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***Review*:**

Stable oxygen isotope composition of plant tissue: a review

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Abstract. With the development of rapid measurement techniques, stable oxygen isotope analysis of plant tissue is poised to become an important tool in plant physiological, ecological, paleoclimatic and forensic studies. Recent advances in mechanistic understanding have led to the improvement of process-based models that accurately predict variability in the oxygen isotope composition of plant organic material ($\delta^{18}O_p$). $\delta^{18}O_p$ has been shown to reflect the isotope composition of soil water, evaporative enrichment in transpiring leaves, and isotopic exchange between oxygen atoms in organic molecules and local water in the cells in which organic molecules are formed. This review presents current theoretical models describing the influences on $\delta^{18}O_p$, using recently published experimental work to outline strengths and weaknesses in the models. The potential and realised applications of the technique are described.

Additional keywords: crop yield, leaf water enrichment, palaeoclimate, Péclet effect, stomatal conductance.

Introduction

Oxygen atoms occur naturally in three stable isotopes, ^{16}O, ^{17}O and ^{18}O, with approximate concentrations of 99.74, 0.05 and 0.21%, respectively. The ^{17}O composition of atmospheric CO_2 is useful in atmospheric chemistry studies (see Eiler and Schauble 2004), but as ^{17}O compositions have not yet been measured in plant material, this review will be limited to interpretation of variation in the ratio of ^{18}O to ^{16}O.

The $^{18}O/^{16}O$ composition of plant tissue is of interest to at least four scientific disciplines, namely: ecophysiological studies of regulation of plant water loss; breeding for improved water use efficiency and yield in crop species; paleoclimatic reconstruction from tree rings; and identification of the origin of plant tissue. These applications stem from the knowledge that the oxygen isotope composition of plant tissue reflects not only that of water taken up by the plant, but also (1) evaporative and diffusional effects during transpiration, and (2) isotopic exchange between organic molecules and plant water (Barbour *et al*. 2005). Recent developments in online measurement techniques (e.g. Farquhar *et al*. 1997) have provided a high degree of automation and rapid sample analysis, allowing experiments involving oxygen isotope analysis of large numbers of plant tissue samples. This review will summarise current theoretical understanding of processes influencing the oxygen isotope composition of plant tissue, draw on published work to illustrate strengths and weaknesses of existing models, and describe potential applications of the technique.

Definitions

The absolute isotope composition of a material is difficult to measure directly, so isotope ratios are generally compared with that of a standard. In the case of $^{18}O/^{16}O$, the standard is commonly Vienna-Standard Mean Oceanic Water (VSMOW), with an isotope ratio of 2.0052×10^{-3} (Gonfiantini 1984). Plant isotope compositions are commonly expressed as relative deviations from VSMOW, and denoted $\delta^{18}O_p$ where $\delta^{18}O_p = R_p/R_{st} - 1$, and R_p and R_{st} are the isotope ratios of the substance of interest and the standard, respectively. Variation in the isotope composition of source (soil) water may be removed from $\delta^{18}O_p$ by presenting the composition as an enrichment above source water ($\Delta^{18}O_p$) given by: $\Delta^{18}O_p = R_p/R_s - 1$, where R_s is the $^{18}O/^{16}O$ ratio of source water. $\Delta^{18}O_p$ may be calculated from $\delta^{18}O_p$ by:

$$\Delta^{18}O_p = \frac{\delta^{18}O_s - \delta^{18}O_p}{1 + \delta^{18}O_s}. \quad (1)$$

Source water oxygen isotope composition

The oxygen isotope ratio of soil water taken up by a plant ($\delta^{18}O_s$) depends, to a first approximation, on the temperature of droplet formation for rain and snow falling at the site. Precipitation becomes more depleted in ^{18}O as temperature decreases at higher latitudes and altitudes. 'Rain-out' effects can also be important across continents, with heavier, more enriched water falling first, followed by progressively more depleted precipitation as the air mass moves across the land surface. There is also an amount effect, with high-precipitation, tropical sites having more depleted water than would be expected from site temperatures. No significant factionation effects for oxygen isotopes are observed during plant uptake of soil water (Wershaw *et al*. 1966; Dawson and Ehleringer 1991). Therefore, $\delta^{18}O_s$ has a very strong influence on $\delta^{18}O_p$. For example, Roden and

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Ehleringer (1999) found that between 96 and 99% of variation in $\delta^{18}O$ of wood cellulose from three temperate tree species grown hydroponically could be explained by variation in $\delta^{18}O$ of the water in the growth tanks. It is expected, and observed (Barbour *et al*. 2001), that plant tissue from high latitudes and elevations is more depleted in ^{18}O than plant tissue from the tropics. That plant tissue reflects source water $\delta^{18}O$, and so (indirectly) site temperature, has been recognised for some time and prompted Libby *et al*. (1976) to suggest that annual tree rings may be 'isotopic thermometers'.

However, $\delta^{18}O$ of water taken up from the soil by plants may vary considerably from that of $\delta^{18}O$ of average precipitation at the site, owing to enrichment in the remaining water by evaporation from the soil surface, temporal separation of precipitation and uptake by plants, and uptake of groundwater that is isotopically distinct from site precipitation (Adams and Grierson 2001). Although variation in $\delta^{18}O$ of source water has seldom been used in studies to date (but see Drake and Franks 2003; Cook and O'Grady 2006), variation in the hydrogen isotope ratio (δD) of source water has been studied in several experimental systems. Parallels between $\delta^{18}O$ and δD of source water may be drawn because the two isotopes share a unique relationship in precipitation: $\delta D = 8\,\delta^{18}O + 10‰$ (i.e. the meteoric water line; Craig 1961). Several studies have exploited large differences between δD of surface soil water and that of groundwater, or seasonal variation in δD of rainfall, to determine the source of plant water within the soil profile (Dawson *et al*. 1998). However, care must be taken when hydrogen isotopes are used to determine plant source water, as hydrogen isotopic fractionation has been observed during uptake of water by mangroves in saline environments (Lin and Ehleringer 1992). No oxygen isotope effects have been reported during plant uptake of water.

Although variation in $\delta^{18}O_s$ may be strongly reflected in $\delta^{18}O_p$ (Jäggi *et al*. 2003), Roden and Ehleringer (1999) demonstrated the influence of relative humidity on $\delta^{18}O_p$. Barbour *et al*. (2004) suggested that removing spatial and temporal variation in source water $\delta^{18}O$ by presenting plant organic matter $^{18}O/^{16}O$ as an enrichment above source water (i.e. $\Delta^{18}O_p$, as described above) simplifies interpretation and enables identification of variation owing to leaf water enrichment and isotopic exchange.

Evaporative enrichment of leaf water

Leaf evaporative conditions influence the isotope composition of plant organic material (Roden and Ehleringer 1999; Barbour and Farquhar 2000), and it is appropriate to present current theoretical understanding of evaporative enrichment in leaves. ^{18}O enrichment of leaf water relative to soil water was first shown by Gonfiantini *et al*. (1965). Craig and Gordon (1965) presented a model of evaporative enrichment of a free water surface that is commonly applied to leaf water, with modifications. This model relates enrichment of leaf water above source water ($\Delta^{18}O_{es}$) to the kinetic fractionation during diffusion through the stomata and leaf boundary layer (ε_k), the proportional depression of water vapour pressure by the heavier $H_2{}^{18}O$ molecule (ε^+), the oxygen isotope composition of water vapour relative to source water ($\Delta^{18}O_v$) and scaled by the ratio of ambient to intercellular water vapour mol fraction (e_a/e_i) (Craig and Gordon 1965; Dongmann *et al*. 1974; Farquhar and Lloyd 1993) by:

$$\Delta^{18}O_{es} = \varepsilon^+ + \varepsilon_k + (\Delta^{18}O_v - \varepsilon_k)e_a/e_i. \qquad (2)$$

In well-mixed conditions, $\Delta^{18}O_v$ is often close to $-\varepsilon^+$, so that $\Delta^{18}O_e$ is proportional to $1 - e_a/e_i$. As a result, $\Delta^{18}O_p$ should be negatively related to relative humidity. Eqn 2 also suggests that at constant e_a, increasing stomatal conductance will result in less enrichment at the sites of evaporation within leaves, owing to the reduction in both leaf temperature and e_i caused by increased transpiration. The equilibrium fractionation factor, ε^+, is dependent on temperature by (Bottinga and Craig 1969):

$$\varepsilon^+ (‰) = 2.644 - 3.206\left(\frac{10^3}{T_l}\right) + 1.534\left(\frac{10^6}{T_l^2}\right), \qquad (3)$$

where T_l is leaf temperature in K. The total kinetic fractionation factor, ε_k, may be calculated from stomatal (g_s) and boundary layer (g_b) conductances to water vapour by (Farquhar *et al*. 1998):

$$\varepsilon_k = \frac{32 g_s{}^{-1} + 21 g_b{}^{-1}}{g_s{}^{-1} + g_b{}^{-1}}. \qquad (4)$$

The fractionation factor for diffusion through air (and stomata) was recently determined to be 32‰ by Cappa *et al*. (2003) and, assuming a 2/3 power effect according to Pohlhausen analysis (Kays 1966), fractionation during diffusion through the boundary layer will be 21‰ (Cernusak *et al*. 2003*b*).

Although Eqn 2 predicts general trends in leaf water enrichment quite well, in some cases measured leaf water $\delta^{18}O$ has been less enriched than expected (e.g. Yakir *et al*. 1989; Flanagan *et al*. 1994; Wang *et al*. 1998), and in others leaf water was more enriched than predicted (Bariac *et al*. 1994*a*; Wang and Yakir 1995; Helliker and Ehleringer 2000). Several approaches have been taken to address these discrepancies, including: (1) discrete pools of water within a leaf with differing isotope compositions (e.g. Yakir *et al*. 1990); (2) unenriched water within veins lowering the bulk leaf water enrichment (e.g. Roden and Ehleringer 2000; Roden *et al*. 2000); (3) a string of inter-connected pools of water within the leaf with differing isotope compositions (e.g. Helliker and Ehleringer 2000; Ghannoum *et al*. 2002); (4) diurnal changes in the evaporative environment and water content of the leaf (e.g. Cernusak *et al*. 2002); and (5) the convection of unenriched water towards the sites of evaporation opposed by back diffusion of enrichment from those sites resulting in a lower bulk leaf water enrichment (e.g. Farquhar and Lloyd 1993). These approaches have recently been combined to provide a coherent model of enrichment in both the lamina tissue and the veins (Ogée *et al*. 2007), and the model development is presented below.

The Péclet effect describes the convection of unenriched water to the evaporating sites opposed by backward diffusion of $H_2^{18}O$, and predicts an increasing discrepancy between enrichment predicted by Eqn 2 and that measured in lamina tissue, with increasing transpiration rate (Farquhar and Lloyd 1993). This predicted trend has been observed rather infrequently, and always under very tightly controlled (and well measured) evaporative conditions at isotopic steady

state (e.g. Flanagan *et al.* 1991*a*, 1994; Barbour *et al.* 2004; M. M. Barbour, unpublished data). In other experiments, particularly those under field conditions, the difference between $\Delta^{18}O_{es}$ and measured enrichment of leaf water does not relate to differences in transpiration rate, presumably because the leaf water was not at isotopic steady state. The Péclet effect is characterised by the dimensionless ratio of convection to diffusion ($\wp$):

$$\wp = \frac{LE}{CD}, \tag{5}$$

where E is the leaf transpiration rate (mol m^{-2} s^{-1}), C is the molar density of water (55.5×10^3 mol m^{-3}), D is the diffusivity of $H_2{}^{18}O$ in water (2.66×10^{-9} m^2 s^{-1}) and L is the length over which the effect is evident (m). The tortuous path of water through the leaf results in the effective length being at least two orders of magnitude larger than the actual distance between the vein and the evaporating sites (Barbour and Farquhar 2004). Average lamina water enrichment above source water at steady state ($\Delta^{18}O_{LS}$) can be modelled by (Farquhar and Lloyd 1993):

$$\Delta^{18}O_{LS} = \frac{\Delta^{18}O_{es}(1 - e^{-\wp})}{\wp}, \tag{6}$$

where $\Delta^{18}O_{es}$ is given by Eqn 2. Eqn 6 was the starting point for a series of theoretical and experimental studies that have attempted to describe patterns in enrichment both within individual leaves and between differing evaporative environments. Observations of increasing enrichment in vein and mesophyll water towards the tip and outside of leaves (Bariac *et al.* 1994*a*, 1994*b*; Wang and Yakir 1995; Helliker and Ehleringer 2000; Gan *et al.* 2002) led to the development of a model that included both longitudinal and radial Péclet effects. Farquhar and Gan (2003) suggested that bulk leaf water enrichment ($\Delta^{18}O_b$) may be described by:

$$\Delta^{18}O_b = \phi_x \overline{\Delta^{18}O_x} + \phi_v \overline{\Delta^{18}O_v} + \phi_L \Delta^{18}O_{LS}, \tag{7}$$

where ϕ is the proportion of total leaf water associated with various tissues, and subscripts x, v and L refer to xylem and surrounding vascular tissue, small 'veinlets', and lamina mesophyll, respectively (i.e. $\phi_x + \phi_v + \phi_L = 1$). $\overline{\Delta^{18}O_x}$ and $\overline{\Delta^{18}O_v}$ are the average enrichments of water in the main veins and veinlets, respectively. Farquhar and Gan (2003) show that:

$$\Delta^{18}O_b = \Delta^{18}O_{es}\left[\phi_x e^{-\wp_r} + \phi_v \frac{e^{\wp_{rv}} - 1}{\wp_{rv} \cdot e^{\wp_r}} + \phi_L \frac{1 - e^{-\wp}}{\wp}\right], \tag{8}$$

where $\wp_r$ is the Péclet effect associated with radial flow (i.e. from the veins to the sites of evaporation) gradients in enrichment in the veinlets ($\wp_{rv}$) and the lamina mesophyll ($\wp$, i.e. $\wp_r = \wp + \wp_{rv}$).

Gan *et al.* (2002, 2003) demonstrated that the longitudinal Péclet effect (of order 10^7) is large in comparison to the radial Péclet effect (of order one), but that the radial Péclet effect determines the diminution of average leaf water enrichment below that predicted by Eqn 2. Further, most studies to date have determined $\wp$ from measurements of bulk leaf water enrichment, for which the radial Péclet effect is most relevant (because the longitudinal Péclet effect largely determines variation in enrichment within a leaf, while the radial Péclet effect determines the difference between $\Delta^{18}O_b$ and $\Delta^{18}O_{es}$).

Evidence in support of the relevance of a Péclet effect to $\Delta^{18}O_p$ was presented by Barbour *et al.* (2000*b*), who found that $\Delta^{18}O$ of sucrose was negatively related to e_a/e_i (as predicted by both the simple Craig–Gordon model and the Péclet effect extension), but that the Craig–Gordon prediction of $\Delta^{18}O$ of sucrose was significantly more sensitive to changes in e_a/e_i than was observed (as would be the case with a Péclet effect). Or, put another way, the proportional difference between water with which phloem sap sucrose exchanges isotopically and the enrichment at the sites of evaporation (i.e. $1 - \Delta^{18}O_{LS}/\Delta^{18}O_{es}$) increased with increasing transpiration rate. In this analysis, the Craig–Gordon predicted $\Delta^{18}O$ of sucrose was calculated from Eqn 2, plus the fractionation factor between carbonyl oxygen and water (taken to be 27‰). Further evidence in support of the Péclet effect was presented by Barbour *et al.* (2004), who also found a positive relationship between $1 - (\Delta^{18}O_p - 27)/\Delta^{18}O_{es}$ and transpiration rate, where $\Delta^{18}O_p$ was measured in cellulose extracted from leaf tissue of three temperate tree species. In both these experiments, the leaf evaporative conditions were carefully controlled and measured, so that leaf water enrichment was likely to be close to isotopic steady state.

However, the steady-state leaf water models (Eqns 2, 6) are unlikely to give accurate predictions of $\Delta^{18}O_{es}$ and $\Delta^{18}O_{LS}$ as stomata respond to rapidly changing environmental conditions (Flanagan and Ehleringer 1991*b*; Harwood *et al.* 1998; Lai *et al.* 2006; Seibt *et al.* 2006). A non-steady-state model has been developed (Farquhar and Cernusak 2005), and shows good agreement with measured leaf water $\delta^{18}O$ (Cernusak *et al.* 2002, 2005; Pendall *et al.* 2005). The non-steady-state enrichment of water at the sites of evaporation ($\Delta^{18}O_e$) is given by (Farquhar and Cernusak 2005):

$$\Delta^{18}O_e = \Delta^{18}O_{es} - \frac{\alpha_k \alpha^+}{g_l e_i} \times \frac{d\left(W \times \frac{1 - e^{-\wp}}{\wp} \times \Delta^{18}O_e\right)}{dt}, \tag{9}$$

where $\alpha_k = 1 + \varepsilon_k$, $\alpha^+ = 1 + \varepsilon^+$, g_l is the total leaf conductance to water vapour (i.e. stomata and boundary layer conductances in series), W is the leaf water content in mol m^{-2}, and t is time in seconds. Similarly, the non-steady-state enrichment within the lamina ($\Delta^{18}O_L$) is given by:

$$\Delta^{18}O_L = \Delta^{18}O_{LS} - \frac{\alpha_k \alpha^+}{g_l e_i} \times \frac{1 - e^{-\wp}}{\wp} \times \frac{d(W\Delta_L)}{dt}. \tag{10}$$

In the field, diurnal variation in leaf water enrichment is expected as a result of diurnal variation in temperature. Ambient vapour pressure usually remains nearly constant over a diurnal cycle, such that variation in the term e_a/e_i will be driven mostly by temporal changes in the saturation vapour pressure within the leaf. Consequently, the extent of diurnal variation in leaf water enrichment depends on environmental conditions (Dongmann *et al.* 1974; Zundel *et al.* 1978; Yakir *et al.* 1990; Walker and Lance 1991; Bariac *et al.* 1994*b*; Cernusak *et al.* 2002, 2005; Ogée *et al.* 2007), and is likely to affect $\Delta^{18}O_p$. In expanding leaves, diurnal variation in environmental conditions will cause variation in the isotopic composition of the water in which new leaf material is

forming. In mature leaves acting as carbohydrate sources, diurnal variation in environmental conditions will cause variation in the $\Delta^{18}O$ of the carbohydrates exported from the leaf. As shown by Cernusak *et al*. (2005), the $\Delta^{18}O_p$ in either case will, therefore, reflect a photosynthesis-weighted average of diurnal variation in leaf water enrichment.

More recently, Ogée *et al*. (2007) combined the Gan *et al*. (2002) approach of separate Péclet effects in the veins and the lamina with the Farquhar and Cernusak (2005) non-steady-state model, and extended the model to include radial diffusion in the veins, longitudinal diffusion in the lamina tissue, and non-uniform transpiration rates. This new model accurately predicted measured variation in $\Delta^{18}O_L$ along field-grown corn leaves, even at the distal end of the leaves, although not at the distal end of the leaves, where the Gan *et al*. (2002) model also overestimated measurement enrichment. Also, leaf length and intervein distance did not explain measured variability in $\Delta^{18}O_L$, as suggested by Helliker and Ehleringer (2000), but rather, that variability in lamina tortuosity between different monocot leaves was implied. Further, it was demonstrated that spatial variability in $\Delta^{18}O_L$ within individual leaves may be easily scaled to the whole leaf using the equations presented by Farquhar and Cernusak (2005), that is, Eqn 10 above. It seems likely that if this new model were parameterised for the C_4 grasses measured by Ghannoum *et al*. (2002), it could reconcile previously unexplained similarities in $\delta^{18}O_p$ between droughted and well-watered plants with different transpiration rates.

One of the weaknesses of the Péclet effect model, and extensions, is that the effective length (L) over which gradients in leaf water develop must be either known or fitted. L is not simply related to the physical distance between veins and stomata, but rather is scaled by a tortuousity factor that increases the effective length by at least two orders of magnitude (Barbour and Farquhar 2004). Wang *et al*. (1998) estimated L from modelled $\Delta^{18}O_e$ and measured enrichment in bulk leaf water in 89 species grown in the Jerusalem Botanical Garden and found a range from 4 to 166 mm. Barbour and Farquhar (2004) suggest that L may vary with distance from the vein to the evaporating sites, and if the pathway of water movement within leaves varies. However, L was unrelated to leaf morphology across the species sampled by Wang *et al*. (1998). Clearly, additional work is required to establish both the degree of variability in L and anatomical dimensions or physiological processes influencing L.

Isotopic exchange between water and organic molecules

Organic molecules reflect the water in which they formed due to isotopic exchange between carbonyl oxygen and water (Sternberg *et al*. 1986). Oxygen atoms in other functional groups, such as hydroxyl, carboxyl and phosphate groups, are not exchangeable at normal cellular temperature and pH. The exchange of oxygen atoms between water and carbonyl groups is possible due to the formation of a short-lived gem-diol intermediate (Samuel and Silver 1965) and, as many intermediates in the biochemical pathways leading to synthesis of structural and non-structural carbohydrates contain carbonyl oxygen groups, this isotopic exchange is important in determining $\Delta^{18}O_p$.

At equilibrium, oxygen atoms in carbonyl groups are between 25 and 30‰ more enriched than the water in which they formed (Sternberg and DeNiro 1983). Acetone, with a single exchangeable oxygen, was found to be 28‰ more enriched than the water with which it exchanged (Sternberg and DeNiro 1983). If a substance contains more than one oxygen atom that has gone through a carbonyl group an average fractionation factor (ε_{wc}) is applicable, even though there may be slight differences in fractionation for different oxygen atoms depending on the proximity of other atoms (Schmidt *et al*. 2001). For example, a recent study by Sternberg *et al*. (2006) reports a fractionation factor of 19.6‰ for the oxygen attached to carbon 2 of the cellulose glucose moiety, compared with an average fractionation of 28.8‰ for oxygen atoms attached to carbons 3–6.

The rate of isotopic exchange between carbonyl oxygen and water varies considerably between molecules, with larger molecules being much slower to reach equilibrium than small molecules. Acetone has a half-time to equilibration of ~10 min, while fructose 1,6-bisphosphate takes 29 min, and fructose 6-phosphate 166 min (Model *et al*. 1968). These rates are likely to be considerably faster *in vivo*, when enzymes such as aldolase would catalyse the reactions (Model *et al*. 1968). Triose phosphates are particularly important, as two of the three oxygen atoms are in carbonyl groups and the half-time to equilibration is fast (Sternberg *et al*. 1986; da Silveira *et al*. 1989; Farquhar *et al*. 1998). Barbour *et al*. (2000*b*) suggested that because a rapid exchange is expected for triose phosphates, exported sucrose should be in full isotopic equilibrium with average leaf water. This has been demonstrated by Cernusak *et al*. (2003*b*), who show that sucrose exported from leaves of castor bean is 27‰ more enriched than average lamina water at isotopic steady-state. That is, enrichment of sucrose above source water ($\Delta^{18}O_{suc}$) is given by (Cernusak *et al*. 2003*b*):

$$\Delta^{18}O_{suc} = 1.027\Delta^{18}O_{LS} + 27‰. \quad (11)$$

Providing further support for this suggestion, Barbour (1999, and see Barbour *et al*. 2005) showed that step changes in [CO_2] and [O_2] were not reflected in $\Delta^{18}O$ of sucrose. Variation in $\Delta^{18}O$ of sucrose was expected after a step change in [CO_2] and [O_2] if full isotopic equilibrium were not attained due to changes in the ratio of oxygenation to carboxylation by ribulose bisphosphate carboxylase-oxygenase and oxygen isotope effects during oxygenation (e.g. Tcherkez and Farquhar 2005).

However, although sucrose has been shown to directly reflect leaf water enrichment, cellulose is some way from full isotopic equilibrium with leaf water, because cellulose from tree rings reflects both relative humidity and source water (Roden *et al*. 2000; Pendall *et al*. 2005). To form cellulose, or other carbohydrate or secondary metabolites, sucrose exported from the source leaf must be broken down into hexose molecules. Consequently, one of the five oxygen atoms in the repeating unit of cellulose become part of a carbonyl group when sucrose is broken down into hexose phosphates during cellulose synthesis. That atom becomes exchangeable with local water, at least for short periods of time. Further, Hill *et al*. (1995) showed that a proportion (y) of hexose phosphates also go through a futile cycle via triose phosphates before cellulose synthesis. Such a

cycle would allow a further three out of five oxygen atoms in the cellulose unit to exchange with local water. This means that with every turn of the futile cycle the probability of an oxygen atom going through an exchangeable carbonyl group increases. This process has been modelled by Barbour and Farquhar (2000):

$$p_{ex} = 0.2 + \left(0.6 + \frac{0.2}{2 - y}\right) y, \quad (12)$$

where p_{ex} is the proportion of exchangeable oxygen in cellulose formed from simple carbohydrates, and $(1 - y)$ is the proportion of hexose phosphate molecules used immediately (i.e. no futile cycling through triose phosphates). Recalculation of published data shows p_{ex} to vary between 0.31 (recalculated from data on *Triticum aestivum*; Luo and Sternberg 1992) and 0.50 (recalculated from data on *Solanum tuberosum*; DeNiro and Cooper 1989). Cernusak *et al*. (2005) demonstrated that p_{ex} was close to 0.40 for both leaf and wood material from *Eucalyptus globulus*, and that p_{ex} values were also fairly consistent among species.

The re-exchange of oxygen atoms with local water in the sink cell forming new cellulose typically acts to dampen the leaf water signal and enhance the source water signal in plant tissue, and has been modelled by (Barbour and Farquhar 2000):

$$\Delta^{18}O_c = \Delta^{18}O_L(1 - p_{ex} p_x) + \varepsilon_{wc}, \quad (13)$$

where $\Delta^{18}O_c$ is the isotopic composition of cellulose and p_x is the proportion of unenriched (source) water in the developing cell. The term p_x may be expected to be less than unity because: (1) phloem water, unloaded with sucrose into the developing cell, has been shown to be intermediate between source water and evaporatively enriched leaf water in both herbaceous species (Cernusak *et al*. 2002) and in trees (Adar *et al*. 1995; Cernusak *et al*. 2005), and (2) there may be some evaporative enrichment in local cells (MM Barbour, unpublished data).

The predictive power of Eqn 13 was tested by Helliker and Ehleringer (2002*a*, 2002*b*) using measurements of $\delta^{18}O$ of cellulose from 10 C_3 and C_4 grass species. As source water $\delta^{18}O$ was constant for this experiment, all variation in $\delta^{18}O_c$ was due to species variation in leaf properties (as they affect leaf water enrichment) and differences in growth humidity. Helliker and Ehleringer (2002*a*) found that a value for $p_{ex}p_x$ of 0.25 fit the data well. Back-calculation of p_x from $\delta^{18}O$ measurements in the developing cell (Helliker and Ehleringer 2002*b*) showed a range for these plants of between 0.50 and 0.62, meaning that p_{ex} ranged from 0.40 to 0.50.

Equation 13 may also be derived in terms of whole tissue ($\Delta^{18}O_p$, Barbour and Farquhar 2000):

$$\Delta^{18}O_p = \Delta^{18}O_L(1 - p_{ex} p_x) + \varepsilon_{wc} + \varepsilon_{cp}, \quad (14)$$

where ε_{cp} is an empirical term describing the difference in $\Delta^{18}O$ between cellulose and whole tissue. As described above, in field conditions Eqns 13 and 14 should be modified to allow for photosynthetic weighting of diurnal variation in leaf water enrichment. Cernusak *et al*. (2005) present appropriate equations, and have found very good agreement between the photosynthesis-weighted model predictions and measured $\Delta^{18}O_{suc}$ for *Eucalyptus globulus*. The models are (Cernusak *et al*. 2005):

$$\Delta^{18}O_c = \frac{\int A\Delta^{18}O_L}{\int A}(1 - p_{ex} p_x) + \varepsilon_{wc}, \quad (15)$$

$$\Delta^{18}O_p = \frac{\int A\Delta^{18}O_L}{\int A}(1 - p_{ex} p_x) + \varepsilon_{wc} + \varepsilon_{cp}, \quad (16)$$

where $\int A\Delta^{18}O_L$ is the daily integral of the product of photosynthesis and leaf water enrichment and $\int A$ is the daily integral of photosynthesis.

Recent work (e.g. Cernusak *et al*. 2005) suggests that p_{ex} varies over quite a small range (0.3–0.5), and averages 0.40. However, the degree of variability in p_x is not as clear and creates a weakness in the model. There is some evidence (Roden *et al*. 2000; Cernusak *et al*. 2005) that p_x is very close to unity for xylem cells in the stems of mature trees, where the sink tissues are some distance from source leaves, allowing significant exchange of water between phloem and xylem (Cernusak *et al*. 2005). However, p_x is known to be less than unity in herbaceous tissues (e.g. Helliker and Ehleringer 2002*b*; Cernusak *et al*. 2005).

A more significant weakness in the model relates to variability in the ε_{cp} parameter. Cernusak *et al*. (2005) found that ε_{cp} increased with leaf age, and varied considerably even among fully expanded leaves. Some of the variability was attributable to variability in the contribution of structural and non-structural leaf components, and to short-term variability in $\Delta^{18}O_{suc}$ (Cernusak *et al*. 2005). However, until a clearer understanding of ε_{cp} is available, it is probably prudent to limit interpretation to a single component of whole-plant tissue. Cellulose seems the logical candidate.

Ecophysiological applications

Many studies of plant and ecosystem function use measurements of shoot stomatal conductance as a basic tool in understanding carbon and water fluxes. However, stomatal conductance itself is difficult and time-consuming to measure in the field, so a simple, one-off, integrative measurement of g_s would be of considerable benefit. Theory outlined above suggests that $\Delta^{18}O_{suc}$ may provide a short-term record of g_s, although $\Delta^{18}O_c$ and $\Delta^{18}O_p$ may provide longer-term records.

A further benefit of stable oxygen isotope measurements to ecophysiological studies lies in the interpretation of variation in the carbon isotope ratio of plant material ($\delta^{13}C_p$). $\delta^{13}C_p$ provides an integrative record of supply and demand for CO_2 (Farquhar *et al*. 1982). As such, variation in $\delta^{13}C$ may be driven by changes in g_s (i.e. supply of CO_2), or in photosynthetic rate (i.e. demand for CO_2), or both. $\Delta^{18}O_p$ is not thought to be strongly influenced by photosynthetic rate, so that combined measurement of both $\delta^{13}C_p$ and $\Delta^{18}O_p$ should allow stomatal and photosynthetic effects on $\delta^{13}C_p$ to be teased apart (Farquhar *et al*. 1994; Yakir and Israeli 1995; Scheidegger *et al*. 2000).

An interesting application of the hypothesis outlined above has developed from the technique of phloem sap bleeding of mature trees first described by Pate and Arthur (1998, and see Pate *et al*. 1998), and later refined by Gessler *et al*. (2001, 2004). Using measurements of both carbon and oxygen isotope

composition of phloem sap sampled from field-grown *Fagus sylvatica* trees at a single location, Keitel *et al*. (2003) were able to show that site aspect and tree density (i.e. intercepted irradiance) had non-significant effects on $\delta^{13}C$ and $\delta^{18}O$ in a season with high water availability. Variation in both $\delta^{13}C$ and $\delta^{18}O_{suc}$ were strongly correlated with g_s, with no evidence of photosynthetic effects on $\delta^{13}C$. However, when phloem sap and leaf tissue from *F. sylvatica* trees collected along a climate gradient across Germany and France was analysed for $\delta^{13}C$ and $\delta^{18}O$, results indicated variability in both stomatal conductance and photosynthesis between sites (Keitel *et al*. 2006). Trees at the cooler northern sites, where the air saturation deficit was lower, tended to have higher stomatal conductance and this was reflected in lower $\delta^{13}C$ and $\Delta^{18}O_{suc}$ values. However, the slope of the relationship between $\delta^{13}C$ and $\Delta^{18}O$ and a significant relationship between $\delta^{13}C$ and sunshine hours indicated that photosynthetic rates also varied between sites. Using the same technique, Cernusak *et al*. (2003*a*) observed a strong, negative correlation between $\Delta^{13}C$ and $\Delta^{18}O$ of phloem sap sugars sampled from *Eucalyptus globulus* along a rainfall gradient in south-western Australia. The relationship indicated that the observed large variation in $\Delta^{13}C$ resulted primarily from variation in stomatal conductance, as confirmed by leaf gas exchange and twig water potential measurements.

The oxygen isotope composition of plant tissue in foliar organs that grow basipetally (i.e. grass blades and conifer needles) is of particular interest because current understanding suggests that a sequential environmental and plant physiological record may be preserved from the tip (oldest tissue) to the base (newest tissue). Helliker and Ehleringer (2002*b*) were the first to recognise the value of this type of analysis, and demonstrated that a step change in relative humidity during the elongation of grass leaves was clearly recorded as a change in $\delta^{18}O$ of leaf tissue. More recently, Wright and Leavitt (2006) developed this technique further in pine needles. With greater spatial resolution (16 samples per needle), and applying process-based models, these authors were able to estimate the timing of needle development by relating $\delta^{18}O$ of portions of needle tissue to the evaporative environment during the growth season.

Stable oxygen isotope ratios have also been important in studies on the ecophysiology and resource aquisition of parasitic plants (Flanagan *et al*. 1993; Cernusak *et al*. 2004). Cernusak *et al*. (2004) surveyed a wide range of Australian parasitic plants and their hosts. Mistletoes were significantly depleted in ^{18}O compared with their hosts (4‰), consistent with higher g_s commonly observed in mistletoe. Root hemiparasites were also more depleted than their hosts, although the difference was only 1‰. This difference was again was interpreted as resulting from higher stomatal conductance in the parasitic plants. In contrast, plant tissue from holoparasites (i.e. those completely reliant on the host plant for carbon) was enriched in ^{18}O compared with their hosts, despite the water in the parasite tissue being depleted compared with that of the host. This suggests that the $p_{ex}p_x$ term may be lower for holoparasites, perhaps because fewer oxygen atoms exchanged with depleted local water during cell development.

More recently, Querejeta *et al*. (2006) used the stable oxygen isotope composition of plant tissue to interpret the effects of mycorrhizal association on plant regulation of water loss. Lower $\delta^{18}O$ of *Olea europaea* and *Rhamnus lyciodes* foliar tissue from plants inoculated with native mycorrhiza, compared with both uninoculated plants and those inoculated with non-native mycorrhiza, suggests that the native mycorrhiza provided more efficient hyphal water uptake, allowing higher stomatal conductance, than the non-native mycorrhiza.

A further application of interest in ecophysiological studies is using $\Delta^{18}O_p$ to interpret studies of ecosystem isofluxes of CO_2 (Bowling *et al*. 2003; Barbour *et al*. 2005; Seibt *et al*. 2006) and water (Lai *et al*. 2006). The stable oxygen isotope ratio of ecosystem CO_2 and H_2O may allow partitioning of the fluxes into the components of photosynthesis and respiration (for CO_2) or transpiration and soil evaporation (for H_2O). However, to interpret variation in $C^{18}O^{16}O$ and $H^{18}O^{16}O$, $\Delta^{18}O_e$ must be known. When combined with estimates of L, $\Delta^{18}O_p$ may provide an integrative estimate of $\Delta^{18}O_e$, and would therefore dispense with time-consuming diurnal sampling of leaf water for isotope analysis.

One of the more difficult components of the plant $^{18}O/^{16}O$ environment to measure is the isotope composition of water vapour. Sampling techniques typically rely on cold-finger traps and, even with high-volume pumps or low-volume trapping devices, the requirement for liquid nitrogen or dry ice has limited both the temporal resolution and the number of samples able to be collected and analysed (Helliker *et al*. 2002). Recent technical developments using tunable diode laser absorption spectrometry hold great promise (Lee *et al*. 2005), but the cost of such systems is probably prohibitive to many ecophysiological experiments. However, a plant-based proxy for $\delta^{18}O$ of water vapour has been proposed by Helliker and Griffiths (2007). These authors exploited the features of Crassulacean acid metabolism and epiphytic habit in Spanish moss (*Tillandsia usneoides*) to develop and test theory describing a causal link between $\delta^{18}O$ of water vapour and $\delta^{18}O$ of leaf water. $\delta^{18}O$ of leaf water was reflected in $\delta^{18}O_p$, as in all plants, so that $\delta^{18}O$ of atmospheric vapour could be reconstructed from contemporary and archived leaf samples.

Plant breeding applications

As plant material has been shown to record leaf evaporative conditions, measurement of $\Delta^{18}O_p$ may provide a powerful tool for plant breeders. Although an integrative record of g_s may be of interest to breeders in its own right, the link between $\Delta^{18}O_p$ and crop yield is likely to spark greater interest. Two important crop plants, cotton and wheat, display strong correlations between g_s and yield when grown in non-limiting environments (e.g. Lu *et al*. 1994; Sayre *et al*. 1997). Barbour *et al*. (2000*a*) have shown that the $\delta^{18}O$ of both whole leaf tissue and cellulose is strongly negatively related to seasonal mean g_s and to grain yield for field-grown wheat. The plants investigated were wheat cultivars released between 1962 and 1988, and crop yield increased from the early to later releases. Most (88%) of the 0.88% yield increase per year of release was explained by variation in g_s (Sayre *et al*. 1997).

As described above, measurement of $\Delta^{18}O_p$ may also help plant breeders interpret variation in carbon isotope discrimination. In the field-grown wheat experiment described

by Barbour *et al.* (2000*a*), most of the variation in $\Delta^{13}C$ was driven by changes in g_s, so a strong, positive correlation between $\delta^{18}O$ and $\delta^{13}C$ (or a negative relationship between $\Delta^{18}O$ and $\Delta^{13}C$) was expected and observed.

However, before encouraging plant breeders to include measurement of $\Delta^{18}O_p$ in their existing carbon isotope breeding programs, or to begin new $\Delta^{18}O$ programs, caution is required for three reasons. First, it is anticipated that not all species display a strong correlation between yield and g_s, and even in species that do, in situations where the correlation does not hold (such as during significant soil water deficit), the correlation between $\Delta^{18}O_p$ and yield will break down. Second, further research into the extent to which leaf water ^{18}O enrichment is recorded in $\Delta^{18}O_p$ (i.e. better estimates of $p_{ex}p_x$ and ε_{cp}) in a range of species and environments is required. For example, Sheshshayee *et al.* (2005) show that $\Delta^{18}O_p$ is actually positively related to g_s and transpiration rate in sunflower, cowpea, rice and groundnut, contrary to theoretical predictions. And finally, several accompanying samples (including measurement of source water and water vapour $\delta^{18}O$) must be taken from the environment of interest to allow full interpretation of variation in the oxygen isotope composition of plant tissue.

Paleoclimatic and archeological applications

Since the mid-1940s, when $\delta^{18}O$ of annual tree rings was first suggested to be an 'isotope thermometer' (Libby *et al.* 1976), palaeoclimatic reconstructions have been attempted using either whole wood or cellulose extracted from tree rings (e.g. Edwards and Fritz 1986). More recently, $\delta^{18}O$ analysis of several other plant tissues has been proposed in palaeoenvironmental reconstruction studies, including plant material from peat bog cores (e.g. Brenninkmeijer *et al.* 1982; Aucour *et al.* 1996; Hong *et al.* 2000; Ménot-Combes *et al.* 2002), subfossil leaf cellulose from rodent middens (e.g. Terwilliger *et al.* 2002), pollen (e.g. Loader and Hemming 2004), and grass cellulose and phytoliths (e.g. Webb and Longstaffe 2006). $\delta^{18}O$ of tree rings has also been used as a record of Asian monsoon intensity (Weiguo *et al.* 2004), as an indicator of relative humidity (when the slope of the δD *v.* $\delta^{18}O$ relationship is compared with the meteoric water line; Shu *et al.* 2005), and in age models for ring-less tropical tree species (Evans and Schrag 2004; Poussart *et al.* 2004; Verheyden *et al.* 2004; Poussart and Schrag 2005). Similarly, a record of Arctic and Northern Atlantic Oscillation phase change has been demonstrated to be preserved in the $\delta^{18}O$ of the long-lived arctic plant *Cassiope tetragona* (Welker *et al.* 2005).

Underlying many of these approaches is the assumption that $\delta^{18}O$ of the plant material in question relates to $\delta^{18}O_s$ which, in turn, varies with temperature. From the theory outlined above it should be clear that this link is indirect, so that simple transfer functions between $\delta^{18}O_p$ and temperature should not be expected. Indeed, simple correlations between tree ring $\delta^{18}O$ and temperature (e.g. Libby *et al.* 1976; Gray and Thompson 1977; Wilson and Grinsted 1977) or relative humidity (e.g. Burk and Stuiver 1981; Ramesh *et al.* 1986; Loader *et al.* 1995; Switsur and Waterhouse 1998) or both (e.g. Danis *et al.* 2006) have met with variable success. Using a simplified version of the models described above and assuming atmospheric vapour to be in isotopic equilibrium with $\delta^{18}O_s$, Anderson *et al.* (2002) suggested that $\delta^{18}O_s$ may be calculated from $\delta^{18}O$ of cellulose ($\delta^{18}O_c$), relative humidity (h), and equilibrium, kinetic and exchange fractionation factors:

$$\delta^{18}O_s \approx \delta^{18}O_c - (1-f)(1-h)(\varepsilon^+ + \varepsilon_k) - \varepsilon_{wc}, \quad (17)$$

where f is a 'dampening factor' (Saurer *et al.* 1997) that effectively combines the Péclet effect, the proportion of unenriched water in veins of leaves, and the $p_{ex}p_x$ term in Eqn 13 into a single fitted value. Note that the evaporative enrichment terms appear on the right-hand side of the expression, so that relative humidity must be known before $\delta^{18}O_s$ may be estimated. Clearly, this approach is a compromise between including processes that have been demonstrated to be important in plant physiological studies, and simplifying the complexity of process-based models so that they may be applied to Holocene climate studies. It seems doubtful that either the physiologists or the palaeoclimatologists would be satisfied.

A 'cleaner' $\delta^{18}O_s$ record is suggested to be present in $\delta^{18}O$ of the phytoliths of grass stems (Webb and Longstaffe 2006). Phytoliths are silica-based deposits formed in the leaf and stem tissues of many grass species. In contrast with stem cellulose, which reflects both leaf evaporative enrichment and stem (source) water, phytoliths have been shown to reflect just the local cellular water during synthesis. Insofar as stem water in grasses reflects $\delta^{18}O_s$ (i.e. is not strongly affected by evaporative enrichment), $\delta^{18}O$ of stem phytoliths should record variation in $\delta^{18}O_s$. Consequently, analysis of leaf and stem phytoliths together with leaf and stem cellulose should allow separation of source water and evaporative effects (Webb and Longstaffe 2006). Another promising technique for separating source water and evaporative influences on $\delta^{18}O_c$ involves chemical separation, before $\delta^{18}O$ analysis, of oxygen atoms that are always in isotopic equilibrium with local water after cellulose synthesis from simple carbohydrates (Sternberg *et al.* 2003). With additional refinement of this technique, it may become standard in palaeoclimatic reconstruction studies.

In a recent review of isotope dendrochronology, McCarroll and Loader (2004) recommended a multi-proxy (i.e. multi-isotope) approach and challenged plant physiologists to develop integrated models that link environmental and physiological controls on tree-ring $\delta^{13}C$, $\delta^{18}O$ and δD. High-resolution intra-annual sampling across tree rings has greatly improved understanding of the combined environmental and physiological influences on the stable isotope composition of stem wood (e.g. Loader *et al.* 1995; Walcroft *et al.* 1997; Barbour *et al.* 2002; Helle and Schleser 2004; Verheyden *et al.* 2004), and integrated, process-based $\delta^{13}C$ and $\delta^{18}O$ models are currently being developed and tested (e.g. J. Ogée, personal communication). The next challenge will be to scale process-based models, which typically integrate over hours to days, to the annual resolution of tree rings.

Stable isotopes of plant tissue are yet to be widely applied to archaeological studies. The geographic origin of cellulose from archeological linen was identified using $\delta^{18}O$ and δD (DeNiro *et al.* 1988). In a recent study, Williams *et al.* (2005) used $\delta^{18}O$ of cellulose extracted from archaeological maize cobs to identify the source water used for maize production in the prehistoric

American south-west. The dependence on irrigation water for cultivation in some localities was particularly clear.

Point of origin and forensic applications

$\delta^{18}O$ of local waters carries a strong geographic signature, because of influences such as temperature of rain droplet formation, continentality, storm-track trajectories and locality of cloud condensation (Craig 1961). Insofar as $\delta^{18}O_p$ reflects $\delta^{18}O_s$, plant material may be used to identify the geographical origin of samples and, in some cases, processing techniques. Two important applications are authenticity of food stuffs and forensic analysis. In an example of the use of $\delta^{18}O$ in food product authenticity, combined $\delta^{18}O$ analysis of sugars, citric acids and water from fruit juice was used to assess whether commercial orange juice was 'freshly squeezed' or reconstituted from concentrates (Houerou *et al*. 1999). In authentic freshly squeezed juices, $\delta^{18}O$ of both sugars and water varied predictably with geographical origin, and sugars were 26 to 34‰ more enriched than water (99% confidence interval), while juices made from concentrate fell outside this range.

It has been suggested that $\delta^{18}O$ analysis of illicit drugs may help determine the region of origin for plant-derived narcotics such as cocaine and heroin (Ehleringer *et al*. 1999, 2000), although the technique is yet to be applied. However, the American anthrax attacks in 2001 have sparked interest in $\delta^{18}O$ as a tool to determine the origin of microbial agents (Kreuzer-Martin *et al*. 2004). $\delta^{18}O$ of *Bacillus subtilis* spores was linearly related to $\delta^{18}O$ of culture water, allowing unambiguous determination of the region of origin of spores cultured in five localities across America (Kreuzer-Martin *et al*. 2003).

Conclusions

Interpretation of variation in the oxygen isotope composition of plant organic material has several exciting potential applications. Current understanding is summarised in models that predict variation in $\delta^{18}O_p$ as a result of variation in source water $\delta^{18}O$, in the leaf evaporative environment, and in isotopic exchange between plant water and organic molecules. These models predict measured variation in $\delta^{18}O$ of cellulose quite accurately, and should allow novel isotope techniques to be used to address a wide range of questions involving plant regulation of water loss.

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