Spatial Variation of Deuterium Enrichment in Bulk Water of Snowgum Leaves[1][OA]

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Deuterium enrichment of bulk water was measured and modeled in snowgum (*Eucalyptus pauciflora* Sieber ex Sprengel) leaves grown under contrasting air and soil humidity in arid and wet conditions in a glasshouse. A map of the enrichment was constructed with a resolution of 4 mm by using a newly designed cryodistillation method. There was progressively increasing enrichment in both longitudinal (along the leaf midrib) and transversal (perpendicular to the midrib) directions, most pronounced in the arid-grown leaf. The whole-leaf average of the enrichment was well below the value estimated by the Craig-Gordon model. The discrepancy between model and measurements persisted when the estimates were carried out separately for the leaf base and tip, which differed in temperature and stomatal conductance. The discrepancy was proportional to the transpiration rate, indicating the significance of diffusion-advection interplay (Péclet effect) of deuterium-containing water molecules in small veins close to the evaporating sites in the leaf. Combined Craig-Gordon and desert-river models, with or without the Péclet number, P, were used for predicting the leaf longitudinal enrichment. The predictions without P overestimated the measured values of δdeuterium. Fixed P value partially improved the coincidence. We suggest that P should vary along the leaf length l to reconcile the modeled data with observations of longitudinal enrichment. Local values of P, $P(l)$, integrating the upstream fraction of water used or the leaf area, substantially improved the model predictions.

The isotopic composition of leaf water and plant cellulose is of considerable interest to plant ecophysiologists, hydrologists, and (paleo-) climatologists. Atmospheric temperature modulates the isotopic composition of precipitation (Ehleringer and Dawson, 1992), while evaporation can substantially enrich the isotopic composition of both leaf and soil surface water (Wang and Yakir, 2000). The isotopic composition of oxygen in leaf water is imprinted on the CO_2 exchanged between a plant and the atmosphere, and in O_2 evolved during photosynthesis (Farquhar and Lloyd, 1993). Thus, ^{18}O in cellulose or deuterium (D) in cuticle wax *n*-alkane integrate the environmental conditions (air temperature, soil and air humidity, evapotranspiration) over the period of their synthesis, with these signals being stored chronologically, e.g. in tree rings (Gray and Thompson, 1977) or sediments (Sachse et al., 2006), until the deposits are biologically or chemically degraded. The isotopic signature of water and leaf organic matter has been used in partitioning the global carbon budget between terrestrial and marine vegetation (Farquhar and Lloyd, 1993) or evapotranspiration between soil evaporation and plant transpiration (Wang and Yakir, 2000; Yakir and Sternberg, 2000; Williams et al., 2004). Recently, it was also shown that the isotopic composition of water and leaf organic matter can reveal further details in the regulation of CO_2 assimilation and water use efficiency (Barbour and Farquhar, 2000; Barbour et al., 2000). The above applications rely on a not yet completed mechanistic understanding of leaf water isotopic enrichment.

The natural abundance of D in environmental water is approximately 0.015%. While there are no changes in isotopic abundance of hydrogen and oxygen during water uptake by plant roots (White et al., 1985), transpiration causes the enrichment of leaf water in heavier isotopes (D and ^{18}O). There are two reasons for this enrichment: first, equilibrium fractionation at evaporating sites, where the lighter $H_2{}^{16}O$ molecules evaporate more easily than their heavier counterparts (D- or ^{18}O-containing water molecules [$DH^{16}O$ or $H_2{}^{18}O$]) and, second, kinetic fractionation due to faster diffusion of lighter water molecules through stomatal pores and the laminar boundary layer. As a result, the lighter water molecules escape the leaf more easily, leaving behind the heavier molecular species. Due to an isotopic exchange of $^{18}OH^-$ and D^+ ions with HCO_3^- in chloroplasts, and due to hydration of carbonyl in cellulose intermediates in cytosol, water enrichment leaves the isotopic signature in primary photosynthates and cellulose.

As the isotopic enrichment of evaporating water obeys physical rules, it can be modeled. Craig and Gordon

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(1965) quantified the enrichment at the surface of a mixed water body and showed that it depends on three environmental variables: vapor pressure in the atmosphere (e) relative to saturated pressure at the surface temperature (e_{Ts}*), isotopic composition of water vapor in the atmosphere, and isotopic composition of the source water. The model was verified for evaporation from free water and bare wet (W) soil (e.g. Zimmermann et al., 1966) and adapted for water transpired from a leaf (Dongmann et al., 1974; Farquhar et al., 1989). However, it was often found that the Craig-Gordon-based predictions of the isotopic enrichment of leaf water overestimated the experimentally estimated values (e.g. Yakir et al., 1990; Flanagan and Ehleringer, 1991; Flanagan et al., 1991). Moreover, the model cannot account for the observed spatial variation of enrichment within the leaf (Wang and Yakir, 1995; Gan et al., 2002). The model's failures have been attributed to mixing of enriched water at evaporating sites and in the symplast with nonenriched xylem water (Yakir et al., 1989), or to non-steady-state conditions (Wang and Yakir, 1995; Farquhar and Cernusak, 2005). However, the two-pool mixing model fails in predicting the negative effect of transpiration on enrichment observed by Walker et al. (1989), Helliker and Ehleringer (2002), and Barbour et al. (2004).

An alternative explanation (Farquhar and Lloyd, 1993) offers the interplay of backward diffusion of the heavier water molecules from the evaporating sites opposed by a convective stream of transpiration flux (Péclet effect). However, neither of the models could properly explain a nonrandom systematic gradient along the leaf blade: water is usually enriched in heavier isotopes toward the leaf tip. Analogous enrichment is observed along a desert river becoming narrower due to high evaporation and, eventually, vanishing in the sand. Desert-river models have been successfully analyzed in hydrology (Gat, 1996). However, the desert river-leaf blade analogy suffers from a lack of radial interactions in the case of the river, i.e. interactions between the main stream and the adjacent land. On the contrary, the main stream in the middle vein of the leaf is isotopically affected by stomatal transpiration and backward diffusion of heavier isotopes that accumulate at the evaporation sites. Recently, progress in understanding longitudinal enrichment along the leaf blade has been achieved by incorporating radial and longitudinal Péclet effects into a desert-river model (Farquhar and Gan, 2003). The model was developed for a simplified leaf with parallel grass-type venation. So far, no similar analytical model is available for a dicotyledonous leaf with reticulate venation.

The objectives of this study were to (1) compare the isotopic composition of leaf water as predicted by the Craig-Gordon formula with those estimated experimentally in snowgum (*Eucalyptus pauciflora* Sieber ex Sprengel) leaves grown under two environmental conditions contrasting in water availability, and (2) contribute to understanding the gradients in leaf water enrichment. Transpiration rate and leaf temperature were measured separately for the base and tip of the leaf lamina to test whether the isotopic gradient along the leaf could be explained by differences in these two parameters. Due to a refinement of the water extraction technique described here, we were able to construct isotopic maps with sufficient spatial resolution. Based on the finer isotopic pattern, we tried to judge which of the submodels improved the correspondence between the Craig-Gordon model predictions and reality. It was found that a desert-river model combined with the Péclet effect can better approximate the longitudinal isotopic enrichment when the Péclet number varies along the leaf blade with respect to the upstream transpiring area.

MODELING OF LEAF WATER ISOTOPIC ENRICHMENT

Whole-Leaf Enrichment

Isotopic enrichment of leaf water was estimated at evaporating sites above source (xylem) water by using the Craig-Gordon model modified for plant leaves (Dongmann et al., 1974; Farquhar et al., 1989):

$$\Delta_e \cong \varepsilon_k + \varepsilon^* + (\Delta_v - \varepsilon_k) \times h \quad (1)$$

where Δ_e stands for the isotopic enrichment of water at evaporating sites above source (i.e. xylem water entering the leaf lamina). The index v denotes atmospheric moisture and its isotopic fractionation, Δ_v, which was determined as the difference between signatures of atmospheric water, δ_{aw}, and petiole water, δ_{pw} ($\Delta_v = \delta_{aw} - \delta_{pw}$). ε^* and ε_k are the equilibrium and kinetic fractionation factors, respectively, and h is the ratio of e_a to e_i, the vapor pressures in ambient atmosphere and the internal leaf air space, respectively. The e_i value was derived from leaf temperature assuming saturation water vapor pressure in the mesophyll air space; e_a was estimated from the volume of air flowing through the glass spiral and the mass of water trapped during the sampling of glasshouse atmospheric moisture. Values of 84‰ and 16.3‰ were used for the δD fractionation factors ε^* and ε_k, respectively (Wang and Yakir, 2000; Cappa et al., 2003). Because of the temperature difference between the leaf base and tip, Δ_e was calculated for both leaf parts separately. The Δ_e values calculated from Equation 1 were compared with the D enrichment of bulk leaf water above the source water, Δ_L, which was calculated as the average of δD across a particular set of leaf discs analyzed: $\Delta_L = (\Sigma\delta_{\text{leaf water}}/n) - \delta_{\text{petiole water}}$. For this study, a set of discs was chosen from the leaf base (23 discs) and another set from the tip (25 discs).

Because the predictions made by Equation 1 often overestimate measured leaf water enrichment Δ_L, several alternative refinements of the Craig-Gordon model have been offered in the past. Two-component mixing models assume that the highly enriched water of δ_e at

evaporating sites is diluted with unenriched xylem water, δ_x, during extraction of the leaf water (Leaney et al., 1985), expressed in Δ notation as the deviations from xylem water ($\Delta_e = \delta_e - \delta_x$ and $\Delta_L = \delta_L - \delta_x$):

$$\Delta_L = \Delta_e(1 - m) \quad (2)$$

where m is the fraction of leaf water not subject to evaporation, being mostly xylem water.

Farquhar and Lloyd (1993) suggested a nonlinear approach describing a transition in enrichment between the evaporating sites and xylem water using the Péclet number, P. Enrichment at evaporation sites, Δ_e, is exponentially decreased by P:

$$\Delta_L = \frac{\Delta_e \times (1 - e^{-P})}{P}. \quad (3)$$

The physical meaning of P is the ratio of the convective flux velocity of nonenriched xylem water (in m s^{-1}) to the backward diffusion conductance of heavier water molecules (also in m s^{-1}; see Eq. 7 for definition of the Péclet number). It should be noted that the two-component model and the Péclet effect model are not mutually exclusive. There is still a portion of xylem water with less or no enrichment that should be subtracted when calculating the Péclet effect.

Longitudinal Pattern of Enrichment

Isotopic water enrichment progressively increases toward the leaf tip. The Craig-Gordon enrichment Δ_e should be identical to the leaf average of transpiration-weighted values of the isotopic enrichment at evaporating sites (Farquhar and Gan, 2003). However, the Craig-Gordon model fails to predict longitudinal enrichment $\Delta(l)$ along the lamina length l. In an attempt to describe the longitudinal redistribution of the enrichment observed in snowgum, the desert-river model was used (equation 5 in Farquhar and Gan, 2003):

$$\Delta_x(l) = \frac{\Delta_e}{h'}\left[1 - \left(1 - \frac{l}{l_m}\right)^j\right] \quad (4)$$

where a humidity-dependent exponent $j = h'/(1 - h')$ and $h' = 1 - (1 + e^*) \times (1 + e_k) \cdot \times (1 - h)$ with all e^*, e_k, and h expressed as fractions between 0 and 1. The index x denotes the xylem water. The modified desert-river model incorporating the radial Péclet number, P (denoted P_r; equations 12 and 13 in Farquhar and Gan, 2003), was used for a better fit:

$$\Delta_x(l) = \frac{\Delta_e}{h'}\left[1 - \left(1 - \frac{l}{l_m}\right)^k\right] \quad (5)$$

where

$$k = \frac{h'}{e^P - h'} \quad (6)$$

with P being the radial Péclet number. In these models, enrichment is a function of leaf length l, with $l = 0$ at the leaf base and maximum $l = l_m$ at the leaf tip, and other spatially independent parameters Δ_e, h', and P.

The radial Péclet number P is a measure of advective dilution of heavier water isotopes at, and their diffusive backward transport from, the evaporating sites (Farquhar and Lloyd, 1993). In the original desert-river model (Eq. 4), the diffusion in radial direction (perpendicular to the main river or xylem stream) dominates the radial convective flow and P is 0. As a result, the desert-river model offers no essential difference between isotopic enrichment in xylem and the leaf lamina (or across the river stream). However, Equation 5 allows for a radial Péclet number higher than 0 and, thus, differentiates between the enrichment of lamina and xylem water. P depends on transpiration rate E (in mol m^{-2} s^{-1}) and the structure of veinlets and apoplastic water pathways, which is expressed by the effective length of the water path from xylem to the evaporating sites L (in m; Farquhar and Lloyd, 1993):

$$P = \frac{E \times L}{C \times D_w} \quad (7)$$

where D_w is the diffusion coefficient (m^2 s^{-1}) of water containing one deuterium atom (DH^{16}O) in the prevailing water isotopic species ($H_2{}^{16}O$) and C is the molar concentration of water (mol m^{-3}). Equation 5 was developed for a leaf with parallel venation and invariable distance between the veins (single tube and

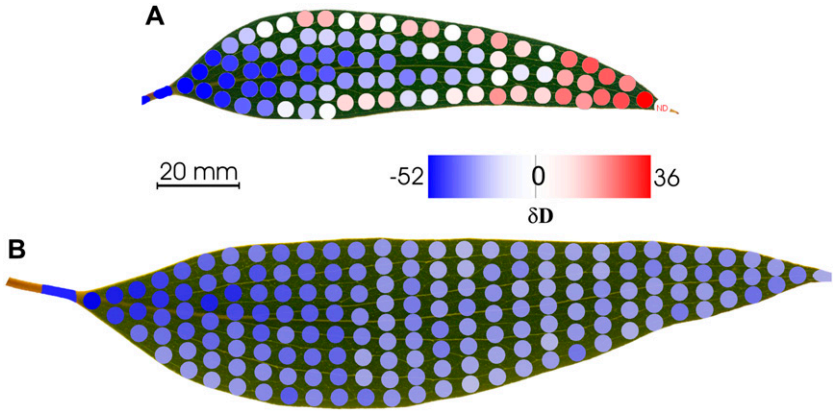

Figure 1. Topology of D enrichment of bulk leaf water in the lamina of snowgum leaves grown for 2 years under soil water deficit and dry air, A climate (A) and at ample water availability and high air humidity, W climate (B). Irradiance and air temperature were similar. Leaf dimensions are proportional to actual size of the leaf. Position and size of the leaf discs match closely the real situation. The color scale is chosen arbitrarily.

Table I. *δD of the leaf bulk water extracted from the discs sampled close to the middle vein and along both leaf edges distinguished by their convexity (see Fig. 1, A and B)*

Significant differences between midvein and edges of a particular third of the leaf lamina are marked by different letters ($\alpha < 0.05$). Means and SDs (in parentheses) were calculated from four to nine discs.

Leaf Transect	Basal Third	Middle Third	Tip Third
A			
Midvein region	−39.7 (6.1)[a]	−17.5 (11.4)[a]	4.8 (8.5)
Less convex edge	−18.7 (16.8)[b]	3.3 (6.5)[b]	11.3 (6.4)
More convex edge	−2.0 (12.0)[c]	5.4 (4.7)[b]	12.3 (11.6)
W			
Midvein region	−37.2 (5.8)[a]	−25.8 (4.1)	−21.0 (2.9)
Less convex edge	−30.0 (5.1)[b]	−21.6 (5.7)	−19.5 (2.1)
More convex edge	−22.1 (4.5)[c]	−21.6 (7.0)	−21.3 (4.8)

attached mesophyll). In snowgum and many other dicotyledonous species, the leaves have pinnate (feather like) venation with reticulate open or closed veinlets. Such leaves represent a much more complex system for analytical modeling of enrichment. Therefore, a semiempirical approach was used. The radial Péclet number P was treated as a variable that changed along the leaf length l: $P(l) = P_f$. The P_f value was zero at the leaf base ($l = 0$) and increased proportionally with the fraction f of the transpiring leaf area upstream of the local value of l (i.e. the fraction of total leaf area lying between imaginary lines drawn perpendicularly to the leaf midrib at the leaf base and l) toward the leaf tip where it became maximal (i.e. 1). The concept follows the idea that (1) the fractionation processes between evaporating sites and the small veins leave an isotopic signature in the xylem water due to the back diffusion of heavier isotopes and (2) the signature (enrichment) is proportional to the number of evaporating sites (leaf area) at a particular distance, l, from the leaf base. However, as noted by one of reviewers of this article, varying P embedded in k in the exponent of the modified desert-river equation is not the same as varying P along the leaf. If P varies along the leaf, mathematically one does not get the form of Equation 5 with P varying exactly the same way in the exponent.

RESULTS

The spatial pattern of δD in bulk leaf water of snowgum leaf showed more progressive enrichment along the leaf grown in arid (A) than W conditions (Fig. 1, A and B). Both W and A leaves had similar δD values in petiole water. The petiole water was enriched by about 25‰ compared to the tap water used for irrigation. Hereafter, δD of petiole water is regarded as the source value. In addition to the longitudinal D enrichment, there was also a δD enrichment from the middle vein toward the leaf margin; again, the differences between middle vein and the leaf margin were more pronounced in the A leaf (Table I).

We wondered whether the differences in δD between the leaf base and tip or those induced by water availability might be explained by the environmental variables involved in the Craig-Gordon model. The leaf from the A climate was significantly warmer than that from the humid glasshouse at the same irradiance. In addition, the leaf bases were warmer than leaf tips for both environments (Fig. 2B). The leaf tip and base temperatures were negatively related to stomatal conductance (Fig. 2A) and transpiration rate (data not shown). Isotopic enrichment of water at evaporating sites above the source water, Δ_e, was calculated for the

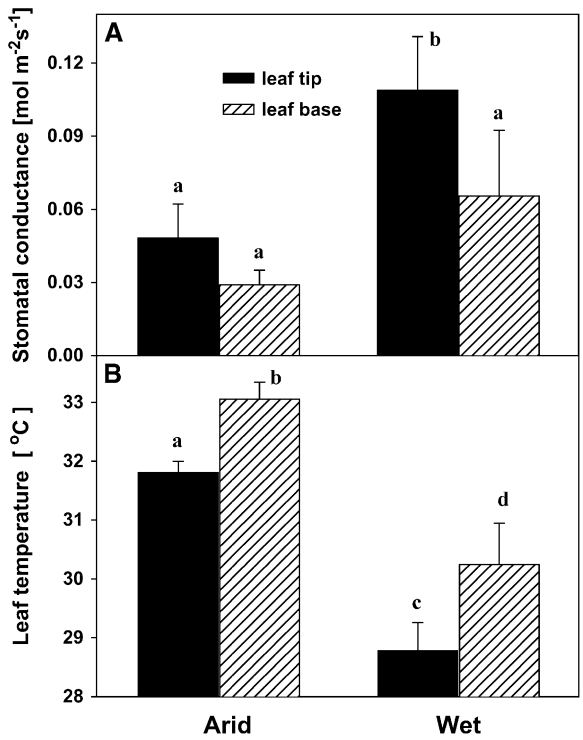

Figure 2. Stomatal conductance and leaf temperature measured during sunny days in leaves of snowgum grown in a glasshouse at low air humidity and limited water supply (A) and high humidity and ample irrigation (W). Stomatal conductance was measured by the gas-exchange technique (LI-6400) on a rectangular leaf area (2 × 3 cm) adjacent to the leaf base or tip. Leaf temperature was measured independently with an infrared sensor and a data logger. Means and SES (bars) from six to 21 measurements are shown. Significantly different means are indicated by different letters ($\alpha = 0.05$).

Table II. *Parameters of leaf water enrichment*

Variables and their values used in calculating D enrichment of leaf water at evaporating sites, Δ_e, using the adaptation of Craig-Gordon model for a plant leaf (Eq. 1). *T* denotes temperature, *e* water vapor pressure, *h* RH at a given leaf temperature, δ relative deviation of the D/H ratio from the standard. The indices a, l, aw, and pw denote free atmosphere, leaf, atmospheric water, and petiole water, respectively. Δ_L is enrichment of extracted leaf lamina water above the petiole water, *E* and *L* are the transpiration rate and effective length of water pathway, which, when used in the Péclet number and combined with the Craig-Gordon model (Eq. 3), eliminates the difference between Δ_e and Δ_L. The values for the leaf base and tip are based on independent measurements; the values located in between these lines were regarded as common for both the base and tip.

Leaf Region	T_a	e_a	T_l	*h*	δ_{aw}	δ_{pw}	*E*	Δ_e	*L*	Δ_L	$1 - \Delta_L/\Delta_e$
	°C	hPa	°C	%	‰	‰	mmol m^{-2} s^{-1}	‰	m	‰	
A											
Base			33.1	30			0.75	76.8	0.812	18.4	0.76
	25.7	15.0			−114.3	−50.9					
Tip			31.8	32			1.09	75.1	0.056	61.5	0.18
Whole leaf							0.92	75.9	0.226	40.9	0.46
W											
Base			30.2	49			1.60	67.0	0.425	14.4	0.79
	25.4	21.2			−99.4	−47.6					
Tip			28.8	53			2.61	64.1	0.121	26.4	0.59
Whole leaf							2.11	65.6	0.189	22.7	0.65

leaf base and tip, and its average for whole A and W leaves, based on Equation 1 and the data shown in Table II. Leaf bulk water enrichment above the source (petiole) water, Δ_L, was determined by averaging δD values over 25 leaf discs at the leaf tip and 23 at the base, or over all discs from a leaf (Table II). Δ_e always overestimated the measured Δ_L. The overestimation was higher at the leaf base and decreased to the tip. The $\Delta_e - \Delta_L$ difference normalized to the modeled value ($1 - \Delta_L/\Delta_e$) was proportional to the transpiration rate for the leaf tips when compared separately from the leaf bases (Fig. 3). The A and W bases were not significantly different in both transpiration rate and the fractional difference. If the enriched leaf water at evaporating sites is mixed with xylem water, then the $1 - \Delta_L/\Delta_e$ difference could represent the fraction of nonenriched xylem water, *m*, in bulk leaf water (Eq. 2). Consequently, the xylem fraction *m* would need to be close to 80% at the leaf base in both the humid and A environment and decreases to 62% and 25% at the leaf tip in the humid and dry conditions, respectively (Fig. 3).

The longitudinal enrichment, modeled by the desert-river model with zero *P* (Eq. 4), yielded a sufficient fit only for the third of the A leaf lamina nearest to the leaf base (Fig. 4A). Differences between modeled and experimental values increased progressively toward the leaf tip. The experimental points represent averages over the leaf discs arranged in rows perpendicular to the leaf middle rib (see Fig. 1, A and B). The discrepancy between the predicted and measured values diminished when a nonzero radial Péclet number *P* was used (Fig. 4B). However, unlike *P* in the exponent k in Equation 5, the variable *P*, denoted $P_f(l)$ here, was allowed to vary with the leaf length, *l*. The P_f value, starting at 0 at the leaf base, increased with the fraction of leaf area between notional lines drawn perpendicularly to the leaf midrib at $l = 0$ and the local *l*. Similarly, the inconsistency between the local average of enrichment observed in the leaf grown in W conditions (white circles in Fig. 4C), and the modeled one (bold line in Fig. 4C), decreased at spatially variable P_f (Fig. 4D).

DISCUSSION

The study showed that water in dicotyledonous eucalyptus leaves becomes isotopically enriched both along the midrib from the leaf base to the tip and usually also across the leaf lamina from the midrib to the leaf edge. The enrichment pattern appeared smooth at

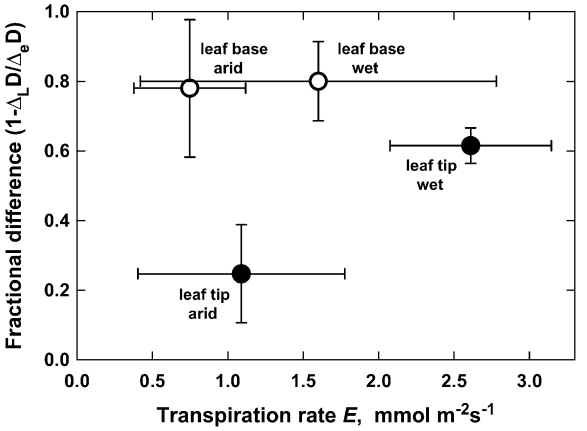

Figure 3. The fractional difference between D enrichment in leaf water calculated using the Craig-Gordon formula (Δ_eD) and that estimated analytically in bulk leaf water (Δ_LD) is plotted against transpiration rate. Both values were estimated under similar conditions in snowgum leaves grown in A or W conditions. The horizontal bars show SEs of six to 10 individual transpiration measurements at the bases and tips of three to five leaves; the vertical bars are SE calculated for 25 and 23 Δ_LD values of water from leaf discs punched at the leaf tip and base, respectively.

the attained resolution (1 pixel = disc of 4 mm diameter). Increasing water enrichment toward the outside and tip of the lamina were observed by Bariac et al. (1994; French bean [*Phaseolus vulgaris*], wheat [*Triticum aestivum*], maize [*Zea mays*]), Wang and Yakir (1995; several C_3 dicots), Helliker and Ehleringer (2000, 2002; C_3, C_4 grasses, sunflower [*Helianthus annuus*]), and Gan et al. (2002, 2003; cotton [*Gossypium hirsutum*], maize), and theoretically analyzed by Farquhar and Gan (2003) and Barnes et al. (2004) for a grass-type leaf. In our case, both the leaf-averaged enrichment and the gradient in enrichment were higher at low air humidity and limited water supply, i.e. in the simulated A climate, compared to leaves grown at high relative humidity (RH; Fig. 1, A and B). A similar humidity effect was theoretically predicted (Craig and Gordon, 1965; Dongmann et al., 1974; Farquhar and Lloyd, 1993). It has also been observed several times in leaves (e.g. Barbour and Farquhar, 2000; Gan et al., 2002; Pendall et al., 2005) and many times as ^{18}O enrichment in the cellulose of tree rings (e.g. Shu et al., 2005).

We attempted to find a suitable model that could describe the mean value and the longitudinal pattern in isotopic enrichment of leaf water. The Craig-Gordon model adapted for plant leaves greatly overestimated the mean found by averaging the isotope ratio of all individual leaf discs. The same was true for the leaf bases and tips, which varied in transpiration rate and temperature. This discrepancy between the model and reality has been noted repeatedly (Yakir et al., 1990; Flanagan et al., 1991), resulting in the development of submodels joined with the Craig-Gordon expression. The existence of two pools of leaf water, the enriched one at evaporating sites, which can be properly characterized by the Craig-Gordon estimates, and the non-enriched pool in xylem vessels, fails to explain the leaf spatial enrichment gradient until we accept that the pools can vary in their relative proportion along the leaf blade. Application of the Péclet number, a measure of the counterbalance between advective dissolution of enriched water at evaporating sites and its backward diffusion, as offered by Farquhar and Lloyd

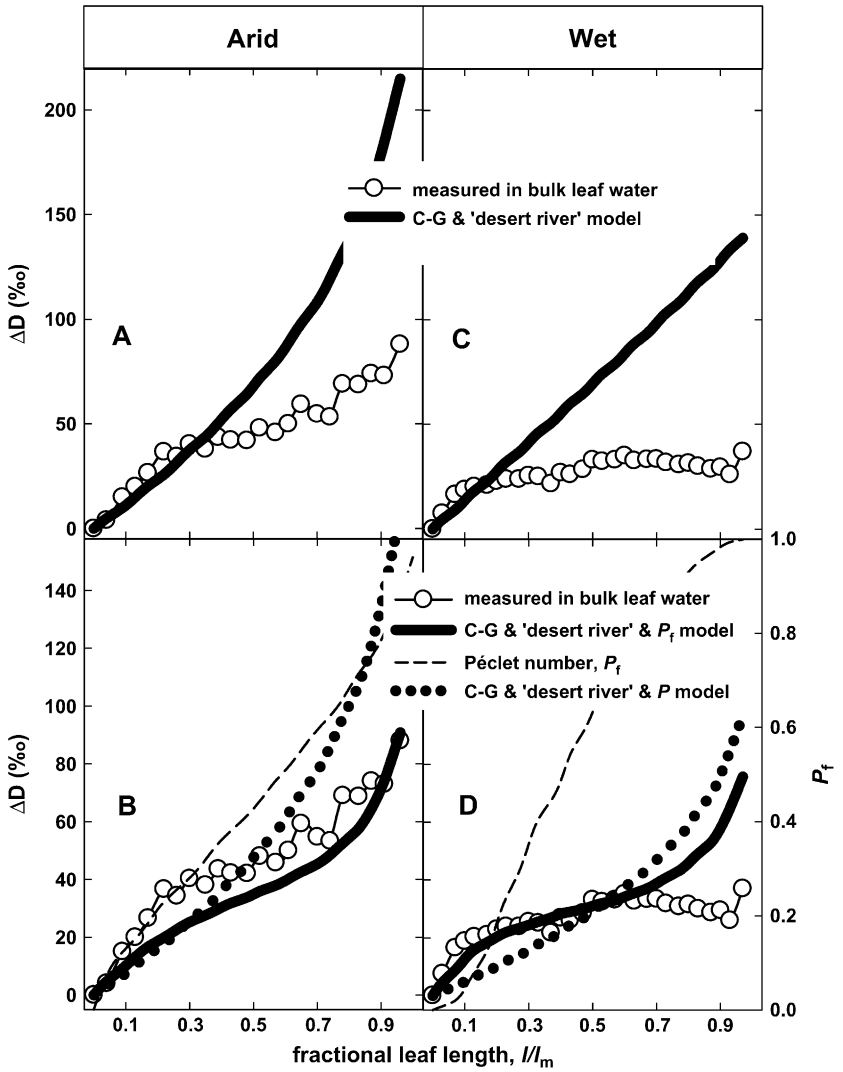

Figure 4. Longitudinal D enrichment (ΔD) of leaf water in snowgum plants grown in A (A and B) or W (C and D) conditions. Fractional leaf length, $l/l_m = 0$ denotes the leaf base, $l/l_m = 1$ is leaf tip. White circles are ΔD averages over the discs shown in Figure 1 and arrayed perpendicular to the leaf longitudinal axis. The patterns of enrichment shown by solid lines were calculated using (1) the combined Craig-Gordon and desert-river model without the radial Péclet effect, Equation 4 (A and C) or (2) the above model with the Péclet number equal to the fractional leaf area, Equation 5 (B and D). Values of the Péclet number (integrating the fractional leaf area) are shown by dashed lines and the right scale. The dotted lines show the same as in 2 with fixed Péclet number $P = 0.31$ (B) and $P = 0.68$ (D).

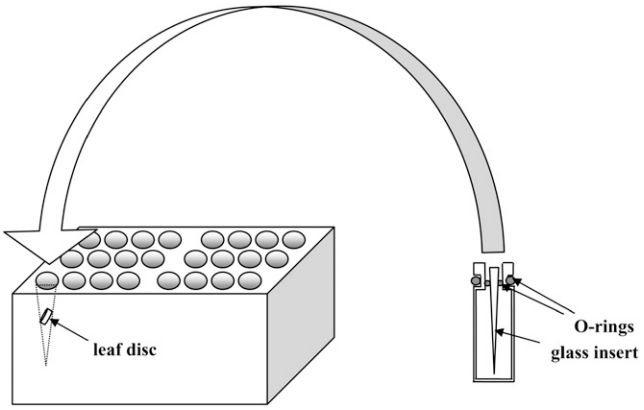

Figure 5. Schematic of the leaf discs cryodistillation block. Each of the 24 conical holes (3 mL volume) in a metal block accommodated one 4 mm diameter leaf disc (left) and was closed by an inverted 2 mL glass vial, the volume of which was reduced by a glass insert (150 μL volume, Supelco, catalog no. 24719), and sealed in the neck of the vial using a small O ring. Four metal blocks with the samples and vials were put in a heater (QBT4, Grant Instruments) and warmed to 80°C. Vial bodies were pushed into dead-end holes drilled from the outer surface in the bottom of a styrofoam box. Liquid nitrogen (around 8.0 dm^3) was poured into the box so as to chill the bottoms of the vials, with an 8 mm layer of the styrofoam remaining between the bottom of the glass vials and the bottom of the box interior. Heat transfer was facilitated by duralumin sticks touching the vial's bottom, so the bottom of the glass vials was cooled down to approximately −20°C. Water vapor from the discs diffused up into the tips of the glass inserts, which were chilled, where it turned to ice. The leaf discs were completely dried after 4 h of cryodistillation. After cooling the metal block, the vials were pulled and tightly sealed with crimple caps.

(1993), represents another means in reducing the overestimated Craig-Gordon value. This concept, scaled for the radial and longitudinal directions and coupled with the analogy of enrichment in a desert river, led to an analytical solution suitable for a simplified grass-like leaf (Farquhar and Gan, 2003). However, the assumption of uniform distance between veins restricts applicability of the solution largely to grasses and other monocotyledonous leaves. An analytical solution for dicots with reticulate venation will probably be more complex. A semiempirical approach was tried in this study.

The desert-river model was taken as the first approximation to test it for the enrichment found in eucalyptus. Eucalyptus has oblong leaves with partly parallel but still pinnate venation typical for dicotyledonous plant species. The predicted values calculated by the desert-river model (Eq. 4) were close to the measured values in bulk leaf water in the basal third of the leaf blade but deviated more and more toward the leaf tip (Fig. 4, A and C). Application of the radial Péclet number P, which could improve model prediction, requires knowledge of its value. While the physical parameters involved in P (C and D_w in Eq. 7) and transpiration rate are usually known, the effective length L of water transport is difficult to assess and specific to species and probably environment. However, it is possible to find an L value that eliminates the deviation between the modeled and analytical estimates. In this way, we found that L should decrease from the leaf base to the leaf tip by 14.5 and 3.5 times in eucalyptus leaves grown in the A and W conditions, respectively (Table II).

As suggested by one of the reviewers of this article, it should be noticed that L calculated in this way is not the real effective length; especially not L at the leaf tip. The concept of Péclet number used in Equation 3 does not involve any spatial variability; it assumes a uniform leaf with no progressive enrichment. There was a longitudinal enrichment, namely in the leaf from dry conditions. So even if L were uniform along the leaf, the values calculated here would appear to be variable since we additionally attributed the effect of longitudinal advection to L. Then, it is possible that L (and the Péclet number) at a distance l from the leaf base could account for the number of evaporating sites or mesophyll area that affected water crossing the line at l toward the leaf tip. This is possibly not the case with the parallel venation of monocotyledons, which have a constant width between the veins, where the number is constant and can be implicitly involved in L. When P was allowed to vary, such that it integrated the leaf area from the leaf base to the tip, the prediction of the model was close to our analytical estimates for both the A and W leaves (Fig. 4, B and D). Although this simplified approach limits the $P(l)$ values to the 0 to 1 interval, and though $P(l) > 1$ values would yield even better coincidence with the experimental values in the W leaf (data not shown), the spatial variability of

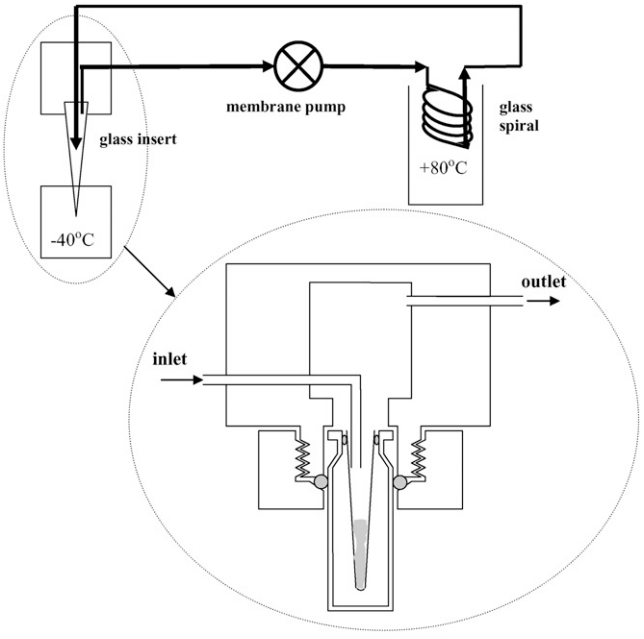

Figure 6. Scheme of the cryodistillation closed circuit (top part) used for transfer of atmospheric moisture, which had been trapped in the glass spiral, into the glass insert embedded in a vial suitable for an isotope ratio mass spectrometer autosampler. The bottom of the vial (see detail in bottom part) was immersed in a liquid nitrogen bath cooling the glass tip walls to about −40°C.

P seems sound. At further refinements, $P(l)$ could account for the real evaporating surface of amphistomatous or hypostomatous leaves. Such a concept would distinguish between the $P(l)$ range 0 to 2 for amphistomatous and 0 to 1 for hypostomatous leaves. For example, when we multiplied the fractional leaf area at particular l by a factor of 2 (taking into account the amphistomatous leaves in snowgum) and by 0.8 (the ratio of stomatal density on adaxial and abaxial leaf sites in our leaves) and subtracted the mean fraction of nonenriched water, we obtained better fit with the measurement data than that shown in Figure 4, B and D. However, this empirical approach requires further testing.

Roden and Ehleringer (1999) and Barbour et al. (2004) found that the Craig-Gordon model, when combined with two-component mixing and/or Péclet effects, was better able to predict leaf water enrichment under a variety of environmental conditions. In three broad leaf plants, these authors were able to find species-specific L values, which reconciled the modeled and measured Δ for leaves grown under various humidity levels. In this study, whole-leaf averages of L equal to 189 and 226 mm were obtained for snowgum leaves grown in W and A conditions, respectively (Table II). These values are 1 order of magnitude higher than those presented for alder (*Alnus incana*), birch (*Betula occidentalis*), and cottonwood (*Populus fremontii;* Barbour et al., 2004). It should be noted that the actual linear distance between evaporating sites close to stoma and end of the vein is mostly less than 100 μm but the effective length, based on leaf projected area, was estimated between 9 and 200 mm in wheat (Barbour and Farquhar, 2003). Our unusually high L may be due to the sclerophyllous anatomy of snowgum leaves. Conversely, high L could strengthen the role of already-implemented (fractional) evaporating leaf area especially if the longitudinal xylem walls are not ideally water proofed and exchange water with mesophyll cell walls.

The fractional difference between ΔD at evaporating sites and in bulk water ($1 - \Delta_L/\Delta_e$) should not be affected by the transpiration rate had the two-component mixing model been sufficient to describe the leaf water fractionation (Barbour et al., 2004). However, the $1 - \Delta_L/\Delta_e$ difference was proportional to E in this study, with the relation being most evident at the leaf tips and much less, if any, at the leaf base (Fig. 3). The lack of variation in fractional differences between the leaf bases from W and A conditions may be explained by a higher ratio of mesophyll/xylem water at the tip than at the base. An alternative explanation may be gleaned from Figures 2 and 3: stomatal conductances and transpiration rates at the leaf bases did not vary significantly in both contrasting environments. It is likely that there is a positive relationship between the fractional difference $1 - \Delta_L/\Delta_e$ and transpiration rate. This indicates that the advection-diffusion interplay, formalized in the Péclet number, plays a significant role in the fractionation of leaf water isotopes. However, this interpretation should be taken with caution, because transpiration was not manipulated solely at one leaf. Leaves grown in different humidity regimes can differ also in leaf anatomy and, thus, in apparent water path length L between veinlets and the evaporating sites.

CONCLUSION

To reconcile the modeled data (Craig-Gordon and desert-river models employing the radial Péclet number) with our observations of longitudinal enrichment required that we vary the radial Péclet number, P, along the length of the leaf. The P magnitude was in the range between 0 and 1, similar to the suggestion by Farquhar and Gan (2003), and increased from the leaf base ($P_{l=0} = 0$) to the tip ($P_{l=\max} = 1$). The local value of P, equal to the fraction of the upstream evaporating area, improved substantially the model prediction. This indicates that isotopic enrichment along the length l of a eucalyptus leaf can be assessed using the desert-river model incorporating the radial Péclet zeffect with the l-specific Péclet number. The application of the Péclet number is also supported by the positive relationship between the transpiration rate E and the fractional difference of experimental and modeled delta values ($1 - \Delta_L/\Delta_e$). A simple low-volume cryodistillation method was designed and tested. The high-throughput technique let us achieve a finer spatial resolution in isotopic distribution.

MATERIALS AND METHODS

Plant Material and Growth Conditions

Snowgum (*Eucalyptus pauciflora* Sieber ex Sprengel) plants were grown from seeds for 2 years in pots filled with a mixture of garden soil, peat, and sand (1:1:1) in glasshouses. During cultivation, the plants were repotted several times and fertilized regularly (Kristalon, N:P:K:Mg [19:6:20:3], with trace elements; Hydro Agri).

From the beginning, the plants were grown in two glasshouses, which differed in air humidity and soil water supply, under natural irradiance and controlled temperature. The atmosphere in the W glasshouse was humidified (ultrasonic Boneco 7136, Plaston) so that the RH ranged from 40% to 80%. Air humidity in the second, A glasshouse was not regulated and RH fluctuated between 20% and 50%. The RH in glasshouse W was usually 20% higher than in A. Air temperatures were roughly the same in W and A, fluctuating between 18°C and 32°C during the year. The plants in the A environment experienced shortages of water; they were watered once a day with a limited volume of water just sufficient to avoid wilting. The plants in W were generously watered two to three times per day. Tap water was used for irrigation. At the time of sampling (September, 2003), the saplings were about 1 year old, growing in 18 × 16 cm (diameter × height) pots, and approximately 60 cm (W) or 40 cm (A) high.

Leaf Sampling and Extraction of Bulk Water

Leaves of approximately equivalent age, at the beginning of maturity, were chosen in W and A plants. Around noon on a sunny day (see Table II for the atmospheric and leaf conditions before cutting the leaves), leaf discs (4 mm in diameter) were punched out with a cork borer from adjacent locations, beginning from the tip and working toward the base of the excised leaf as fast as possible. For this procedure, the leaf blade was gradually withdrawn from a plastic wrapper. The discs were immediately dropped into conical holes (3 cm^3 volume, 1 disc per hole) in a duralumin block. The holes were quickly covered

with 2 cm^3 glass vials (Supelco, catalog no. 27058) and sealed with rubber O rings, and the block placed upside down (Fig. 5). The block was heated to 80°C and the bottom of the vials was cooled to about −20°C by liquid nitrogen. After 4 h of cryodistillation, the vials were closed and stored in a freezer until the hydrogen isotope analyses were carried out. Water from up to 4 × 24 samples could be processed in a single run when using four blocks. See Figure 5 for technical details of the cryodistillation device.

The distillation process was tested by adding a known amount of water (about 50 mg) to paper tissue placed in the sample holes. Gravimetrical measurements showed that 1.1% to 2.9% (±SD 2.6%, $n = 20$) of water was lost during the distillation. A greater loss (2.9%) occurred over night than during the 4 h distillation. A significant reduction in loss was achieved when two instead of one external O ring were used. Tests on isotopic fractionation showed that water became isotopically heavier during the distillation, depending on the amount of water in the sample: 40 mg samples with a δD of −75.2‰ ± 1.0‰ were enriched to −74.1‰ ± 1.5‰ during the distillation; however, 10 mg samples were enriched, on average, to −69.0‰ ± 1.2‰ ($n = 20$, with one external O ring only).

Sampling of Atmospheric Water Vapor

Air was drawn from the glasshouse at a flow rate of less than 300 cm^3 min^{-1} by a membrane pump (KNF Neuberger, type NMP 30 KNDC) and pumped through a moisture freezing trap. The trap, a spiral glass tube, was immersed in ethanol precooled to −80°C by liquid nitrogen. At this temperature, only a negligible amount of water vapor remains in the outgoing air stream; the water vapor saturation pressure is around 0.01 Pa. The length of the submerged tube was at least 1 m. The spiral was removed from the ethanol bath after 5 min of sampling and incorporated in a closed loop for transfer of the trapped water into a glass vial suitable for the autosampler of the isotope analyzer. Apart from the glass spiral, the loop consisted of a membrane pump and a 2 mL glass vial with a conical glass insert and inlet capillary tube directing the stream of moist air into the insert (Fig. 6). The bottom of the vial was immersed in liquid nitrogen to cool the insert. The spiral was heated by a hot-air blast to accelerate the water transfer. The water evaporated from the spiral and condensed in the glass tip at a slow circulation rate of air in the loop (<300 cm^3 min^{-1}). A recovery test indicated that complete distillation of 15 mm^3 of water took 15 min. After the distillation, the vial was capped and stored in a freezer. The sampling of atmospheric moisture was repeated six times for both the W and A glasshouses during three sunny days in September, 2003.

Hydrogen Isotope Analyses

The hydrogen isotope ratio (D/H) in the collected water was assessed with a Deltaplus XL isotope ratio mass spectrometer coupled to a TC/EA high-temperature conversion elemental analyzer (both ThermoFinnigan). A 0.5 mm^3 volume of the individual water samples was taken for the analyses. The water injected into the helium carrier stream was pyrolyzed in the elemental analyzer at 1,450°C and hydrogen isotope species entered the mass spectrometer where the D/H ratio in the sample was compared with that in a working standard. This standard was calibrated against Vienna-Standard Mean Ocean Water. For monitoring the reliability of measurements, Greenland Ice Sheet Precipitation standard and OH-4 (an International Atomic Energy Agency water standard) were also included in the analyses. Relative D content was expressed as δD: $\delta D\ [‰] = (R_{sample}/R_{standard} - 1) \times 1{,}000$, where R is the respective D/H ratio.

Leaf Temperature and Transpiration Rate Measurements

Transpiration rate was measured with an open gas-exchange system (LI-6400P, LI-COR). The base or tip of an attached eucalyptus leaf (6 cm^2) was clamped in the leaf chamber with measuring conditions corresponding to ambient ones at the time of leaf sampling (leaf temperature 33°C, CO_2 concentration 370 μmol $[CO_2]$ mol^{-1} [air], irradiance by natural sunlight 600–900 μmol [photons] m^{-2} s^{-1}). The inlet air humidity was that typical for the A (45%) or humid (65%) glasshouse. Transpiration rates averaged over 30 s intervals were recorded for 10 min.

Leaf temperature was recorded during the transpiration measurements with the leaf chamber thermocouple of the LI-6400. In addition, surface temperature was measured on the bases and tips of fully expanded eucalyptus leaves in situ using a Minikin I infrared sensor and data logger (EMS). Five-second readings were averaged over the 30 s intervals. The base and tip measurements were carried out consecutively on a given leaf and repeated for six leaves of three different plants each from the A and W environments.

Statistical Treatment

In preliminary experiments, the isotopic composition of each sample was analyzed in triplicate. The SEs of δD means (SE) were no greater than 2.29‰. To reduce the expenditure of time and money for the analyses, the isotope determinations shown here were carried out only in one replicate for each sample. The transpiration rate, conductance, and temperature data are given as means ± SE from six up to 21 independent measurements. Two-way ANOVA and Student's t test were used to analyze the variations in response to the growth conditions (W versus A) and the measuring positions on the leaves (tip versus base, middle vein versus leaf edge). A probability level $\alpha = 0.05$ was used as the critical value for determining statistical significance of the differences. The Microsoft statistical packages of Excel 2000 (9.0.2812) and Statistica (StatSoft) were used.

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