Pergamon

PII: S0031-9422(96)00538-9

Phytochemistry, Vol. 43, No. 6, pp. 1183–1188, 1996

0031-9422/96 $15.00 + 0.00

HETEROTROPHIC WHOLE PLANT TISSUES SHOW MORE ^{13}C ENRICHMENT THAN THEIR CARBON SOURCES

VALERY J. TERWILLIGER* and JUN HUANG

Gas Source Stable Isotopes Laboratory, Departments of Geography and Botany, University of Kansas, Lawrence, KS, 66045, U.S.A.; National Research Council Canada, Plant Biotechnology Institute, Saskatoon, SK S7N 0W9, Canada

(*Received in revised form* 6 *May* 1996)

Key Word Index—$\delta^{13}C$ Heterotrophic growth; translocated carbon; *Lycopersicon esculentum*; Solonaceae; *Nicotiana tabacum*

Abstract—An inhibitor of carotene synthesis was applied to produce chlorophyll-free heterotrophic leaves on some branches of photoautotrophic tomato (*Lycopersicon esculentum*) plants and to produce fully heterotrophic *in vitro* cultured tobacco (*Nicotiana tabacum*) plants. The $\delta^{13}C$ values of heterotrophic leaves were 1–3‰ less negative than those of the adjacent, simultaneously produced photoautotrophic leaves that would have provided the source of organic carbon for growth of tomato plants. The $\delta^{13}C$ values of heterotrophic tobacco plants were >2‰ less negative than the sucrose or any other assimilable carbon compound in the organic growth media in which they were grown. These results indicate that isotope effects associated with the use of translocated organic carbon for growth can cause an enrichment in ^{13}C of whole tissue. Furthermore, mass balance considerations indicate that the enrichment does not simply reflect the $\delta^{13}C$ value of the organic substrate for growth. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

The two principal influences on the stable carbon isotope composition ($\delta^{13}C$) of whole plant tissues are considered to be diffusion and carbon fixation by Rubisco. In C_3 plants, both factors result in higher rates of incorporation of $^{12}CO_2$ than $^{13}CO_2$ and the action of Rubisco discriminates more than diffusion [1, 2]. These two factors influence the intercellular concentration of $^{13}CO_2$ relative to $^{12}CO_2$ by controlling the mean residence time of internal CO_2 as described by the expression (modified from Farquhar *et al.* [1])

$$\delta^{13}C_{plant} = \delta^{13}C_{CO_2,a} - \alpha_1 - \left\{(\alpha_2 - \alpha_1)\frac{[CO_2]_i}{[CO_2]_a}\right\} \quad (1)$$

where subscripts a and i denote atmospheric and intercellular, respectively, α_1 is the carbon fractionation during diffusion (maximum 4.4‰ [3]), and α_2 is the carbon fractionation by Rubisco (max = 29‰ [4]). Environmental stimuli (e.g. light, temperature, humidity) contribute to the $\delta^{13}C$ of plant tissue by affecting $[CO_2]_i/[CO_2]_a$.

Lowdon and Dyck [5] found the $\delta^{13}C$ values of leaf tissue to decrease by as much as 5‰ from the time a bud breaks until a leaf matures in woody angiosperms; a change far in excess of the 0.5–0.7‰ variation in the $\delta^{13}C$ of air during the growing season of their study area [6]. As they expand, leaves develop more effective barriers to diffusion, higher chlorophyll concentrations and higher photosynthetic capacities [7], features that would result in lower $[CO_2]_i/[CO_2]_a$ values during carbon fixation. By the logic of Eq. (1), their $\delta^{13}C$ values should thus become less, not more, negative over time. Some field experiments suggest that observed decreases in $\delta^{13}C$ from leaf emergence to maturity are a result of seasonal changes in microclimate that stimulate an increase in $[CO_2]_i/[CO_2]_a$ over time, or the use for leaf emergence of carbohydrate reserves the $\delta^{13}C$ values of which have been affected by different environmental conditions [8–10]. In either explanation, the $\delta^{13}C$ value of whole tissue is the result of an interplay between diffusion rates and Rubisco activities at some point during the plant's growing season.

In contrast, however, other field studies suggest that the ^{13}C enrichment of low or non-photosynthetically active tissues relative to fully developed photosynthetic tissue may be a result of influences in addition to the interplay between diffusion rates and Rubisco activities. For example, Leavitt and Long [11] found the $\delta^{13}C$ of both whole tissue and purified cellulose of (heterotrophic) wood to be less negative than the corresponding $\delta^{13}C$ values of simultaneously produced (photoautotrophic) leaves. Terwilliger [12] found decreases in $\delta^{13}C$ from the emergence to full expansion

*Author to whom correspondence should be addressed.

of leaves to occur in many tropical species with widely differing leaf life cycles in the absence of environmental stimuli that could logically have produced this consistent trend. These observations suggest a third explanation for the ^{13}C enrichment of tissue growth that is fuelled by heterotrophic inputs. This explanation, tested herein, is that growth from translocated organic sources of energy may have less negative $\delta^{13}C$ values than growth from the immediate products of photosynthesis, regardless of environment. We tested this hypothesis by producing heterotrophic leaves developing simultaneously with photoautotrophic leaves on tomato (*Lycopersicon esculentum* Mill. cv Rutger) plants and entirely heterotrophic tobacco (*Nicotiana tabacum* L. cv. Wisconsin 38) plants. The heterotrophic growth was achieved by preventing photosynthesis in lighted environments via an inhibitor of carotene synthesis.

RESULTS

Heterotrophic leaves have less negative $\delta^{13}C$ than photosynthesizing leaves

Tomato leaves that were administered the carotene synthesis inhibitor (see Experimental) during development produced tissue that was entirely white. Untreated photoautotrophic leaves on inhibitor treated and control plants were green. Significant differences in $\delta^{13}C$ occurred between leaves of different ages and trophic states (Kruskal–Wallis Anova, $P < 0.0001$) (Fig. 1). The $\delta^{13}C$ values of both older (20–30 days after treatment) and younger ($\leq$ 9 days) white leaves were significantly (1–3‰) less negative than those of green leaves of both ages on treated tomato plants (Mann–Whitney U test, $P \leq 0.01$). Both younger and older white leaves of treated plants had significantly less negative $\delta^{13}C$ values than older green leaves of untreated control plants ($P < 0.01$) but did not differ significantly in $\delta^{13}C$ from younger green leaves of control plants. Green leaves of both ages on treated plants had significantly more negative $\delta^{13}C$ values than leaves of any age on control plants (Mann–Whitney U tests, $P < 0.05$).

$\delta^{13}C$ of heterotrophic tissue relative to its carbon source

Heterotrophic tobacco plants had $\delta^{13}C$ values (-21.7 ± 0.2 SE) that were at least 2‰ less negative than those of their growth medium or its individual assimilable carbon compounds (Fig. 2). Carbon compounds in the growth medium of the completely heterotrophic, *in vitro* cultured tobacco plants were sucrose ($\delta^{13}C = -24.2$‰) and a small amount of inositol ($\delta^{13}C = -29.1$‰). The $\delta^{13}C$ of the total growth medium was the same as that of the sucrose.

Gas exchange of heterotrophic and photoautotrophic tomato

Young heterotrophic leaves of tomatoes exhibited no measurable CO_2 uptake and had significantly lower transpiration rates than did photoautotrophic leaves of the same age (Mann–Whitney U test, $P \leq 0.006$) (Table 1). Transpiration rates of heterotrophic leaves did not differ from those of older green leaves, however. Ratios of intercellular to ambient [CO_2] were significantly higher in heterotrophic leaves than in green leaves of any age ($P < 0.009$). Young green leaves did not differ significantly from older green

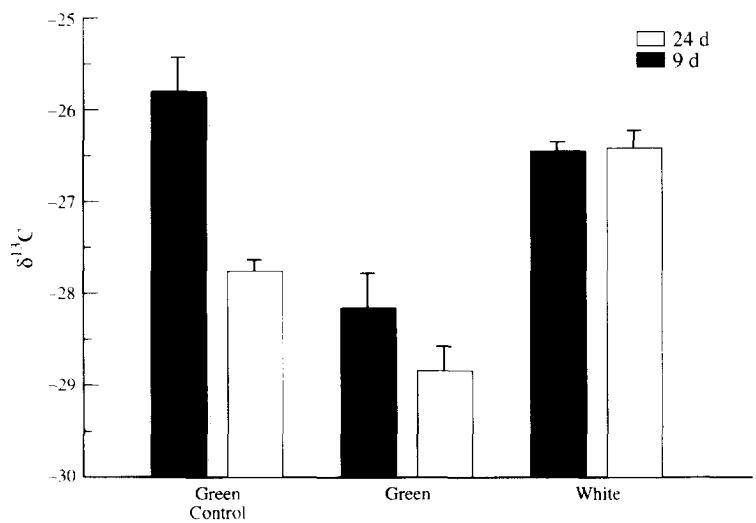

Fig. 1. Average (±SE) $\delta^{13}C$ values of leaves of tomato. Closed bars represent leaves ≤9 days after treatment (young) and open bars represent leaves 20–30 days after treatment (old). 'White' leaves had been administered an inhibitor of carotene synthesis that prevented photosynthesis and hence were heterotrophic ($n = 10$ each of young and old leaves). 'Green' leaves were photoautotrophic but on the same plant as inhibitor treated leaves ($n = 10$ each of young and old leaves). 'Green Control' leaves were on untreated plants ($n = 4$ each of young and old leaves).

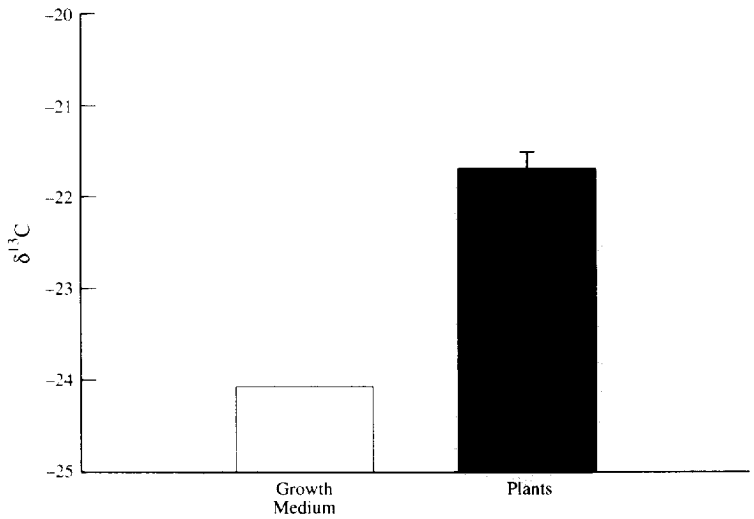

Fig. 2. Average (+S.E.) $\delta^{13}C$ values of five completely heterotrophic tobacco plants compared to the $\delta^{13}C$ value of carbon substrate for growth (mostly sucrose).

leaves in photosynthesis rate, transpiration rate or in $[CO_2]_i/[CO_2]_a$. Gas exchange rates did not differ between green leaves on treated plants and leaves on control plants.

DISCUSSION

In practice, most applications of stable carbon isotope methods to phytological research assume that $\delta^{13}C_{air}$ and influences on the balance between diffusion and CO_2 uptake by Rubisco are the only factors that will leave a noticeable imprint on the $\delta^{13}C$ of whole tissue. The possibility that 'other influences' (α_3) merit further elucidation has been acknowledged, however, as given by the following expansion of Eq. (1) (modified from Farquhar *et al.* [2]):

$$\delta^{13}_{C\ plant} = \delta^{13}C_{air} - \alpha_1 - \left\{(\alpha_2 - \alpha_1)\frac{[CO_2]_i}{[CO_2]_a}\right\} + \alpha_3 \quad (2)$$

Resolving whether effects other than diffusion and carboxylation by Rubisco can influence the $\delta^{13}C$ of whole plant tissue is therefore a contribution to defining α_3 that would improve the phytological interpretations made from stable carbon isotopic analyses. This requires preventing photosynthesis, and hence any effect of CO_2 diffusion and carboxylation by Rubisco on the $\delta^{13}C$ of emerging tissue. Luo and Sternberg [13] pursued this by sprouting seeds in total darkness that contained different major substrates for growth. Our study differs in that a method was achieved of comparing photosynthetic growth with simultaneously produced growth on the same plant where photosynthetic carboxylation was eliminated. Our work also differs from previous studies in that we obtained entirely heterotrophic growth from an external pool of a substrate with known isotopic composition rather than a likely compound among many in a seed.

The $\delta^{13}C$ values of heterotrophic leaves were, in every instance, at least 1‰ less negative than any photoautotrophic leaves of treated tomato plants in our study (Fig. 1). Furthermore, the $\delta^{13}C$ values of heterotrophic tobacco plants were at least 2‰ less negative

Table 1. Median (first quartile range), net photosynthesis or carbon evolution rates (P), transpiration rates (E), and $[CO_2]_{intercellular}/[CO_2]_{air}$ (c_i/c_a) ratios in ≤9 days heterotrophic (white), ≤9 days photoautotrophic (green), and 20–30 days photoautotrophic (old green) leaves of tomato

Gas exchange Char.	Leaf status			Comparison[a]		
	White	Green	Old green	White vs green	White vs old green	Green vs old green
P (μmol m^{-2} s^{-1})	−0.8 (0.7)	4.0 (3.3)	2.3 (1.4)	<*	<*	NS
E (mmol m^{-2} s^{-1})	0.9 (0.7)	3.2 (2.7)	2.7 (1.6)	<*	NS	NS
c_i/c_a	1.1 (1.0)	0.9 (0.8)	0.9 (0.8)	>*	>*	NS

[a]Pairwise comparisons of gas exchange characteristics between leaf types are shown as <*, >*, (U test, $P < 0.05$, $n = 18$) or NS not significant; ($P > 0.05$), where the first leaf type has a significantly greater, lesser or not significantly different, respectively, gas exchange characteristic than the second leaf. Green leaves of controls did not exhibit differences in gas exchange from green leaves on treated plants and are combined.

than those of any carbon source that could have significantly contributed to their growth (Fig. 2). We emphasize that the carbon sources to the *in vitro* cultured tobacco were solely from a growth medium that would not have been subjected to isotopic fractionations outside of the plants. Photoautotrophic leaves would thus have been the original source of carbon for simultaneously expanding heterotrophic leaves of tomato. Growth of both tomato and tobacco plants was sufficient to have diluted unknown carbon isotopic contributions from their small initial propagules. If α_s is solely a result of diffusion properties and photosynthetic carboxylation rates when the carbon source to be tapped for growth elsewhere is first produced, then there would have been no difference in $\delta^{13}C$ between heterotrophic and photoautotrophic leaves of tomato or between tobacco plants and growth medium in our study. Heterotrophic leaves had higher $[CO_2]_i/[CO_2]_a$ than did photoautotrophic leaves (Table 1). If an undetected amount of CO_2 fixation by Rubisco occurred in the apparently heterotrophic leaves then, according to Eq. (1), $\delta^{13}C$ values would have been more negative in heterotrophic leaves than in photoautotrophic leaves. The most logical conclusion from our observed results is that ^{13}C enrichment can occur when new tissue grows from a translocated energy source.

This work directly corroborates more indirect indications that factors other than those affecting the interplay between diffusion and carboxylation by Rubisco can contribute to the carbon isotopic composition of leaf tissue [4, 13, 14]. Three logical hypotheses about the reason for ^{13}C enrichment in heterotrophic growth emerge from our results. One possibility is that the substance imported for heterotrophic tissue synthesis had a less negative $\delta^{13}C$ than that of a whole photoautotrophic leaf. A second possibility is that a loss of respired carbon that was depleted in ^{13}C relative to remaining tissue occurred during heterotrophic growth. Thirdly, the activity of phosphoenolpyruvate carboxylase fixed enough CO_2 to cause the ^{13}C enrichment of heterotrophic growth.

Starch, a common form of stored carbon, and sucrose, a common form in which carbon is translocated, can be enriched in ^{13}C relative to the primary products of photosynthesis [15]. Possible kinetic isotope effects and metabolic fluxes leading to these enrichments have been proposed [16, 17]. Export of ^{13}C enriched metabolites from green to white leaves may be a reason why the ^{13}C of green leaves on treated plants was more depleted than in leaves of control plants. Export of ^{13}C enriched metabolites from older to younger leaves may also explain why younger leaves had less negative $\delta^{13}C$ values than older leaves of untreated plants.

Translocation of ^{13}C enriched metabolites does not provide a convincing sole mechanism for the ^{13}C enrichment of tobacco plants relative to their substrate (sucrose) for growth. There should be no change in the isotopic composition of sucrose as a direct result of its ascent to sink tissues through xylem cells. Isotope effects associated with production of metabolites would occur thereafter. Nonetheless, according to Eq. (1), the sum of the proportional isotopic contributions of all metabolic products could not differ from the $\delta^{13}C$ of the original carbon source. Assuming that its sole carbon source came from its growth medium, the ^{13}C enrichment of whole tobacco plant tissue relative to its carbon source thus suggests that a carbon product from the metabolism of sucrose escapes the plant. Most logically, this product would be ^{13}C depleted CO_2 that evolved during respiration.

Some studies of respired CO_2 from growth in CO_2 free air indicate that respiration causes no changes in the $\delta^{13}C$ of whole leaves [18]. However, Jacobson *et al.* [19] found respired CO_2 to differ in $\delta^{13}C$ from whole tissue. They concluded that no fractionation of carbon occurs during respiration but that the $\delta^{13}C$ of respired CO_2 will equal that of the specific substrate being respired, not the CO_2 fixed by Rubisco. Gebauer and Schulze [14], however, proposed that cumulative measurable isotope effects from respiration caused twigs (heterotrophic) to become progressively enriched in ^{13}C over time compared to needles of the branch. Luo and Sternberg [13] also concluded that respiration can cause a measurable isotope effect after they found heterotrophic sprouts from seeds that had been treated with an uncoupler of respiration to differ in $\delta^{13}C$ from sprouts with normal respiration rates. Assessing the effect, if any, of respiration on the $\delta^{13}C$ of entire leaves requires identifying the $\delta^{13}C$ values of all possible carbon substrates for growth and this has been problematic [4, 20]. If growth medium supplied the only carbon for heterotrophic tobacco growth, then our results and mass balance considerations directly imply that preferential loss of $^{12}CO_2$ during respiration contributed to the ^{13}C enrichment observed in heterotrophic whole plant tissue.

CO_2 hydration precedes its fixation by phosphoenolpyruvate carboxylase. The hydrated HCO_3^- will be about 9‰ enriched in ^{13}C over atmospheric CO_2 if diffusion occurs with minimal resistance [21]. PEPC will then exhibit only sufficient preference for ^{12}C to cause a 2‰ reduction in $\delta^{13}C$ [22]. Given that the $\delta^{13}C$ value of atmospheric CO_2 is about -7.8 to -8‰ [23], the $\delta^{13}C$ of carbon fixed by PEPC is close to zero. The mass balance equation

$$\delta^{13}C_{PLANT} = p\,\delta^{13}C_{PEPC\ fixed\ CO_2} + (1-p)\delta^{13}C_{GROWTH\ MEDIUM} \quad (3)$$

where $\delta^{13}C_{GROWTH\ MEDIUM} = -24.2$‰ and $\delta^{13}C_{PLANT} = -21.7$‰ suggests that the proportional contribution of PEPC fixed CO_2 ($p \times 100$) to all carbon in plant tissue would have had to be a minimum of about 10%, assuming that diffusion rates were high. Our gas exchange measurements suggest that diffusion rates were probably not high. Where diffusion rates are at a minimum, a tendency for ^{12}C to diffuse more readily than ^{13}C produces about a 4.4‰ decrease in $\delta^{13}C$ [23]. Isotope effects associated with carboxyla-

tion would not be measurable in this case and PEPC fixed carbon would have a $\delta^{13}C$ of about $-12‰$. In this case, PEPC fixed CO_2 would have had to contribute closer to 20% of the carbon to have produced the $\delta^{13}C$ values that we obtained in heterotrophic tobacco, assuming that the CO_2 fixed had the global atmospheric $\delta^{13}C$ value. The contributions of PEPC fixed carbon to heterotrophic tobacco may have had to be yet higher than 20% since the contributions of respired CO_2 to the internal gaseous carbon pool would have been considerable given that the tobacco was grown in sealed containers.

There have been very few efforts to examine this anapleurotic effect of PEPC in C_3 plants. The most relevant study that we could find tracked the activities of PEPC from seed to seedling in apples (*Malus pumila* Mill. cv Golden Delicious) [24]. Cotyledons and primary leaves had the highest PEPC activities (281 and 206 nmol min^{-1} g^{-1} fresh weight, respectively), while secondary leaves had extraordinarily low PEPC activity (3 nmol min^{-1} g^{-1} fresh weight). Cotyledons and primary leaves showed little to no net photosynthesis, whereas secondary leaves did; thus PEPC activities appeared to be inversely related to degree of autotrophy. Efforts to determine if $\delta^{13}C$ values of C_3 plant tissue can be affected by PEPC fixed CO_2 have been made in CO_2 free chambers. This approach is inconclusive, however, owing to the possibilities for internal recycling of respired CO_2 [20].

Our results thus provide direct evidence that heterotrophic plant growth can be enriched in ^{13}C compared to its organic carbon source and point to possibilities for using stable carbon analyses to detect when growth is not photoautotrophic. Enrichment seems to occur during respiration, either as fractionation of the organic substrate for growth or by CO_2 fixation in anapleurotic reactions.

EXPERIMENTAL

Plant material. Pairs of heterotrophic and photoautotrophic leaves on tomato (*L. esculentum* Mill. cv Rutger) plants were grown for isotopic analyses (14 plants) or gas exchange measurements (9 plants) in a greenhouse on a 28°/25° day/night cycle. Tomato plants were grown at natural photosynthetic photon flux densities during summer and lighting was supplemented with halogen lamps for 14 hr day^{-1} in the early autumn. Plants were either propagated hydroponically in an aerated solution with an inorganic fertilizer (20–18–18, Fisons Horticulture) or in well-watered, inorganically fertilized pots. One of each pair of emerging petioles was treated by inserting a cotton wick wrapped in cotton batting with 10^{-4} M solution of 1-methyl-3-phenyl-5[3-(trifluoromethyl)phenyl]-4(1H)-pyridinone (Fluridone, Elanco) dissolved in the surfactant Polyoxyethylenesorbitan which facilitated its uptake into developing treated leaves. This inhibitor induces chlorosis by interfering with phytoene desaturase activity. The resulting increase in phytoene concentrations inhibits carotenoid synthesis which, in turn, causes photooxidation of chlorophyll [25]. Studies have failed to show any effect of this inhibitor on the assimilation, translocation or respiration of carbon following fixation [26]. The other emerging petiole of each pair was not treated. As a result, both photoautotrophic and completely heterotrophic leaves developed on the same plant. Four control plants received no treatment. Tomatoes were chosen for this experiment because their paired branching pattern makes them particularly suitable for this form of treatment.

Five completely heterotrophic tobacco (*N. tabacum* L. cv. Wisconsin 38) plants were propagated for 6 weeks from meristems. Tobacco, rather than tomato, was chosen for this second experiment because it was important to grow sufficient tissue from a meristem to dilute the isotopic input of the propagule. The plants were cultured in a sterile growth medium with a pH 5.8, containing Murashige and Skoog salts, inositol (0.1 g l^{-1}), sucrose (30 g l^{-1}), and a 10^{-2} M solution of the aforementioned carotenoid inhibitor dissolved in DMSO then diluted to 10^{-6} M in the final solution.

Analyses of $\delta^{13}C$. Tomato leaves were dried in an oven at 60° for 24 hr. Individual tobacco plants were lyophilized. Dried plant material was ground using an agate mortar and pestle. Growth medium for the heterotrophic tobacco plants was desiccated at 60°, as were individual samples of assimilable organic compounds used in the medium. Samples (2–3 mg) were combusted in an on-line system (Heraeus CHN-O Rapid) which automatically directed the CO_2 into an isotope ratio mass spectrometer (Finnigan Delta-S, Laboratory for Isotope Biodynamics, Department of Biochemistry, University of Nebraska, Lincoln) for analysis of $\delta^{13}C$. The $\delta^{13}C$ values were measured in per millitre (‰) using the equation

$$\delta^{13}C = 1000 \times \left(\frac{[^{13}C]/[^{12}C]_{sample}}{[^{13}C]/^{12}C]_{standard}} - 1 \right) \quad (4)$$

where the results are calculated with respect to a Pee Dee Belemnite (PDB) [27] standard by use of an acetanilide intermediate house standard.

Gas exchange. Instantaneous gas exchange rates were evaluated for photoautotrophic and heterotrophic tomato leaves as these rates could have affected $\delta^{13}C$ values. Specifically, CO_2 assimilation and transpiration rates of leaves were measured for 1 day using an infra-red gas analyser with an open system cuvette that exposed a 6.25 cm^2 leaf area to light (CID-301PS, Vancouver, WA). The photosynthetic photon flux densities during measurement averaged 450 μmol m^{-2} s^{-1} (±15 SE).

Acknowledgements—This work was funded by the National Science Foundation and Kansas Science and Technology Advanced Research programme K*STAR-NSF-EPSCoR-OSR-92-55-22-3 and the University of Kansas Graduate Research Fund. We thank Dr Michael Christianson for developing and showing us the tech-

nique for inhibiting carotene synthesis. Drs Marion O'Leary, Craig Martin and Michael Christianson provided intellectual input into this study and S. Madhavan obtained the isotope ratios. Drs S. Madhavan, Todd Swanson and Steven Leavitt reviewed the manuscript.

REFERENCES

1. Farquhar, G. D., O'Leary, M. H. and Berry, J. A., *Australian Journal of Plant Physiology*, 1982, **11**, 539.
2. Farquhar, G. D., Ehleringer, J. R. and Hubick, K. T., *Annual Reviews of Plant Physiology and Molecular Biology*, 1989, **40**, 503.
3. Craig, H., *Journal of Geology*, 1954, **62**, 115.
4. O'Leary, M. H., *Bioscience*, 1988, **38**, 328.
5. Lowdon, J. A. and Dyck, W., *Canadian Journal of Earth Sciences*, 1974, **11**, 79.
6. Mook, W. G., Koopmans, M., Carter, A. F. and Keeling, C. D., *Journal of Geophysical Research*, 1983, **88**, 10915.
7. Kursar, T. A. and Coley, P. D., *Plant, Cell and Environment*, 1992, **15**, 901–909.
8. Francey, R. J., Gifford, R. M., Sharkey, T. D. and Weir, B., *Oecologia*, 1985, **66**, 211.
9. Fu, Q. A., Boutton, T. W., Ehleringer, J. R. and Flagler, R. B., in *Stable Isotopes and Plant Carbon–Water Relations*, ed. J. R. Ehleringer, A. E. Hall, and G. D. Farquhar, Academic Press, San Diego, CA, 1993, p. 297.
10. Sobrado, M. A., in *Proceedings of the VI International Congress of Ecology*, INTECOL, Manchester, 1994, p. 325.
11. Leavitt, S. W. and Long, A., *Nature*, 1982, **298**, 742.
12. Terwilliger, V. J., *American J. Botany* (in press).
13. Luo, Y. H. and Sternberg, L. D. S. L., *Phytochemistry*, 1994, **35**, 877.
14. Gebauer, G. and Schulze, E.-D., *Oecologia*, 1991, **87**, 198.
15. Brugnoli, E., Hubick, K. T., von Caemmerer, S., Wong, S. C. and Farquhar, G. D., *Plant Physiology*, 1988, **88**, 1418.
16. Bartley, G. E., Scolnik, P. A. and Giuliano, G., *Annual Review of Plant Physiology and Plant Molecular Biology*, 1994, **45**, 287.
17. Gleixner, G., Danier, H. J., Werner, R. A. and Schmidt, H. L., *Plant Physiology*, 1993, **102**, 1287.
18. Hsu, J. C. and Smith, B. N., *Plant and Cell Physiology*, 1972, **13**, 689.
19. Jacobson, B. S., Laties, G. G., Smith, B. N., Epstein, S. E. and Laties, B., *Biochimica et Biophysica Acta*, 1970, **216**, 295.
20. O'Leary, M. H., *Phytochemistry*, 1981, **20**, 553.
21. Mook, W. G., Bommerson, J. C. and Staverman, W. H., *Earth and Planetary Science Letters*, **22**, 1974, 169.
22. O'Leary, M. H., Rife, J. E. and Slater, J. D., *Biochemistry*, 1981, **20**, 7308.
23. O'Leary, M. H., *BioScience*, 1988, **38**, 328.
24. Blanke, M. M., and Ebert, G., *Journal of Experimental Botany*, 1992, **43**, 965.
25. Weed Science Society of America, *Herbicide Handbook of the Weed Science Society of America*, Weed Science Society of America, Champaign, IL, 1979.
26. Sandmann, G. and Böger, P., in *Target Sites of Herbicide Action*, ed. P. Böger and G. Sandmann, CRC Press, Boca Raton, FL, 1989, p. 25.
27. Craig, H., *Geochimica et Cosmochimica Acta*, 1953, **3**, 53.