$</td>
<td>22 Feb.</td>
<td>75</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Panicum texifolium Hack.</td>
<td>12</td>
<td>$-10.6$ ($-10.6$)</td>
<td>22 Feb.</td>
<td>74</td>
<td></td>
</tr>
<tr>
<td></td>
<td>* Urochloa mosambicensis (Hack.) Dandy</td>
<td>34</td>
<td>$-11.4$</td>
<td>27 Feb.</td>
<td>69</td>
<td></td>
</tr>
<tr>
<td></td>
<td>U. panicoides Beauv.</td>
<td>15</td>
<td>$-12.2$</td>
<td>22 Feb.</td>
<td>47</td>
<td></td>
</tr>
<tr>
<td>Chloridoid</td>
<td>* Bouteloua curtipendula (Michx.) Torr.</td>
<td>31</td>
<td>-12.8</td>
<td>22 Feb.</td>
<td>119</td>
<td></td>
</tr>
<tr>
<td>---------------------</td>
<td>----------------------------------------</td>
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<td>---------</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>* Chloris gayana Kunth</td>
<td>26</td>
<td>-12.2</td>
<td>22 Feb.</td>
<td>34</td>
<td></td>
</tr>
<tr>
<td></td>
<td>* Eragrostis curvula (Schrad.) Nees</td>
<td>11</td>
<td>-11.6</td>
<td>22 Feb.</td>
<td>75</td>
<td></td>
</tr>
<tr>
<td></td>
<td>E. philippica Jedw.</td>
<td>18</td>
<td>-11.8</td>
<td>22 Feb.</td>
<td>75</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sporobolus elongatus R. Br.</td>
<td>16</td>
<td>-11.8 (-11.8)</td>
<td>22 Feb.</td>
<td>75</td>
<td></td>
</tr>
<tr>
<td></td>
<td>* S. jimbriatus Nees</td>
<td>23</td>
<td>-12.3</td>
<td>27 Feb.</td>
<td>70</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>NAD-ME Eu-panicoid</th>
<th>* Panicum capillare L.</th>
<th>21</th>
<th>-13.1 (-13.1)</th>
<th>22 Feb.</th>
<th>74</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>* P. decompositum R. Br.</td>
<td>30</td>
<td>-12.4</td>
<td>—</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>P. dichotomiflorum Michx.</td>
<td>13</td>
<td>-12.4</td>
<td>18 Mar.</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>* P. miliaceum L.</td>
<td>14</td>
<td>-12.6</td>
<td>22 Feb.</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>* P. stephianum Fourc.</td>
<td>4</td>
<td>-11.6</td>
<td>16 Apr.</td>
<td>65</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chloridoid</th>
<th>* Bouteloua gracilis (H.B.K.) Lag. ex Steud.</th>
<th>8</th>
<th>-13.2</th>
<th>22 Feb.</th>
<th>90</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>* Cynodon dactylon (L.) Pers.</td>
<td>10</td>
<td>-13.5</td>
<td>—</td>
<td>47</td>
</tr>
<tr>
<td></td>
<td>* Eleusine coracana (L.) Gaertn.</td>
<td>17</td>
<td>-12.2 (-12.2)</td>
<td>22 Feb.</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>* Eragrostis ciliaris (All.) Lutati</td>
<td>2</td>
<td>-13.3</td>
<td>22 Feb.</td>
<td>75</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Dicotyledons</th>
<th>Amaranthaceae</th>
<th>6</th>
<th>-12.2</th>
<th>25 Feb.</th>
<th>32</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>* Amaranthus edulis Spec.</td>
<td>9</td>
<td>-12.5</td>
<td>25 Feb.</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>Chenopodiaceae</td>
<td>5</td>
<td>-14.7</td>
<td>25 Feb.</td>
<td>56</td>
</tr>
</tbody>
</table>

* This species has been classed as NAD–ME-type biochemically (e.g. Gutierrez et al. 1974), but material used is PCK-type anatomically (see Hattersley and Browning 1981).
Fig. 1. Means and ranges of $\delta^{13}C$ values for $C_4$ types in grasses. Each bar represents the 95% confidence interval for its $C_4$ type population mean, $\mu$. $t$-tests give $P(t)$ values of $<0.00001$ (NADP-ME v. NAD-ME), $<0.01$ (NADP-ME v. PCK), and $<0.01$ (PCK v. NAD-ME). Confidence intervals (95%) for differences between true means are $0.85-1.84\%$ (NADP-ME v. NAD-ME), $0.13-1.08\%$ (NADP-ME v. PCK), and $0.16-1.33\%$ (PCK v. NAD-ME).

Table 2. Comparison of mean $\delta^{13}C$ values of different photosynthetic types of grasses

All analyses were performed by the author. For Vogel et al. (1978), typing of species was taken from Ellis (1977). Brown (1977) typed his own species. Species and genera in Ziegler et al. (1981) were typed, where possible (25 out of 31 spp.), by the author from Ellis (1977) and own data. For Brown (1977) and Ziegler et al. (1981), it was only possible to compare NADP-ME-type with non-NADP-ME-type $C_4$ species. $n$, number of species sampled. $D$, $P(t)$ indicates differences between means, and their statistical significance.

<table>
<thead>
<tr>
<th>Source</th>
<th>NADP-ME-type</th>
<th>PCK-type</th>
<th>NAD-ME-type</th>
<th>$D$, $P(t)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>This study</td>
<td>-11.35</td>
<td>-11.95</td>
<td>-12.70</td>
<td>1.35&lt;sup&gt;b&lt;/sup&gt;, $P(t) &lt; 0.001$</td>
</tr>
<tr>
<td></td>
<td>($n = 11$)</td>
<td>($n = 11$)</td>
<td>($n = 9$)</td>
<td></td>
</tr>
<tr>
<td>Vogel et al. (1978)</td>
<td>-12.38</td>
<td>-13.36</td>
<td>-13.69</td>
<td>1.31&lt;sup&gt;b&lt;/sup&gt;, $P(t) = 0.004$</td>
</tr>
<tr>
<td></td>
<td>($n = 11$)</td>
<td>($n = 7$)</td>
<td>($n = 10$)</td>
<td></td>
</tr>
<tr>
<td>This study</td>
<td>-11.35</td>
<td>-12.29</td>
<td></td>
<td>0.94, $P(t) = 0.002$</td>
</tr>
<tr>
<td></td>
<td>($n = 11$)</td>
<td>($n = 20$)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brown (1977)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-12.22</td>
<td>-13.26</td>
<td></td>
<td>1.04, $P(t) = 0.001$</td>
</tr>
<tr>
<td></td>
<td>($n = 21$)</td>
<td>($n = 42$)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ziegler et al. (1981)</td>
<td>-11.59</td>
<td>-12.95</td>
<td></td>
<td>1.36, $P(t) &lt; 0.001$</td>
</tr>
<tr>
<td></td>
<td>($n = 12$)</td>
<td>($n = 13$)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> U.S. species only.  <sup>b</sup> Between NADP-ME and NAD-ME.
values by amounts greater than the differences I am reporting between C₄ type means. Such factors include (i) the isotopic composition of the source CO₂, which varies due to industrial activity, soil organism respiration, growing plants in non-open environments, time of year, etc.; and (ii) all aspects of the environment which affect plant assimilation rate, such as temperature, light intensity, water availability, salinity, etc. (see review by O'Leary 1981).

It is unlikely that the differences I have observed between types were due to differences in environment experienced by each type, since all plants for the experiment were grown in the same source CO₂, and the majority were sown at the same time (Table 1). Omitting the eight species which were ‘late starters’ from the data does not alter the outcome. Plants were harvested, of necessity, at different times after sowing (Table 1). However, there is no correlation between time after sowing and δ¹³C value (r = -0.002). In any case, since only mature leaves were sampled, and since species germinated at different times, the actual ‘age’ range of leaves in the sample is probably more uniform than ‘time after sowing’ implies.

The overall range of values obtained (-10.6‰ to -13.5‰), is narrower than that reported for over 400 C₄ grasses in the literature (-9.2‰ to -19.3‰; Bender 1971; Smith and Epstein 1971; Troughton et al. 1974; Sankhla et al. 1975; Winter et al. 1976; Brown 1977; Vogel et al. 1978). The latter constitutes a sample of C₄ species from many varied habitats and geographical locations, measured in a variety of laboratories. I interpret the narrow range I obtained (2.9‰) as reflecting that all species in the experiment experienced the same overall environment. Differences in means for C₄ types have emerged even within this narrow range. From theoretical considerations, O'Leary (1981) expects narrow ranges for the ‘photosynthetic options’, C₃, C₄ and crassulacean acid metabolism.

Since the C₄ type samples include both eu-panicoid and chloridoid species where applicable, the differences in δ¹³C values found are not due to a correlation of δ¹³C value with some unknown taxonomically correlated factor. The mean δ¹³C value of eu-panicoids (-11.72) is indeed statistically significantly different from the mean for chloridoids (-12.47), but this seems to be due to only the eu-panicoids containing NADP-ME-type species. If mean δ¹³C values are compared for eu-panicoid NAD-ME plus PCK-type species (-12.11) and for chloridoid NAD-ME plus PCK-type species (-12.47), the difference is not significant, implying that there is no or little difference in δ¹³C values between these subfamilies which is not attributable to C₄ type.

It seems likely that the differences in mean δ¹³C values for types are caused by differences in those intrinsic leaf processes which affect discrimination against ¹³C. One such process is CO₂-HCO₃⁻ leakage from PCR tissue. The more leaky PCR tissue is, the more will discrimination against ¹³C by RuP₂ carboxylase have some influence on the plant δ¹³C value. The carbon isotope composition of the CO₂ and HCO₃⁻ pools in the PCR tissue will be more positive when there is no leakage than when there is some. This is because these pools will equilibrate more with the equivalent pools in PCA tissue (and via them, with the atmospheric CO₂ pool), when the conductance of PCR tissue to CO₂ and HCO₃⁻ is greater. The δ¹³C value of the CO₂ pool available to RuP₂ carboxylase in PCR tissue, which must be more positive than atmospheric δ¹³C (say -7‰), will more nearly approach -7‰ the more leakage there is. With respect to the ¹³C discriminated against by RuP₂ carboxylase, the effect of approaching equilibrium, even if to a small extent, between the carbon
isotope compositions of the CO$_2$ and HCO$_3^-$ pools in PCR and PCA tissue, is to make the $\delta^{13}$C values of the PCA pools more positive than they would be if leakage had not occurred. The $\delta^{13}$C value of PCA intercellular CO$_2$ also becomes more positive than if RuP$_2$ carboxylase discrimination had not occurred. Bearing in mind that the PCA intercellular CO$_2$ concentration is lower than that of the atmosphere, this means that the concentration gradient between the atmosphere and PCA tissue for $^{13}$CO$_2$, relative to $^{12}$CO$_2$, will not be as great. Therefore, less $^{13}$CO$_2$, relative to $^{12}$CO$_2$, will diffuse into the leaf, i.e. plant $\delta^{13}$C will be more negative. An equation derived by G. D. Farquhar (personal communication) describing the discrimination against $^{13}$C in C$_4$ plants (see Appendix), encompasses this prediction (that the more PCR CO$_2$ leakage there is, i.e., the larger $\phi$, the more negative will be the $\delta^{13}$C value of the plant). Leakage, however, affects more than just how much discrimination by RuP$_2$ carboxylation is realized (relative to the atmosphere) (see Appendix).

In Farquhar’s C$_4$ equation, $\delta$ depends on the absolute intercellular CO$_2$ concentration ($c_i$), as well as $\phi$. It is not possible to conclude at this time, whether $\delta$ differences between C$_4$ types reflect primarily PCR leakage differences, and/or different ‘average’ $c_i$ values or, indeed, other factors (see below). Also, it is important to realize that leakage itself is one of the factors determining $c_i$ (that $\phi$ and $c_i$ are dependent variables). Experiments are required where $c_i$ and instantaneous $\delta_{plant}$ are monitored simultaneously.

The absence of a ‘suberized lamella’ in PCR walls of NAD–ME-type grass species could mean that apoplastic CO$_2$ leakage occurs in this type, unlike the other two types. However, data on other anatomical parameters (e.g. PCR tissue surface area to volume ratio, intra-PCR site of C$_4$ acid decarboxylation: see Hattersley and Browning 1981) suggest that NAD–ME-type leaf anatomy may compensate for this apparent ‘extra’ leakage potential. While it is possible that leakage rates may not differ significantly between C$_4$ types, it is interesting in this context to note that the three dicotyledon $\delta^{13}$C values (Table 1) are as negative as or more negative than those for NAD–ME-type grasses. *Amaranthus edulis* does not possess a PCR wall ‘suberized lamella’ (Laetsch 1971), nor probably do the other two species since no dicotyledon has yet been found with such a lamella (see references in Hattersley and Browning 1981). On the other hand, PCK and NADP–ME-type C$_4$ grasses both have PCR cell walls with suberized lamellae, and yet their $\delta^{13}$C value means still differ significantly (though with a lower probability than between NAD–ME and NADP–ME types). *Apoplastic* CO$_2$ leakage, therefore, cannot be the only cause of $\delta^{13}$C value differences between C$_4$ types.

It is possible that other as yet unknown fractionation processes are responsible for differences in C$_4$ type values, e.g. fractionation associated with enzyme reactions specific to each C$_4$ type, or differences between C$_4$ types in cellulose composition (see Deleens and Garnier-Dardart 1977), or fractionation associated with diffusion of CO$_2$ and HCO$_3^-$ in the liquid phase in plant tissue. Whether or not discrimination against $^{13}$C occurs during the process of diffusion in liquid phase in plants (tracer diffusion in this case: see Mills and Harris 1976) is as yet unknown. If it is important, then it needs to be incorporated into the expression of Farquhar et al. (1982a) for C$_3$ species, and into expressions for C$_4$ species (see Appendix), with respect to the diffusion of CO$_2$ and HCO$_3^-$ from mesophyll (or PCA) cell surfaces to the sites of RuP$_2$ carboxylation (C$_3$ plants) or PEP carboxylation (C$_4$ plants). In C$_4$ plants, fractionation during leakage (by diffusion) of CO$_2$ and HCO$_3^-$ from PCR tissue
would also occur. Vogel's (1980) estimate is that the discrimination factor associated with liquid diffusion may be similar to that associated with gaseous diffusion.

It may also be invalid to assume that $b_3$ (discrimination factor during RuP$_2$ carboxylation: see Appendix) is constant, both with time for any one species or plant individual, and with species or photosynthetic type (see also Vogel 1980). Species differences for non-angiosperms have been found even within a single study (Estep et al. 1978; $b_3 = 31.4 - 38.7$). In vitro discrimination by RuP$_2$ carboxylase isolated from a C$_4$ plant has only been measured once (Whelan et al. 1973), so it is not possible to evaluate whether $b_3$ differs between C$_3$ and C$_4$ plants. Differences in $K_m$(CO$_2$) for enzyme isolated from C$_3$ and C$_4$ plants have been demonstrated for

Fig. 2. $\delta$ notation for CO$_2$ and HCO$_3^-$ pools and for carbon in CO$_2$ and HCO$_3^-$ fluxes during C$_4$ photosynthesis. Apoplastic leakage from PCR to PCA tissue may or may not occur, or be relevant. Photosynthesis and respiration have been omitted. A $\delta$ value is not given for C$_4$ acids because only C-4 of dicarboxylic acids is relevant to PEP carboxylation and C$_4$ acid decarboxylation. $\delta_e$ is, therefore, the $\delta^{13}$C value of C-4 in C$_4$ acids, as well as the $\delta^{13}$C value for HCO$_3^-$ in PEP carboxylation and for CO$_2$ produced by decarboxylation. PCA, primary carbon assimilation; PCR, photosynthetic carbon reduction; PEP, phosphoenolpyruvate; RuP$_2$, ribulose 1,5-bisphosphate; St, stomata; Pd, plasmodesmata.

grasses (Yeoh et al. 1980), but it is unknown whether the differences between the enzymes which determine $K_m$(CO$_2$) also affect $b_3$. In order to avoid the dependence on the 'constants' $b_3$ and $b_4$ in equation (1) (Appendix), I have derived expressions which incorporate instead $\delta^{13}$C values of the various CO$_2$ and HCO$_3^-$ pools shown in Fig. 2 for C$_4$ plants [Appendix, equations (3) and (7)]. The relationships between $c_i$, $c_m$, $c_k$, $\delta_i$, $\delta_k$, and $\delta_T$ are complex, and it remains to be seen whether all these parameters can be measured experimentally and, thereby, whether species differ with respect to the CO$_2$ concentrating mechanism and leakage [equations (6) and (8), Appendix]. Farquhar (personal communication) has suggested an independent test for whether PCR leakage differs between C$_4$ types in grasses: CO$_2$–HCO$_3^-$ leakage differences should be reflected in differences in quantum yield.
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Appendix

Theoretical Aspects

Farquhar *et al.* (1982b) have recently derived an equation which describes the discrimination against ¹³C in C₃ plants (see Farquhar 1980), and which seems to be validated by recent data (Farquhar *et al.* 1982a). Farquhar (personal communication) presents an analogous equation for C₄ species:

\[ \delta = \delta_{\text{atm}} - a - (b^* - a)(c_i/c_a), \]

where \( \delta \) = plant carbon δ¹³C value, \( \delta_{\text{atm}} \) = atmospheric δ¹³C value, \( a \) = discrimination factor during diffusion of CO₂ in air (expressed as a Δ of 4·4%/°C), \( c_i \) and \( c_a \) = the

\[ \Delta \] is the notation for the difference between δsource and δproduct. It is an adequate approximation to the discrimination factor (see O’Leary 1981).
intercellular and atmospheric concentrations of CO₂, and \( b^* \) = discrimination that occurs between intercellular CO₂ in PCA tissue and fixed carbon in the plant.

\[
b^* = b_4 + b_3 \phi ,
\]

where \( b_4 \) = net discrimination factor during the CO₂--HCO₃⁻ conversion \textit{and} PEP carboxylation (expressed as a \( \Delta \) of \(-5\%\)), \( b_3 \) = discrimination factor during RuP₂ carboxylation (expressed as a \( \Delta \) of \(27\%\)), and \( \phi \) = proportion of CO₂ produced by C₄ acid decarboxylation, which leaks from the PCR compartment to the PCA compartment.

As implicit in the equation, RuP₂ carboxylation does have some influence on the \( \delta^{13}C \) values of C₄ plants (cf. model by Reibach and Benedict 1977).

Using the mean \( \delta^{13}C \) values for C₄ types from this study, a \( c_i \) of 100 ppm, a \( c_a \) of 330 ppm, a \( \delta_{\text{atm}} \) of \(-7\%\), and a \( b_3 \) of \(27\%\), \( \phi \) for the C₄ types becomes: NAD–ME-type, 0.53; PCK-type, 0.44; NADP–ME-type, 0.37.

This would say that NAD–ME species are generally the most ‘leaky’, and NADP–ME species the least. Large differences in \( \phi \) appear to cause only small shifts in plant \( \delta^{13}C \) value. However, \( \delta \) is dependent not only on the variable \( \phi \), but also on the variable \( c_i \), and it is not valid to choose a specific single \( c_i \) to estimate values for \( \phi \) from different plant \( \delta^{13}C \) values. It also should be emphasized that \( \phi \) will vary with changing net assimilation rates, stomatal conductance, etc. As with \( \delta \) and \( c_i \), one can refer to an ‘instantaneous’ \( \phi \) value, for any given moment in time, or an ‘average’ \( \phi \) value, for the value of \( \phi \) averaged over an individual plant’s existence.

General differences in leaf \( \delta^{13}C \) values for C₄ types could reflect differences in average \( c_i \) during photosynthesis, therefore, as well as differences in average \( \phi \). Taking \( \phi \) as 0.5, and using other values as in the previous example, \( c_i \) would be 126 ppm for the NAD–ME-type, 53 for PCK, and -5 for the NADP–ME-type. The latter is, of course, impossible, and illustrates that \( \phi \) and \( c_i \) are not independent variables. (If \( \phi \) is \(<0.37\), then the trend of the differences in \( c_i \) for the three C₄ types will be in the opposite direction, viz. NAD–ME < PCK < NADP–ME for \( c_i \) values.) Although these examples, like the previous one, are strictly invalid, they serve to show that potentially large differences in average \( c_i \) would be reflected in only small changes in the \( \delta^{13}C \) value of the plant. Both examples suggest that apparent small differences detected in \( \delta^{13}C \) values could reflect large physiological differences.

I have derived alternative expressions describing the \( \delta^{13}C \) value of a plant using the \( \delta^{13}C \) values of the various CO₂ and HCO₃⁻ pools in leaves, instead of \( b_3 \) and \( b_4 \). These expressions are derived using some of the same fundamental bases as Farquhar \textit{et al.} (1982b) (and see Farquhar 1980). Thus, for C₃ plants:

\[
\delta = \frac{\delta_a - a - (\delta_i - a)(c_i/c_a)}{1 - c_i/c_a} ,
\]

where \( \delta_i \) is the \( \delta^{13}C \) value of the CO₂ pool in the intercellular gas spaces of the leaf mesophyll and \( \delta_a \) is the \( \delta^{13}C \) value of CO₂ in the atmosphere; \( c_i \) is the concentration of CO₂ in the same intercellular gas spaces.

For C₄ plants, the expression becomes:

\[
\delta = \frac{\delta_a - a - [\delta_a c_i + \delta_a (c_m - c_a) - ac_i](1/c_a)}{1 - c_m/c_a} ,
\]

where \( \delta_a \) is the \( \delta^{13}C \) value of the CO₂ pool in the intercellular gas spaces of the leaf mesophyll and \( \delta_a \) is the \( \delta^{13}C \) value of CO₂ in the atmosphere; \( c_i \) is the concentration of CO₂ in the same intercellular gas spaces.
where the $\delta^{13}C$ values with subscripts refer to carbon in the CO$_2$ pools and fluxes given in Fig. 2, $c_m$ and $c_k$ are the concentrations of CO$_2$ in PCA and PCR cells respectively, and $c_i$ is the concentration of CO$_2$ in the PCA tissue (mesophyll) intercellular gas spaces.

The $\delta_k$ CO$_2$ pool must be in equilibrium, or have some kinetic relationship, with a HCO$_3^-$ pool in PCR tissue (Fig. 2). Presumably HCO$_3^-$ will also leak from PCR tissue, via the symplast (and apoplast?). These considerations do not alter the validity of equation (3) for CO$_2$ only. An alternative expression to equation (3) could be derived, considering only HCO$_3^-$ leakage from PCR tissue, with values for the fractionation between intracellular CO$_2$ and HCO$_3^-$ in both PCA and PCR tissues included.

The fractionation ($\alpha$), or the 'isotope effect', associated with the CO$_2$-to-HCO$_3^-$ conversion in PCR tissue, can be given in terms of the $\delta^{13}C$ values of the PCR CO$_2$ pool ($\delta_k$) and the PCR HCO$_3^-$ pool ($\delta_j$) (refer to Fig. 2):

$$\alpha = \frac{1 + \delta_k / 1000}{1 + \delta_j / 1000}. \quad (4)$$

The $\delta^{13}C$ value of CO$_2$ plus HCO$_3^-$ ($\delta_T$) which leaks from PCR to PCA tissue, is given by:

$$\delta_T = \delta_N - \rho(\delta_N - \delta_L), \quad (5)$$

where $\delta_L = \delta^{13}C$ value of leaking CO$_2$ (Fig. 2), $\delta_N = \delta^{13}C$ value of leaking HCO$_3^-$ (Fig. 2), and $\rho$ = the proportion of total leaking carbon which leaks as CO$_2$.

Plant $\delta$ can also be described, in fact, in terms of $\delta_T$ and $\phi$. Since

$$\phi = (\delta - \delta_c)/(\delta - \delta_T) \quad (6)$$

$$\delta = (\delta_T \phi - \delta_c)/(\phi - 1) \quad (7)$$

and

$$\delta_T = \delta - (\delta - \delta_c)/\phi, \quad (8)$$

where $\delta_c$ is the $\delta^{13}C$ value of CO$_2$ released in C$_4$ acid decarboxylation (see Fig. 2), and $\delta_T$ is the $\delta^{13}C$ value of total carbon atoms in the CO$_2$ and HCO$_3^-$ which leak from PCR and PCA tissue. $\phi$, here, is the proportion of CO$_2$ produced by C$_4$ acid decarboxylation which leaks from PCR to PCA tissue as CO$_2$ and HCO$_3^-$; or it is the proportion of CO$_2$ produced by C$_4$ acid decarboxylation which is not fixed by RuP$_2$ carboxylase. When leakage is zero, $\delta_{\text{plant}}$ will equal $\delta_c$. When leakage is 100%, $\delta_T$ will equal $\delta_c$ [see equation (6)].

At all values of $\phi$ (except 1), $\delta_T > \delta_c$. This is because RuP$_2$ carboxylase in the PCR compartment discriminates against $^{13}$CO$_2$ in the incoming CO$_2$ from C$_4$ acid decarboxylation. Leakage of $^{13}$C-rich CO$_2$ into the $\delta_m$, $\delta_b$, and $\delta_l$ pools must have a contributory effect on the proportion of $^{13}$CO$_2$, relative to $^{12}$CO$_2$, entering the leaf (i.e. reduce it, thereby lowering $\delta_{\text{plant}}$). This effect can be referred to as the 'PCR leakage RuP$_2$ carboxylase effect'. Once leakage has occurred, $\delta_{\text{plant}}$ is affected. This is because $\delta_{\text{plant}}$ is determined by the $\delta^{13}C$ value of carbon which actually enters the stomata. It is not relevant whether actual leaked CO$_2$ and HCO$_3^-$ are refixed by PEP carboxylase or not.
There is, however, a second effect of leakage, which I shall call the 'PCR leakage short-circuit effect'. For every carbon atom which leaks out of PCR tissue during photosynthesis, there has to be a carbon atom which enters as C-4 of a C₄ acid. The result is that $c_m$ and $c_e$ will be higher, for a given PEP carboxylation rate, when leakage rates are higher.

PCA CO₂ concentrations affect the amount of discrimination (relative to atmospheric CO₂) due to gaseous diffusion (factor $a$) and that due to biochemical fractionation [factor $b^*$ in Farquhar's equation (1)]. The higher $c_t$, the more is biochemical discrimination realized, and the less is gaseous diffusion fractionation realized. When the term $-(b^* - a)$ is negative (i.e. discrimination against $^{13}$C), then the effect of increasing $c_m$ due to increased leakage will be to lower the $\delta^{13}$C value of net CO₂ entering the leaf, and therefore to lower $\delta_m$ (and $\delta_i$). Under these conditions the 'PCR leakage short-circuit' affects $\delta_{\text{plant}}$, in the same direction as the 'PCR leakage RuP₂ carboxylase effect' (makes it more negative). Using Farquhar's equation (1) and values given there, the above applies when $\phi > 0.37$ (and using a $\delta_a$ of $-7$, when $\delta_{\text{plant}}$ is $\leq -11.4$). The effect acts as it does because, under these conditions, $\delta_i \geq \delta_a$.

When $-(b^* - a)$ is positive, then the 'PCR leakage short circuit' will act in the opposite direction (when $\phi < 0.37$, and $\delta_{\text{plant}}$ is $> -11.4$). Thus increasing $\phi$ from, say, 0.3 to 0.35 will increase $c_m$, and make $\delta_{\text{plant}}$ more positive. This is because $\delta_i \leq \delta_a$ when $\phi < 0.37$. But increasing $\phi$ from 0.3 to 0.35 will also make $\delta_{\text{plant}}$ more negative due to the 'PCR leakage RuP₂ carboxylase effect'. The net balance of these two effects (for $\phi < 0.37$) seems to be to make $\delta_{\text{plant}}$ more negative since, using equation (1), it can be shown that, for any given $c_m$, increasing $\phi$ makes $\delta_{\text{plant}}$ more negative over the whole range of possible $\phi$ values (except that when $c_i = 0$, $\delta_{\text{plant}}$ is $-11.4$ whatever $c_i$ is). It seems that the increase in $c_m$ due to any given increase in $\phi$ would have to be quite large to counteract the increase in discrimination by RuP₂ carboxylation due to leakage. To date, all C₄ plant $\delta^{13}$C values are more negative than $\delta_a$, i.e. they all exhibit a net discrimination against $^{13}$CO₂ in the air. Using equation (1), it can be shown that only when $\phi < 0.21$ are values $< -7$ even possible.

When $\phi > 0.37$, $\delta_i \geq \delta_a \geq \delta_{\text{plant}}$. When $\phi < 0.37$ but $> 0.21$, $\delta_a \geq \delta_i > \delta_{\text{plant}}$, and when $\phi < 0.21$, $\delta_a \geq \delta_{\text{plant}} > \delta_i$. It is also possible to establish the relativities of $\delta^{13}$C values of other CO₂ and HCO₃⁻ pools under a plant's 'average' growing conditions. Thus $\delta_k > \delta_1 > \delta_2 > \delta_m > \delta_i$, and $\delta_b > \delta_e$ (see Fig. 2). The $\delta^{13}$C value of the PCR HCO₃⁻ pool ($\delta_j$) will be more positive than $\delta_k$ because of the discrimination against $^{12}$C associated with the CO₂ $\rightarrow$ HCO₃⁻ conversion. For HCO₃⁻ leakage, by analogy with CO₂ leakage, we have

$$\delta_N > \delta_j > \delta_b,$$

where $\delta_N$ is the $\delta^{13}$C value of HCO₃⁻ which leaks from PCR tissue, and $\delta_b$ is the $\delta^{13}$C value of the PCA HCO₃⁻ pool (Fig. 2).

The effect of dark respiration and photorespiration on the $\delta^{13}$C values of C₃ plants is somewhat analogous to the 'PCR leakage short-circuit effect' in C₄ plants. (In C₃ plants, there is no parallel with the 'PCR leakage RuP₂ carboxylase effect'.) It is simpler however, since $-(b - a)$ is always negative (always discrimination against $^{13}$C; see Farquhar et al. 1982b).
Consider a C₃ plant photosynthesizing in 2% oxygen (no photorespiration). When O₂ is increased to 21%, cᵢ will increase. Since δ₁ > δₕ, this will make the δ¹³C value of net CO₂ entering the stomates more negative, which will lower the δ¹³C value of the mesophyll CO₂ pool (the source pool for RuP₂ carboxylase) and, thereby, the δ¹³C value of CO₂ fixed by RuP₂ carboxylase. A new equilibrium will be established, with a higher cᵢ, lower δ₁, and a lower instantaneous δₚₐₙₐₙ. 

Considering the average conditions under which an individual C₃ plant has been growing, the δ¹³C value of total respired CO₂ will be δₚₐₙₐₙ (ignoring possible intrinsic fractionation in photorespiratory and dark respiratory processes). The δ¹³C value of net carbon entering the leaf will, of course, be δₚₐₙₐₙ. The relative rates of these two processes do not need to be known, therefore, when modelling C₃ plant δ¹³C values, since the δ¹³C value of carbon fixed in RuP₂ carboxylation itself must also be δₚₐₙₐₙ. One would need to know, however, the effect 'total respiration' has on cᵢ.

Considering the modelling of C₃ δₚₐₙₐₙ at any given instant, however, dark respiratory (but not photorespiratory) CO₂ may be of a different δ¹³C value than instantaneous δₚₐₙₐₙ. Relative rates of gross photosynthesis and dark respiration would have to be taken into account, as well as cᵢ effects, to accommodate the 'C₃ respiratory short circuit effect' in any instantaneous model.

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