Functional Ecology 1991, **5**, 270–277

ESSAY REVIEW

Stable isotope composition of stem and leaf water: applications to the study of plant water use

L. B. FLANAGAN and
J. R. EHLERINGER
Department of Biology, University of Utah, Salt Lake City, Utah 84112, USA

Key-words: Ground water, humidity, hydrogen, oxygen stable isotopes, precipitation, water-use efficiency

Introduction

Much of the past research in plant physiological ecology has focused on gas-exchange responses of individual leaves (Pearcy *et al.*, 1987). There has been great progress in developing models that link leaf biochemical properties with leaf gas-exchange characteristics (Farquhar & von Caemmerer, 1982). Success has also come in understanding the physiological basis for the ecological differentiation of plants with C_3, C_4 and CAM photosynthetic pathways (Osmond, Winter & Ziegler, 1982). It has been recognized, however, that further progress in linking leaf-level, instantaneous responses to longer-term whole-plant growth would require new approaches to extend the temporal and spatial scale of physiological measurements (Ehleringer, Pearcy & Mooney, 1986). It has also been suggested that more emphasis should be placed on studies of below-ground resource acquisition (Ehleringer *et al.*, 1986). Stable-isotope techniques offer methods to address some of these long-standing problems in plant physiological ecology (Rundel, Ehleringer & Nagy, 1988).

In this paper we describe how measurements of the isotopic composition of stem and leaf water can be applied to studies of plant water use. The first application we discuss takes advantage of the different isotopic compositions of summer rain and ground water to trace the relative uptake of these two water sources by different plant species. This first technique can be used, in a relatively non-destructive manner, to study aspects of root function under field conditions. The second application we outline involves the use of known fractionation events that occur during transpiration to study the leaf–air water vapour pressure gradient, an important parameter influencing photosynthetic gas exchange. It has been suggested that this second technique could potentially be used to study canopy level interactions in photosynthetic gas exchange over longer time-scales than are possible using conventional techniques (Farquhar *et al.*, 1988; Sternberg, Mulkey & Wright, 1989).

Plant water sources

There is no fractionation of isotopes during water uptake by plant roots (White *et al.*, 1985). The isotopic composition of water in roots and stems, therefore, reflects the isotopic composition of water available to the plant. There are two potential sources of water for plant roots. Water may be taken up from the deep, ground-water reservoir, or water may be obtained from recent precipitation. The isotopic composition of ground water represents a long-term average of the isotopic composition of precipitation that falls in an area. At our study site in southern Utah (see Dawson, Ehleringer & Marshall, 1990, for a site description), the δD value of ground water does not change throughout the year (Table 1; for a definition of δ notation and a discussion of the values for international isotope standards, see Ehleringer & Osmond, 1989). The isotopic composition of precipitation, however, varies seasonally (Table 1). At mid-latitudes there are two dominant factors affecting the stable-isotope composition of precipitation (Dansgaard, 1964). The first is a temperature effect, and results because there is a positive relationship between the condensation temperature and the heavy isotope content of precipitation (Dansgaard, 1964). Precipitation that falls during cool times of the year has a low δD value (e.g. February, Table 1), while precipitation occurring during the warmer months of the year has a high δD value (e.g. July, Table 1). The second effect, an amount effect, occurs because there is a negative relationship between the amount of precipitation that falls and the heavy isotope content of that precipitation (Dansgaard, 1964). As water

Table 1. The hydrogen isotopic composition (δD) of ground water and precipitation at the Coral Pink Sand Dune site in southern Utah. $\delta = ([R_{sample}/R_{SMOW} - 1) \times 1000$. (Data of L.B. Flanagan, J.D. Marshall & J.R. Ehleringer, unpublished observations)

Date	δD (‰)
Isotopic Composition of Ground Water	
4 September 1988	−95
18 March 1989	−95
10 June 1989	−94
24 June 1989	−95
22 July 1989	−96
3 August 1989	−95
28 August 1989	−95
18 November 1989	−95
Isotopic Composition of Precipitation	
1 September 1988	−32
12 February 1989	−153
9 June 1989	−67
22 July 1989	−10
25 July 1989	−18

condenses from an air mass, the first precipitation formed is enriched in heavy isotopes. Consequently, the remaining water vapour in the air mass becomes progressively more depleted as more condensation occurs. Total precipitation, during a given rain event, will be relatively more depleted in heavy isotopes, therefore, when a large amount of water vapour condenses from a given air mass (Dansgaard, 1964). Rain will be relatively enriched in heavy isotopes when only a small amount of water vapour condenses from an air mass. The important point for our purpose is that, since ground water and individual precipitation events have different isotopic compositions, it is possible to determine the relative uptake of these two sources by measuring the isotopic composition of plant stem water (White *et al.*, 1985; White, 1988).

During extended periods of time between rains, plants at our study site in southern Utah have stem water isotopic compositions similar to that of ground water. After it rains, however, there are differences among species in the relative use of ground water and precipitation (Tables 2 and 3). Comparing the two major tree and two major shrub species at the study site, *Juniperus osteosperma* (Torr.) Little and *Chrysothamnus nauseosus* (Pallas) Britt. show no direct uptake of precipitation, while the isotopic composition of stem water indicates that rain water is directly taken up by *Pinus edulis* Engelm. and *Artemisia tridentata* Nutt. (Table 3). Using a two-end, linear mixing model (White, 1988), the stem water δD values in Table 3 indicate a 23% contribution of rain water in *P. edulis* and a 30% contribution of precipitation in *A. tridentata* (Table 3).

Table 2. Comparison of the hydrogen isotopic composition (δD, ‰) of ground water, precipitation and stem water in *Juniperus osteosperma* and *Artemisia tridentata* during June 1989 in southern Utah. Isotopic compositions are expressed relative to SMOW. (Data of L.B. Flanagan, J.D. Marshall & J.R. Ehleringer, unpublished observations.)

	9 June	10 June	11 June
Precipitation	−67		
Ground water		−94	−95
Juniperus osteosperma		−94	−97
Artemisia tridentata		−77	−83

Table 3. Comparison of the hydrogen isotopic composition (δD, ‰) of ground water, precipitation and stem water in the two major tree and two major shrub species at the Coral Pink Sand Dunes site in southern Utah during July 1989. Isotopic compositions are expressed relative to SMOW. (Data of L.B. Flanagan, J.D. Marshall & J.R. Ehleringer, unpublished observations.)

	22 July	23 July
Precipitation	−10	
Ground water	−96	
Trees		
Juniperus osteosperma		−97
Pinus edulis		−76
Shrubs		
Artemisia tridentata		−70
Chrysothamnus nauseosus		−96

The stem water δD data suggest that there are significant differences, among the species compared in Table 3, for the distribution pattern of roots in the soil. Reynolds & Fraley (1989) have shown that the maximum root depth is similar in both *Artemisia* and *Chrysothamnus*. The lateral distribution of roots is significantly different, however, between the two species. *Artemisia* has a much larger lateral distribution of roots than does *Chrysothamnus*, particularly in soil layers near the ground surface (Reynolds & Fraley, 1989). One would expect, therefore, a higher uptake of precipitation in *Artemisia* than in *Chrysothamnus*.

Leaf water isotopic enrichment as an indicator of the leaf–air water vapour pressure gradient

While there is no fractionation of isotopes during water uptake by plants, fractionation does occur

during transpiration. The lighter isotopes of water escape from the leaf more readily during transpiration so that leaf water becomes enriched in heavy isotopes (Dongmann *et al.*, 1974). The extent of the enrichment of heavy isotopes in leaf water depends on the leaf–air water vapour pressure gradient, as will be explained below. The isotopic composition of hydrogen and oxygen in cellulose synthesized by a leaf is influenced by the isotopic composition of leaf water (Sternberg, 1988; White, 1988). Leaf cellulose contains, therefore, a long-term integrated value of the short-term changes in leaf water enrichment that result because of variation in environmental conditions. It has been suggested that measurements of the isotopic composition of leaf cellulose could be used to infer the long-term, integrated nature of the leaf–air water vapour pressure gradient (Farquhar *et al.*, 1988; Sternberg *et al.*, 1989). Such a procedure would be similar to using measurements of the carbon isotopic composition of leaf tissue to obtain integrated information about the ratio of intercellular CO_2 to ambient CO_2 (Farquhar, Ehleringer & Hubick, 1989). In the following sections we outline the theory for isotope effects during transpiration and describe experiments we have done to test the model of leaf water enrichment. A final section reviews recent studies of the relationship between the isotopic composition of leaf water and leaf cellulose.

Isotope effects during transpiration: theory

There are two isotope effects during transpiration:
1 An equilibrium effect resulting from the phase change from liquid water to water vapour.
2 A kinetic effect caused by the different diffusion rates of the light and heavy isotopes.

The equilibrium fractionation factor (α^*) is defined as:

$$\alpha^* = \frac{R_L}{R_v} \qquad \text{Equation 1}$$

where R is the molar ratio of the heavy and light isotope (i.e. D/H or $^{18}O/^{16}O$) and the subscripts L and V refer to liquid water and water vapour, respectively. In the above definition it is assumed that the air is saturated with water vapour and that the liquid and vapour are at the same temperature. These conditions are satisfied for a leaf where water vapour in the intercellular air spaces is in equilibrium with leaf cell water. The equilibrium fractionation factor has been measured over a range of temperatures by Majoube (1971). The regression equations, listed by Majoube (1971), were used to calculate values for the parameter α^*.

The kinetic fractionation factor (α_k) is defined as the ratio of the diffusion coefficients for water molecules containing the light and heavy isotopes. For our purposes α_k can be defined as:

$$\alpha_k = \frac{g}{g'} \qquad \text{Equation 2}$$

where g and g' refer to the stomatal conductance to water vapour molecules containing the light and heavy isotopes, respectively. Merlivat (1978) has measured the relative rates of diffusion in air of water molecules containing the light and heavy isotopes of hydrogen and oxygen. The values for α_k are: H/D = 1·025 and $^{16}O/^{18}O$ = 1·0285 (Merlivat, 1978).

The transpiration rate of a leaf can be described as:

$$E = g\frac{(e_i - e_a)}{P} \qquad \text{Equation 3}$$

where e_i and e_a are the partial pressures of water vapour in the leaf intercellular air spaces and the atmosphere, respectively and P is the total air pressure. A similar equation can be written for water vapour molecules containing heavy isotopes:

$$E' = g'\frac{(R_v e_i - R_a e_a)}{P} \qquad \text{Equation 4}$$

where e_i is multiplied by R_v, the molar ratio of the heavy and light isotopes of water vapour in the intercellular air spaces, and e_a is multiplied by R_a, the molar ratio of the heavy and light isotopes of water vapour in the air outside the leaf. The ratio of the fluxes of the heavy (E') and the light (E) isotopes will describe the isotopic composition of water transpired by a leaf (R_T). Inserting the above-defined fractionation factors, the equation for the isotopic ratio of transpired water is:

$$\frac{E'}{E} = R_T = \frac{1}{\alpha_k}\frac{\left(\frac{R_L}{\alpha^*}e_i - R_a e_a\right)}{(e_i - e_a)} \qquad \text{Equation 5}$$

At steady state the isotopic composition of transpired water is the same as the source or stem water (R_s) (Craig & Gordon, 1965). At steady state, therefore, R_s is equal to:

$$R_s = \frac{1}{\alpha_k}\frac{\left(\frac{R_L}{\alpha^*}e_i - R_a e_a\right)}{(e_i - e_a)} \qquad \text{Equation 6}$$

Rearranging equation 6 to solve for R_L one obtains the following:

$$R_L = \alpha^* \left[\alpha_k R_s \left(\frac{e_i - e_a}{e_i} \right) + R_a \left(\frac{e_a}{e_i} \right) \right] \quad \text{Equation 7}$$

Craig & Gordon (1965) originally developed the theory for the evaporative enrichment model described above (see also Dongmann *et al.*, 1974; White, 1983). Equation 7 assumes that there is no significant influence of boundary layer effects and, therefore, the partial pressure of water vapour at the leaf surface (e_s) is the same as e_a. Boundary layer effects are included in the equation listed below:

$$R_L = \alpha^* \left[\alpha_k R_s \left(\frac{e_i - e_s}{e_i} \right) + \alpha_{kb} R_s \left(\frac{e_s - e_a}{e_i} \right) + R_a \left(\frac{e_a}{e_i} \right) \right] \quad \text{Equation 8}$$

where α_{kb} is the ratio of the conductance of the light and heavy isotopes in a boundary layer.

Based on Pohlhausen analysis, the kinetic fractionation factor in a boundary layer is calculated by taking the value of the kinetic factor for molecular diffusion and raising it to the two-thirds power (Kays & Crawford, 1980). The values for α_{kb} are: H/D = 1·107 and $^{16}O/^{18}O$ = 1·0189. Previous studies (Dongmann *et al.*, 1974; White, 1983) have attempted to include boundary layer effects by adjusting the value of α_k in equation 7. Such a procedure is technically incorrect since diffusion through the stomatal pore is a molecular process (Sharkey *et al.*, 1982) and turbulence is only encountered outside the stomatal pore. The influence of boundary layer conditions on leaf water isotopic enrichment can be calculated by comparing values predicted by equations 7 and 8. The magnitude of the difference between the predictions of the two equations depends on boundary layer conductance, leaf temperature, the leaf–air vapour pressure difference, and the particular element being considered (L.B. Flanagan, unpublished data).

Isotope effects during transpiration: experimental results

Comparisons have been made of the isotopic enrichment predicted by equations 7 and 8 and the measured leaf water isotopic composition. Measurements of leaf water isotopic enrichment were made under a range of conditions:

1 While the leaf environment was held constant in an environmentally controlled gas exchange chamber.

2 At midday under greenhouse conditions.

3 At midday under natural conditions in the field.

There is close agreement between the enrichment predicted by equation 8 and that measured in leaves exposed to a range of water vapour pressure gradients in a gas exchange chamber (Fig. 1). The model, however, slightly overestimates the degree of isotopic enrichment observed in bean leaves. There are two possible reasons for the discrepancy between the observed and modelled enrichment. The first reason is that water extracted from leaves by vacuum distillation removes water from leaf vein tissue in addition to water from leaf mesophyll cells. Vein water should be unfractionated and have the same isotopic composition as root and stem water. This would cause the observed isotopic enrichment of the vacuum extracted leaf water to fall below that predicted by equation 8. The extent of the discrepancy would depend on the relative amounts of vein water and mesophyll water that are present in a leaf. The amount of vein water should be directly proportional to the volume of vein tissue in a leaf which is often approximately 5% or less (Parkhurst, 1982; L.B. Flanagan, unpublished observations). The discrepancy between the modelled and observed data in Fig. 1 is greater than 5%, however.

A second reason for the lack of complete agreement between the modelled and measured data in Fig. 1, is that leaf water had not yet reached steady state isotopic enrichment. As outlined in the theory section, equations 7 and 8 assume that leaf water isotopic composition has reached steady state. This assumption has not yet been verified in our on-going gas exchange experiments. Leaves were allowed to equilibrate under constant conditions for approximately 45–60 min before the experiments were initiated. Experiments are currently underway to test the time required to reach isotopic steady state.

During the approach to steady state, the observed isotopic composition of leaf water can be modelled using equation 9 (White, 1983).

$$\delta_{Leaf} = \delta_{SS} \times f + \delta_{Stem} \times (1 - f) \quad \text{Equation 9}$$

where δ_{Leaf} is the measured isotopic composition of leaf water, δ_{SS} is the modelled isotopic composition of leaf water at steady state, δ_{Stem} is the isotopic composition of stem water. The parameter f in equation 9 describes how close the observed leaf water is to isotopic steady state. Calculations of f for the data in Fig. 1 range between 0·68 and 0·88. Other workers, assuming that steady state had been reached, have used equation 9 to estimate the fraction of vein water $(1 - f)$ in a leaf (Leaney *et al.*, 1985; Walker *et al.*, 1989). The estimates of vein water using this technique range

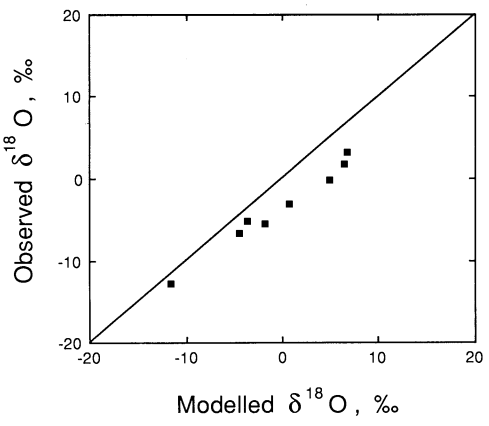

Fig. 1. Comparison of the modelled and observed oxygen isotope composition of water, vacuum distilled, from *Phaseolus vulgarus* leaves maintained under a range of leaf–air water vapour pressure gradients from 1·0 to 3·0 kPa in a controlled environment gas exchange chamber. Environmental conditions were: leaf temperature 30 °C, light intensity 1400 μmol m^{-2} s^{-1} (400–700 nm); boundary layer conductance to water loss 2 mol m^{-2} s^{-1}; $\delta^{18}O_{Stem}$ = −15·8‰. Atmospheric water vapour was varied by using water with different oxygen isotope compositions in the humidifying device of the gas exchange system. The $\delta^{18}O$ value of atmospheric water vapour ranged from −27·6‰ to −17·4‰. Modelled values were calculated using equation 8. Isotopic compositions are expressed relative to SMOW. (Data of L.B. Flanagan, J.P. Comstock & J.R. Ehleringer, unpublished observations.)

from 0·22 to 0·59. These values seem too large to represent vein water only and we suggest that it is unlikely that isotopic steady state had been reached under the conditions in which leaf collections were made.

Under greenhouse conditions and particularly in the field under natural conditions, environmental conditions may change too rapidly during the course of a day for isotopic steady state to be reached. Comparison of the modelled and observed leaf water isotopic composition for plants grown in a greenhouse and under field conditions is shown in Fig. 2. During the approach to steady state, leaf water values should fall on a line connecting the stem water isotopic composition and the modelled steady state isotopic composition, in a plot of δD and $\delta^{18}O$ (White, 1983). Our data suggest that the observed leaf water isotopic compositions were not at steady state (Fig. 2). Similar conclusions about leaf water isotopic composition not being at steady state under field conditions have been made by Zundel *et al.* (1978) and White (1983).

If leaf water has not reached isotopic steady state, it is expected that different fractions of water pushed out of a leaf using a pressure chamber would have a range of isotopic values. The first water extracted from a leaf should have an isotopic composition similar to stem water. The next series of samples collected should have a progressively more enriched isotopic composition. Such a pattern in the isotopic values of water extracted using a pressure chamber has been observed by Yakir, DeNiro & Rundel (1989). Yakir and co-workers have argued, based in part on their measurements of water expressed using a pressure chamber, that distinct compartments of water with different isotopic compositions are isolated in different tissues within a leaf (Yakir *et al*; 1989., Yakir, DeNiro & Gat, 1990). We suggest that a range of isotopic compositions is expected, in water expressed using a pressure chamber, if leaf water is not at isotopic steady state. It may not be necessary

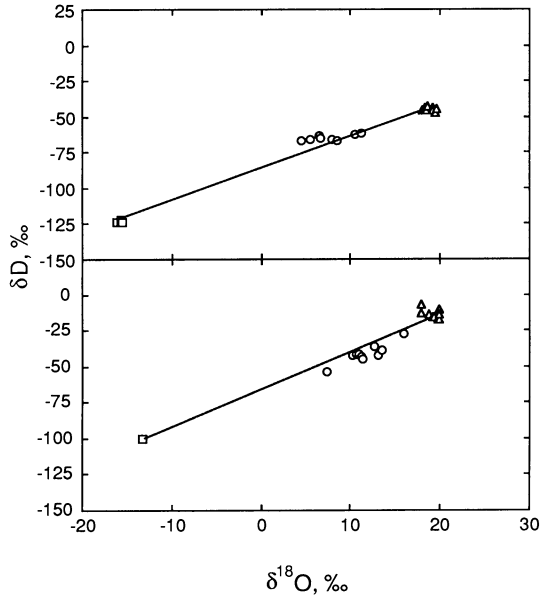

Fig. 2. Relationship between the oxygen and hydrogen isotopic compositions of stem water, leaf water and modelled leaf water at midday under glasshouse and field conditions. In the top panel, data are from experiments with *Cornus stolonifera* under glasshouse conditions: light intensity 1270 μmol m^{-2} s^{-1}; air temperature 27·7 °C, leaf temperature 31·7 °C, relative humidity 22%; atmospheric water vapour δD = −149‰, $\delta^{18}O$ = −22·6‰. (Data of L.B. Flanagan & J.R. Ehleringer, unpublished observations.) In the bottom panel, data are from experiments with *Phoradendron juniperinum* under field conditions: light intensity 1600 μmol m^{-2} s^{-1}; air temperature 26 °C; leaf temperature 26 °C; relative humidity 11%; atmospheric water vapour δD = −210‰; $\delta^{18}O$ = −28·5‰. (Data of L.B. Flanagan, J.D. Marshall & J.R. Ehleringer, unpublished observations.) In both panels the symbols represent: □ stem water; ○ measured leaf water; △ modelled leaf water using equation 7. Isotopic compositions are expressed relative to SMOW.

to propose that distinct, isolated water compartments exist within a leaf, aside from vein water which should be unfractionated. Further experiments using leaves exposed to constant conditions in an environmentally controlled gas exchange chamber should resolve this point.

The precise application of the evaporative enrichment model, to studies of the leaf–air water vapour pressure gradient, requires that the isotopic composition of leaf water be at steady state during the whole course of the day. Since it appears that under field conditions leaf water may not be isotopic steady state, it seems improbable that the leaf water enrichment model can be applied to studies of e_a/e_i in the same quantitative manner that carbon isotope data are applied to studies of c_i/c_a (Farquhar *et al.*, 1989). Qualitative information may still be obtained, however, if two leaves exposed to widely different water vapour gradients have approximately similar *f*-values. Biological factors that will influence the time required to reach isotopic steady state are the transpiration rate and the total water volume of a leaf.

A second consideration in applying the leaf water enrichment model in field situation is that it is necessary to measure the isotopic composition of atmospheric water vapour. This parameter varies during the course of a season as environmental conditions change (White & Gedzelman, 1984). It is not correct to simply assume that atmospheric water vapour is in equilibrium with local ground water. This assumption may be valid in some humid, continental locations but it is certainly incorrect in arid environments and coastal locations (Gat, 1980).

A final complication, in applying the leaf water enrichment model, is that stem water may vary during the course of the season in some plant species that directly take up summer precipitation. Since stem water is a parameter in the leaf water enrichment model, it is necessary to know how it varies over the season if long-term information about changes in leaf water are sought.

Relationship between the isotopic composition of leaf water and leaf cellulose

Sternberg (1988) and White (1988) have recently reviewed studies of the fractionation events during the formation of plant organic material. Our discussion, therefore, will be confined only to points relevant to studies of the leaf–air water vapour pressure gradient.

The various different organic fractions within plant tissue (lipids, resins, cellulose) all have different isotopic compositions (White, 1988). In order to compare results among species that potentially have different proportions of these components, it was suggested by Epstein, Yapp & Hall (1976) that the isotopic analysis be done on a single, purified component of plant tissue (Sternberg, 1988). Since cellulose is the major structural carbohydrate in plants, it is the component most often used for isotopic analysis of organic material. For hydrogen isotope analysis, cellulose nitrate is used. The nitration procedure removes all the hydroxyl hydrogens which are readily exchangeable and often do not give a proper representation of the isotopic composition of hydrogens incorporated during the formation of cellulose (Sternberg, 1988).

For the purpose of estimating the long-term, leaf–air water vapour pressure gradient, there are several factors that limit the use of hydrogen isotopic analysis of cellulose nitrate. For example, the biochemical fractionation events relating leaf water and cellulose nitrate differ among species and are temperature dependent (Sternberg, 1988). These species effects can be useful, however, for studying variations in photosynthetic metabolism (Sternberg, 1988). The relationship between the oxygen isotopic composition of leaf water and leaf cellulose appears to be much less complicated. With some small variations (±3‰), the oxygen isotopic compostion of cellulose is 27‰ higher than the source water present during synthesis (Sternberg, 1988). This relationship is not temperature dependent. Present evidence suggests that complete oxygen isotope exchange occurs between water and glyceraldehyde-3-phosphate (Sternberg & DeNiro, 1983). Starch, sucrose or cellulose produced in a leaf from glyceraldehyde-3-phosphate will retain the same isotopic signature that is developed during carbonyl hydration of glyceraldehyde-3-phosphate (Sternberg, 1988; Yakir & DeNiro, 1990). Cellulose produced in stem tissue, however, may have a different oxygen isotope composition because sucrose translocated to the stem can undergo further carbonyl hydration with stem water (Sternberg, 1988; DeNiro & Cooper, 1989). It is necessary, therefore, to analyse cellulose synthesized in the presence of leaf water to obtain information about the isotopic composition of the leaf water (DeNiro & Cooper, 1989).

Conclusions

Measurements of the isotopic composition of ground water, recent precipitation and plant stem

water offer great promise for ecological studies of species differences in rooting pattern and below-ground resource acquisition. It is possible that this technique may also have agricultural applications such as determining the efficiency of irrigation water uptake.

The application of the evaporative enrichment model to studies of the long-term, leaf–air water vapour pressure gradient is more complicated. For precise, quantitative applications of the model, it is necessary to know how close leaf water isotopic composition is to isotopic steady state. Measurements of the seasonal variation in the isotopic composition of atmospheric water vapour and plant stem water are also required. If it is possible to accomplish the above-mentioned requirements, studies of the oxygen isotope composition of cellulose offer the most promise for estimating the integrated value for leaf water isotopic composition.

Acknowledgments

LBF was supported by a postdoctoral fellowship from the Natural Sciences and Engineering Research Council of Canada. Research is funded by a grant from the Ecological Research Division, Office of Health and Environmental Research, US Department of Energy.

References

Craig, H. & Gordon, L.I. (1965) Deuterium and oxygen-18 variations in the ocean and the marine atmosphere. In *Proceedings of a Conference on Stable Isotopes in Oceanographic Studies and Paleotemperatures, Spoleto* (ed. E. Tongiorgi), pp. 9–130. Lischi & Figli, Pisa.

Dansgaard, W. (1964) Stable isotopes in precipitation. *Tellus,* **16,** 436–468.

Dawson, T.E., Ehleringer, J.R. & Marshall, J.D. (1990) Sex-ratio and reproductive variation in the mistletoe *Phoradendron juniperinum* (Visaceae). *American Journal of Botany,* **77,** 584–589.

DeNiro, M.J. & Cooper, L.W. (1989) Post-photosynthetic modification of oxygen isotope ratios of carbohydrates in the potato: implications for paleoclimatic reconstruction based upon isotopic analysis of wood cellulose. *Geochimica et Cosmoschimica Acta,* **53,** 2573–2580.

Dongmann, G., Nurnberg, H.W., Forstel, H. & Wagener, K. (1974) On the enrichment of $H_2{}^{18}O$ in the leaves of transpiring plants. *Radiation and Environmental Biophysics,* **11,** 41–52.

Ehleringer, J.R. & Osmond, C.B. (1989) Stable isotopes. In *Plant Physiological Ecology. Field Methods and Instrumentation* (eds. R.W. Pearcy, J.R. Ehleringer, H.A. Mooney & P.W. Rundel), pp. 281–300. Chapman & Hall, London.

Ehleringer, J.R., Pearcy, R.W. & Mooney, H.A. (1986) Future development in plant physiological ecology. *Bulletin of the Ecological Society of America,* **67,** 48–58.

Epstein, S., Yapp, C.J. & Hall, J. (1976) The determination of the D/H ratio of non-exchangeable hydrogen in cellulose extracted from aquatic and land plants. *Earth Planetary Science Letters,* **30,** 241–251.

Farquhar, G.D. & von Caemmerer, S. (1982) Modelling of photosynthetic response to environmental conditions. In *Encyclopedia of Plant Physiology,* vol. 12B, *Physiological Plant Ecology II. Water Relations and Carbon Assimilation* (eds. O.L. Lange, P.S. Nobel, C.B. Osmond & H. Ziegler), pp. 549–588. Springer-Verlag, Berlin.

Farquhar, G.D., Hubick, K.T., Condon, A.G. & Richards, R.A. (1988) Carbon isotope fractionation and plant water-use efficiency. In *Stable Isotopes in Ecological Research* (eds. P.W. Rundel, J.R. Ehleringer & K.A. Nagy), pp. 21–40. Springer-Verlag, Berlin.

Farquhar, G.D., Ehleringer, J.R. & Hubick, K.T. (1989) Carbon isotope discrimination and photosynthesis. *Annual Review of Plant Physiology and Plant Molecular Biology,* **40,** 503–537.

Gat, J.R. (1980) The isotopes of hydrogen and oxygen in precipitation. In *Handbook of Environmental Isotope Geochemistry* (eds. P. Fritz & J-Ch. Fontes), vol. 1, pp. 24–48. Elsevier, New York.

Kays, W.M. & Crawford, M.E. (1980) *Convective Heat and Mass Transfer,* 2nd edn. McGraw-Hill, New York.

Leaney, F.W., Osmond, C.B., Allison, G.B. & Zeigler, H. (1985) Hydrogen-isotope composition of leaf water in C_3 and C_4 plants: its relationship to the hydrogen-isotope composition of dry matter. *Planta,* **164,** 215–220.

Majoube, M. (1971) Fractionnement en oxygene-18 et en deuterium entre l'eau et sa vapeur. *Journal de Chimie et Physique,* **58,** 1423–1436.

Merlivat, L. (1978) Molecular diffusivities of $H_2{}^{18}O$ in gases. *Journal of Chemical Physics,* **69,** 2864–2871.

Osmond, C.B., Winter, K. & Ziegler, H. (1982) Functional significance of different pathways of CO_2 fixation in photosynthesis. In *Encyclopedia of Plant Physiology,* vol. 12B, *Physiological Plant Ecology II. Water Relations and Carbon Assimilation* (eds. O.L. Lange, P.S. Nobel, C.B. Osmond & H. Ziegler, pp. 479–549. Springer-Verlag, Berlin.

Parkhurst, D.F. (1982) Stereological methods for measuring internal leaf structure variables. *American Journal of Botany,* **69,** 31–39.

Pearcy, R.W., Bjorkman, O., Caldwell, M.M., Keeley, J.E., Monson, R.K. & Strain, B.R. (1987) Carbon gain by plants in natural environments. *Bioscience,* **37,** 21–37.

Reynolds, T.D. & Fraley, L. (1989) Root profiles of some native and exotic plant species in southeastern Idaho. *Environmental and Experimental Botany,* **29,** 241–248.

Rundel, P.W., Ehleringer, J.R. & Nagy, K.A. (1988) *Stable Isotopes in Ecological Research.* Springer-Verlag, Berlin.

Sharkey, T.D., Imai, K., Farquhar, G.D. & Cowan, I.R. (1982) A direct confirmation of the standard method of estimating intercellular CO_2. *Plant Physiology,* **69,** 657–659.

Sternberg, L.S.L. (1988) Oxygen and hydrogen isotope ratios in plant cellulose: mechanisms and applica-

tions. In *Stable Isotopes in Ecological Research* (eds. P.W. Rundel, J.R. Ehleringer & J.A. Nagy), pp. 124–141. Springer-Verlag, Berlin.

Sternberg, L.S.L. & DeNiro, M.J. (1983) Biogeochemical implications of the isotopic equilibrium fractionation factor between the oxygen atoms of acetone and water. *Geochimica et Cosmochimica Acta,* **47,** 2271–2274.

Sternberg, L.S.L., Mulkey, S.S. & Wright, S.J. (1989) Oxygen isotope ratio stratification in a tropical moist forest. *Oecologia,* **81,** 51–56.

Walker, C.D., Leaney, F.W., Dighton, J.C. & Allison, G.B. (1989) The influence of transpiration on the equilibrium of leaf water with atmospheric water vapour. *Plant, Cell and Environment,* **12,** 221–234.

White, J.W.C. (1983) *The climatic significance of D/H ratios in White Pine in the northeastern United States.* PhD dissertation, Columbia University, New York.

White, J.W.C. (1988) Stable hydrogen isotope ratios in plants: a review of current theory and some potential applications. In *Stable Isotopes in Ecological Research* (eds. P.W. Rundel, J.R. Ehleringer & K.A. Nagy), pp. 142–162. Springer-Verlag, Berlin.

White, J.W.C. & Gedzelman, S.D. (1984) The isotopic composition of atmospheric water vapor and the concurrent meteorological conditions. *Journal of Geophysical Research,* **89,** 4937–4939.

White, J.W.C., Cook, E.R., Lawrence, J.R. & Broecker, W.S. (1985) The D/H ratios of sap in trees: implications for water sources and tree ring D/H ratios. *Geochimica et Cosmochimica Acta,* **49,** 237–246.

Yakir, D. & DeNiro, M.J. (1990) Oxygen and hydrogen isotope fractionation during cellulose metabolism in *Lemna gibba* L. *Plant Physiology,* **93,** 325–332.

Yakir, D., DeNiro, M.J. & Rundel, P.W. (1989) Isotopic inhomogeneity of leaf water: evidence and implications for the use of isotopic signals transduced by plants. *Geochimica et Cosmoschimica Acta,* **53,** 2769–2773.

Yakir, D., DeNiro, M.J. & Gat, J.R. (1990) Natural deuterium and oxygen-18 enrichment in leaf water of cotton plants grown under wet and dry conditions: evidence for water compartmentation and its dynamics. *Plant, Cell and Environment,* **13,** 49–56.

Zundel, G., Miekeley, W., Grisi, B.M. & Forstel, H. (1978) The $H_2{}^{18}O$ enrichment in the leaf water of tropic trees: comparison of species from the tropical rain forest and the semi-arid region of Brazil. *Radiation and Environmental Biophysics,* **15,** 203–212.