The triple isotopic composition of oxygen in leaf water

A. Landais [a,*], E. Barkan [a], D. Yakir [b], B. Luz [a]

[a] *Institute of Earth Sciences, Hebrew University, Givat Ram, 91904 Jerusalem, Israel*
[b] *Department of Environmental Science and Energy Research, Weizmann Institute of Science, 76100 Rehovot, Israel*

Abstract

The isotopic composition of atmospheric O_2 depends on the rates of oxygen cycling in photosynthesis, respiration, photochemical reactions in the stratosphere and on $\delta^{17}O$ and $\delta^{18}O$ of ocean and leaf water. While most of the factors affecting $\delta^{17}O$ and $\delta^{18}O$ of air O_2 have been studied extensively in recent years, $\delta^{17}O$ of leaf water—the substrate for all terrestrial photosynthesis—remained unknown. In order to understand the isotopic composition of atmospheric O_2 at present and in fossil air in ice cores, we studied leaf water in field experiments in Israel and in a European survey. We measured the difference in $\delta^{17}O$ and $\delta^{18}O$ between stem and leaf water, which is the result of isotope enrichment during transpiration. We calculated the slopes of the lines linking the isotopic compositions of stem and leaf water. The obtained slopes in $\ln(\delta^{17}O + 1)$ vs. $\ln(\delta^{18}O + 1)$ plots are characterized by very high precision ($\sim$0.001) despite of relatively large differences between duplicates in both $\delta^{17}O$ and $\delta^{18}O$ (0.02–0.05‰). This is so because the errors in $\delta^{18}O$ and $\delta^{17}O$ are mass-dependent. The slope of the leaf transpiration process varied between 0.5111 ± 0.0013 and 0.5204 ± 0.0005, which is considerably smaller than the slope linking liquid water and vapor at equilibrium (0.529). We further found that the slope of the transpiration process decreases with atmospheric relative humidity (h) as $0.522–0.008 \times h$, for h in the range 0.3–1. This slope is neither influenced by the plant species, nor by the environmental conditions where plants grow nor does it show strong variations along long leaves.
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1. Introduction

Atmospheric oxygen is produced by photosynthesis and consumed by respiration on Earth. In addition to its participation in the biogeochemical cycle, atmospheric O_2 is also affected by photochemical reactions in the stratosphere. Concerning its isotopic composition, the ratios $^{18}O/^{16}O$ and $^{17}O/^{16}O$ in atmospheric O_2 depend on the isotopic composition of the source water from which photosynthetic O_2 is produced, i.e., seawater for the oceanic and leaf water for terrestrial productions, respectively. In both land and ocean, the isotopic composition of the O_2 produced by photosynthesis is the same as that of the source water (Guy et al., 1993; Yakir et al., 1994; Helman et al., 2005). In contrast, respiration is a fractionating process during which $^{18}O/^{16}O$ and $^{17}O/^{16}O$ increase in the remaining O_2. The respiratory fractionation is mass-dependent such that the

increase in the ratio $^{17}O/^{16}O$ is about 0.52 of the increase of the $^{18}O/^{16}O$ ratio (Luz et al., 1999; Luz and Barkan, 2000, 2005).

In addition to biological factors, the ratios $^{17}O/^{16}O$ and $^{18}O/^{16}O$ of atmospheric oxygen are affected by photochemical reactions among O_2, CO_2 and O_3 in the stratosphere. These reactions fractionate oxygen isotopes in a mass-independent way and result in equal increase in $\delta^{17}O$ and $\delta^{18}O$ of CO_2 (Thiemens et al., 1991). The same reactions result in mass-independent decrease of $\delta^{17}O$ and $\delta^{18}O$ of atmospheric O_2 and ^{17}O becomes depleted in atmospheric O_2 in comparison to O_2 affected by biological photosynthesis and biological uptake alone (Bender et al., 1994; Luz et al., 1999). The magnitude of this depletion in ^{17}O depends on the rate of oxygen cycling on Earth through mass-dependent biological fractionations and the rate of the stratospheric photochemical reactions. The magnitude of the ^{17}O depletion was used by Luz et al. (1999) and Blunier et al. (2002) to infer global biological productivity using the triple isotopic composition of atmospheric oxygen.

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* Corresponding author. Fax: +972 2 5662581.
  E-mail address: landais@vms.huji.ac.il (A. Landais).

It should be noted that variations in the extent of the ^{17}O depletion are quite small and in order to meaningfully interpret past variations observed in, for example, ice cores, it is necessary to know the exact relationships among the ratios $^{17}O/^{16}O$ and $^{18}O/^{16}O$ in all the relevant processes on Earth. In recent years, these relationships have been studied extensively in various biological processes (Angert et al., 2003; Helman et al., 2005). However, the $^{17}O/^{16}O$ vs. $^{18}O/^{16}O$ trends in leaf water—the substrate of all terrestrial photosynthesis, which is associated with large ^{18}O evaporative enrichment (Yakir, 1997)—remained unknown. Angert et al. (2003, 2004) derived a crude estimate of the mean global relationship between $^{18}O/^{16}O$ and $^{17}O/^{16}O$ during evapotranspiration from a global budget of the triple isotopic composition of atmospheric O_2 and the different effects of marine and terrestrial photosynthesis. Expressed in a $\ln(\delta^{17}O + 1) - \ln(\delta^{18}O + 1)$ plot, the slope calculated by Angert et al. (2004) is 0.512 ± 0.005 and is considerably smaller than 0.528 obtained for meteoric waters (Meijer and Li, 1998; Barkan and Luz, 2005). Because, the error in the indirect approach of Angert et al. (2004) is relatively large, it is desirable to obtain the evapotranspiration slope from direct measurements of leaf water. To this end, we studied $\delta^{17}O$ and $\delta^{18}O$ of water and triple isotope transpiration effects in leaves in field experiments in Israel and in a European survey. This is the first study of $\delta^{17}O$ in leaf water and it was possible due to the recent development of a high precision method that allows the analysis of small water samples by fluorination (Barkan and Luz, 2005).

Variations of $\delta^{18}O$ in leaf water have been studied extensively over the past 30 years (e.g., Wang et al., 1998; Roden and Ehleringer, 1999; Helliker and Ehleringer, 2000; Barbour et al., 2004, see Yakir, 1991, 1997, for reviews). In general, the isotopic composition of leaf water depends on that of plant source water and on the effect of transpiration (evaporation from leaves). It is therefore possible to determine $\delta^{17}O$ of leaf water if we know the triple isotopic composition of oxygen of the source water, $\delta^{18}O$ of leaf water and the relationship between the ratios $^{17}O/^{16}O$ and $^{18}O/^{16}O$ during transpiration.

Here, we report the results of our study on transpiration isotope effects that include variations along a leaf, daily variations, the influence of plant species and the effects of environmental and climatic conditions. We then compare our data with the current model of leaf water isotopic composition (Dongmann et al., 1974; Farquhar et al., 1989; Flanagan et al., 1991).

2. Site description and sampling

For the study of the variations of isotopic composition along a leaf, we chose a long leaf of maize (*Zea mays* sp.) sampled in Israel at the end of spring 2004 in a commercial cornfield in central Israel at midday. The leaf was divided into eight sections along its length.

To investigate the influence of short-term changes in environmental conditions, we sampled tree leaves during a diurnal cycle (6 a.m. to 7 p.m., May 2005, encompassing large variations in temperature and relative humidity). Note, that for brevity we write humidity instead of relative humidity in the following text. The selected trees were: a bougainvillea (*Bungainvillea x buttiana* Holt. & Stand.), usually found in tropical environments and that typically shows high stomatal conductances, and a coral tree (*Erythrina corallodendron* L.), a deciduous tree usually found in temperate climates. While the majority of the leaves were exposed to sun light, some leaves were in the shade. For each plant, we sampled one branch in order to provide source water.

To study the influence of plant species on the leaf transpiration relationship between $^{18}O/^{16}O$ and $^{17}O/^{16}O$, we selected common trees in the Mediterranean region that have clear difference in leaf morphology and phenology: (a) three different oaks: one deciduous—*Quercus pedunculiflora* from Turkey—and two evergreen species—*Quercus alnifolia* from Cyprus and *Quercus ilex* from Southeast France to Turkey; (b) three different cedar trees: *Cedrus libani* from Lebanon, *Cedrus brevifolia* from the Cyprus mountains and *Cedrus atlantica* from the Atlas mountains; and (c) one chestnut tree (*Castanea sative* sp.). All leaves and stems were sampled around midday in the Jerusalem Botanical Garden in April 2005.

The effect of environmental and climatic conditions was studied by midday sampling of branches and leaves of chestnut trees (*Castanea sative* sp.), at the same season (flowering season), in Jerusalem (April 2005), in the Budapest hills (Hungary, in May 2005) and in the western French countryside (Bouère, Mayenne, in May 2005). In addition, we used stem and leaf water obtained from a range of European sites participating in the WP5 of the Carboeuflux project (www.weizmann.ac.il/ESER/wp5/) that covered a large range of environmental and climatic conditions.

3. Experimental

3.1. Leaf water extraction

Extraction of leaf water was done following Wang and Yakir (2000). Leaves and stems or branches were collected in the field in 15 mL gastight vials after removing the petiole and the central vein from the leaves. Water was then quantitatively extracted by vacuum distillation at 60 °C directly from the vials. According to our tests, the distillation was completed after 3 h. In order to remove volatiles from the extracted water, we added few granules of activated charcoal and slowly stirred the water for 12 h. Tests showed that the addition of charcoal did not modify the isotopic ratio of the water.

3.2. Isotopic measurements

The analytical method for determination of the oxygen isotopic ratios of water is detailed in Barkan and Luz (2005). Summarizing, 2 μl from the extracted water samples are converted into oxygen by fluorination using CoF_3 reagent. The produced oxygen is transferred to a stainless steel tube on a collection manifold immersed in liquid helium. After all samples are processed, the manifold is warmed to room temperature and connected to a Finnigan-MAT Delta^{plus} mass spectrometer. $\delta^{17}O$ and $\delta^{18}O$ of O_2 were measured simultaneously in dual inlet mode by multi-collector mass spectrometry. As described in Barkan and Luz (2003, 2005), each mass spectrometric measurement consists of three separate runs during which the ratio of sample to reference was determined 30 times. The pressures of the sample and reference gas were balanced before each of the three runs. Such procedure was chosen in order to decrease the analytical error. The reported δ-values are averages of three runs. The analytical errors (standard error of the mean ($n = 90$) multiplied by Student's t-factor for a 95% confidence limits) in $\delta^{18}O$ and $\delta^{17}O$ are 0.004‰ and 0.008‰, respectively. All measurements were run against a working O_2 standard calibrated against V-SMOW.

4. Notation for triple isotope fractionation

For mass-dependent isotope fractionations, the equation that relates $^{17}O/^{16}O$ fractionation ($^{17}\alpha$) and $^{18}O/^{16}O$ fractionation ($^{18}\alpha$) is of the form:

$$^{17}\alpha = (^{18}\alpha)^{\lambda} \tag{1}$$

(e.g., Criss, 1999; Mook, 2000). The exponent λ is the slope of the trend line of isotopic ratios generated by mass-dependent fractionations, in a $\ln(\delta^{17}O + 1) - \ln(\delta^{18}O + 1)$ plot, where $\delta^*O = (^*R/^*R_{ref} - 1)$ (note that the factor 1000 is omitted but the δ^*O values are reported in ‰). The slope (λ) varies slightly depending on the isotope fractionation processes (kinetic or steady state) and among the different biological processes (Young et al., 2002; Angert et al., 2003; Helman et al., 2005; Luz and Barkan, 2005).

For steady state, which is generally assumed for evapotranspiration of leaf water (Dongmann et al., 1974; Flanagan et al., 1991), the magnitude of λ is expressed as (Angert et al., 2003; Luz and Barkan, 2005):

$$\lambda = \frac{\ln(^{17}\alpha_{eff})}{\ln(^{18}\alpha_{eff})} \tag{2}$$

where $^*\alpha_{eff}$ is the effective isotope fractionation factor in evapotranspiration (R_{vapor}/R_{liquid}). Note, that $^*\alpha_{eff}$ does not correspond to a single physical process, equilibrium or kinetic, but results from a combination of them, with possible effects of additional processes (e.g., isotopic exchange).

The slope of the liquid–vapor equilibrium (λ_{equil}) has been experimentally determined by Barkan and Luz (2005) and equals 0.529. This value is identical to the one calculated in Barkan and Luz (2005) from theoretically derived mass-dependent fractionations for water-isotope partitioning at liquid–vapor equilibrium (Van Hook, 1968). There are no experimental determinations of the slope of the diffusion of water vapor in air (λ_{diff}). Therefore, at present its magnitude can be estimated only theoretically. From the kinetic theory of gas diffusion (e.g., Stewart, 1975) it follows that the fractionation factor between two isotopes is proportional to their diffusion coefficients, D, ratio such that $\alpha_{diff} = (D_H/D_L)^n$, where subscripts L and H denote light and heavy isotopes, and parameter n ranges from 0 (completely turbulent transport) to 1 (molecular diffusion). In the case of diffusion of water vapor in air, Eq. 2 can be rewritten in the form:

$$\lambda_{diff} = \frac{\ln((^{17}D/^{16}D)^n)}{\ln((^{18}D/^{16}D)^n)} = \frac{\ln(^{17}D/^{16}D)}{\ln(^{18}D/^{16}D)} \tag{3}$$

The ratio of diffusivities is expressed according to the kinetic theory of gases as (e.g., Marrero and Mason, 1972):

$$\frac{D_H}{D_L} = \left(\frac{M_L(M_H + M_G)}{M_H(M_L + M_G)}\right)^{1/2}\left(\frac{\Gamma_L + \Gamma_G}{\Gamma_H + \Gamma_G}\right) \tag{4}$$

where M is molecular mass; subscript G refers to the gas in which the water vapor diffuses (air in our case); and Γ stands for the molecule collision diameter. We assume in a first approximation that the collision diameters for the different isotopes species are identical. In this case, we calculate $\lambda_{diff} = 0.518$ for the diffusion of water vapor in air. It should be noted, however, that according to Merlivat (1978) and Angert et al. (2004) the assumption about equal collision diameters may not be correct and that more experimental measurements are needed in order to determine λ_{diff} with high precision.

5. Leaf transpiration model applied to the triple isotopic composition of oxygen in water

The classical modeled expression for the leaf water enrichment above source water (Δ_e) was derived from the Craig and Gordon (1965) concept of evaporation from large bodies of water and includes leaf boundary layer effects and diffusion through stomata (Dongmann et al., 1974; Farquhar et al., 1989; Flanagan et al., 1991). Δ_e is therefore expressed as (Farquhar and Lloyd, 1993):

$$\Delta_e = \frac{R_e}{R_s} - 1 = \alpha_{L/V}^{eq}\left[\alpha_k^* + (\Delta_V - \alpha_k^* + 1)\frac{e_a}{e_i}\right] - 1 \tag{5}$$

where R_e and R_s are the oxygen isotopic ratios of leaf water at the sites of evaporation and source water, respectively; Δ_v is the isotopic composition of surrounding water vapor with respect to source water; e_a and e_i are the vapor pressure of bulk air and intercellular air spaces, calculated from humidity and temperature (von Caemmerer and Farquhar,

1981; Ball, 1987); $\alpha_{L/V}^{eq}$ is the equilibrium fractionation coefficient for liquid–vapor; and α_k^* is the kinetic fractionation coefficient factor associated with water vapor diffusion through the stomata and the boundary layer. The latter parameter, α_k^*, is defined as (Farquhar et al., 1989):

$$\alpha_k^* = \frac{(\alpha_k - 1) \times rs + (\alpha_{kb} - 1) \times rb}{rs + rb} + 1 \quad (6)$$

where α_k is the kinetic fractionation factor for water diffusion in air; α_{kb} is the kinetic fractionation factor for water diffusion in the boundary layer; and rs and rb are the stomatal and boundary layer resistances to water flux. According to Dongmann et al. (1974) and Farquhar et al. (1989), $\alpha_{kb} = \alpha_k^{2/3}$.

As shown in various studies, the traditional model for leaf water isotopic composition (Eq. 5) overestimates bulk leaf water enrichment (e.g., Allison et al., 1985; Leaney et al., 1985; Yakir et al., 1990; Flanagan et al., 1991; Wang and Yakir, 1995). This discrepancy is due to the fact that bulk leaf water (measured) is a mixture of enriched water at the evaporating surfaces (expected to obey Eq. 5, but cannot be directly measured), and depleted source water in the veins and in the leaf tissue. Recently, the isotopic mixing within the leaf tissue was described in terms of a 'Peclet' system incorporating the opposing effects of the diffusion of fractionated water from, and the convection of source water to, the evaporating surfaces (Farquhar and Lloyd, 1993). The Peclet effect is therefore a way to link the isotopic composition of the bulk leaf water to that in evaporating surfaces, which is predicted by Eq. 5. The Peclet effect is usually combined with the Craig and Gordon approach through a Peclet number, P, such that the isotopic enrichment of the, mixed, bulk leaf water, Δ_{LW}, with respect to source water (i.e., $\Delta_{LW} = R_{LW}/R_s - 1$) is (Farquhar and Lloyd, 1993):

$$\Delta_{LW} = \Delta_e \times \frac{1 - e^{-P}}{P} \quad (7)$$

where $P = LE/CD_w$, L is the effective mixing length, E is the evaporation rate, C is the molar density of water and D_w is the diffusivity of $H_2^{18}O$ or $H_2^{17}O$ in water. Note, however, that some controversy is still associated with the application of the Peclet effect to leaves, and mixing with source water in the leaf veins may require more complex parameterization (Roden and Ehleringer, 1999; Gan et al., 2002).

Taking into account the Peclet effect for calculating bulk leaf water isotopic enrichment, λ_{transp} can be expressed as:

$$\lambda_{transp} = \frac{\ln(^{17}\Delta_{LW} + 1)}{\ln(^{18}\Delta_{LW} + 1)} = \frac{\ln(^{17}\Delta_e \times {}^{17}f + 1)}{\ln(^{18}\Delta_e \times {}^{18}f + 1)} \quad (8)$$

with $f = \frac{1 - e^{-P}}{P}$.

In order to obtain λ_{transp}, we need to know the values for ^{17}P and ^{18}P. While the variations of ^{18}P among different leaves were estimated in numerous previous studies (e.g., Wang et al., 1998; Barbour et al., 2004), ^{17}P is not known. From the Peclet number definition above, it follows that

the ratio $^{17}P/^{18}P$ equals the ratio of diffusivity ratio: $^{18}D_w/^{17}D_w$. Although this ratio has not been directly determined, it is probably close to 1. Indeed, Wang et al. (1953) showed that the difference between the relative diffusion coefficients of $H_2^{18}O$, HDO and HTO in $H_2^{16}O$ is less than 10%. More recently, Mills (1973); Mills and Harris (1976), and Harry and Woolf (1980) showed that the effective diffusivities of the different water isotopic species are within 1% of the self diffusion coefficient for liquid water. Moreover, because the mechanism of diffusion in water is dominated by hydrogen bonding (Gillen et al., 1972), we do not expect significant difference between the relative diffusivities of $H_2^{17}O$ and $H_2^{18}O$ in water. Therefore, ^{17}P should be very close to ^{18}P.

In previous studies, it was shown that ^{18}P is variable in the range 0 and 1.6 (Wang et al., 1998). In this case, numerical calculations with Eq. 8 showed that λ_{transp} is not significantly influenced by the Peclet effect and can be approximated with high accuracy (better than 0.001) as:

$$\lambda_{transp} = \frac{\ln(^{17}\Delta_e + 1)}{\ln(^{18}\Delta_e + 1)} \quad (9)$$

For calculation of Δ_e (Eq. 5) the values of $^{18}\alpha_{L/V}^{eq}$ and $^{17}\alpha_{L/V}^{eq}$ were taken from Majoube (1971) and Barkan and Luz (2005). $^{17}\alpha_k$ was calculated using the given $^{18}\alpha_k$ and the theoretical value of λ_{diff} (0.518). The value of $^{18}\alpha_k$ was taken from the recent work of Cappa et al. (2003); however it should be mentioned that the effect of this parameter on λ_{transp} is small, and if one uses the previous value determined by Merlivat (1978), λ_{transp} will not change significantly. rs and rb were not measured but, according to Roden and Ehleringer (1999), rb varies between 0.3 and 5 m^2 s mol^{-1} and rs between 1 and 1000 m^2 s mol^{-1}. Sensitivity tests showed that λ_{transp} is not sensitive to rs and rb values, and the possible errors due to uncertainties of these parameters are less than 0.001. We therefore took values of 1 for rb and 4 for rs corresponding to the data of Barbour et al. (2004).

6. Results

All the experimental results are given in Tables 1–5. As discussed in Section 4, λ_{transp} is the slope of the line connecting the isotopic composition of leaf water to the isotopic composition of stem water. Values of λ_{transp} are then obtained from linear regression over the measurements of leaf and stem water. We took into account the internal mass spectrometer errors in the linear regression using the classical statistical approach of York (1969) as incorporated in the Isoplot 3.00 software (Ludwig, 2003).

The average absolute difference between leaf and stem water duplicates (Tables 1–5) is around 0.013‰ for $\delta^{17}O$ and 0.023‰ for $\delta^{18}O$ while the maximum difference in $\delta^{18}O$ is about 0.05‰ and in $\delta^{17}O$ about 0.03‰. These differences are considerably greater than the mass

Table 1
Influence of tree species on the transpiration slope

Tree species		Replicate	$\delta^{17}O$	$\delta^{18}O$	λ_{transp}
Quercus	Leaf	1	11.960	23.312	0.5170 ± 0.0003
		2	11.982	23.334	
	Stem	1	−2.642	−5.071	
		2	−2.641	−5.062	
Cedrus libani	Leaf	1	3.941	7.622	0.5183 ± 0.0006
		2	3.964	7.661	
	Stem	1	−2.524	−4.870	
		2	−2.511	−4.831	
Cedrus brevifolia	Leaf	1	2.390	4.480	0.5249 ± 0.0026[a]
		2	2.378	4.441	
	Stem	1	−3.021	−5.823	
		2	−3.030	−5.858	
Quercus alnifolia	Leaf	1	8.399	16.171	0.5201 ± 0.0003
		2	8.432	16.214	
	Stem	1	−3.330	−6.441	
		2	−3.310	−6.410	
		3	−3.313	−6.409	
Cedrus atlantica	Leaf	1	2.893	5.553	0.5203 ± 0.0017
		2	2.872	5.518	
	Stem	1	−2.580	−4.960	
		2	−2.559	−4.941	
		3	−2.578	−4.950	
Quercus ilex	Leaf	1	11.158	21.599	0.5195 ± 0.0008
		2	11.155	21.566	
	Stem	1	−3.171	−6.092	
		2	−3.148	−6.048	
		3	−3.146	−6.063	
Chestnut	Leaf	1	9.293	17.971	0.5185 ± 0.0011
		2	9.277	17.961	
	Stem	1	−2.567	−4.968	
Average	Stem	2	−2.587	−4.997	0.5190 ± 0.0010

$\delta^{17}O$ and $\delta^{18}O$ are in ‰ vs. V-SMOW. All samples were collected on the same day at temperature 25 °C and humidity 32%. The isotopic composition of the irrigation water is −2.79‰ and −5.36‰ for $\delta^{17}O$ and $\delta^{18}O$, respectively.

[a] This outlier point was not included in the calculated average.

Table 2
Variation of the transpiration slope along a maize leaf

Sample	Replicate	$\delta^{17}O$	$\delta^{18}O$	λ_{transp}
1 (Leaf base)	1	5.529	10.638	0.5204 ± 0.0005
	2	5.521	10.622	
2	1	6.178	11.917	0.5192 ± 0.0004
	2	6.171	11.932	
3	1	8.186	15.808	0.5196 ± 0.0003
	2	8.174	15.782	
4	1	8.379	16.170	0.5199 ± 0.0002
	2	8.362	16.139	
5	1	8.692	16.788	0.5196 ± 0.0004
	2	8.700	16.802	
6	1	8.630	16.672	0.5196 ± 0.0003
	2	8.621	16.651	
7	1	10.031	19.408	0.5192 ± 0.0003
	2	10.039	19.424	
8 (Leaf top)	1	11.338	22.004	0.5181 ± 0.0002
	2	11.352	22.038	
Source water	1	−2.370	−4.561	
	2	−2.361	−4.546	
Average				0.5195 ± 0.0005

$\delta^{17}O$ and $\delta^{18}O$ are in ‰ vs. V-SMOW. The temperature was 22 °C and humidity 40%.

spectrometer errors, and at the first sight it is unclear how very small errors in the λ_{transp} (0.0015 or less) can be achieved.

Fig. 1 depicts the differences in $\delta^{17}O$ and $\delta^{18}O$ between pairs of replicate analyses. It shows that the shifts in $\delta^{18}O$ and $\delta^{17}O$ within pairs of duplicates are not independent: when $\delta^{18}O$ increases, there is a parallel increase, but in general of a smaller magnitude, in $\delta^{17}O$. Such a result is not surprising because both $\delta^{17}O$ and $\delta^{18}O$ are derived from the same water sample prepared to yield O_2 gas which is then measured on the mass spectrometer. We thus expect that any bias due to sample handling during preparation and measurement will be mass-dependent. In this case, any shift in $\delta^{17}O$ is expected to be about 0.52 that of $\delta^{18}O$ and Fig. 1 confirms such an expectation. As a result, for transpiration slopes, which are always close to 0.52, the $\delta^{17}O$ and $\delta^{18}O$ errors tend to cancel out and the derived λ_{transp} values are very robust as is indeed evident in Tables 1–5.

6.1. Soil to leaf effect

Many studies have already shown that the difference in $\delta^{18}O$ and δD between irrigation and stem water is small compared to the isotopic enrichment between stem and leaf water (e.g., Bariac et al., 1994; Wang et al., 1998; Ohte et al., 2003). Our new measurements of $\delta^{17}O$ and $\delta^{18}O$ for different tree species (Table 1) lead to the same conclusion and confirm that there are no changes in the isotopic composition of oxygen of water during uptake and transport in roots and stems (Gonfiantini et al., 1965; Wershaw et al., 1970). They also confirm that the evaporation from soil is a second order effect in the water isotopic enrichment between source and leaf (e.g., Saurer et al., 1997; Yakir and da Silveira Lobo Sternberg, 2000). As a consequence, in cases where we did not have measurements of stem water we used the results from soil water instead.

6.2. Variations among different species in the same site

The results for the survey over different tree species are given in Table 1. While there is a large variety of isotopic enrichments of leaf water with respect to stem water, λ_{transp} varies only between 0.517 and 0.520, except for *Cedrus brevifolia* ($\lambda_{transp} = 0.5249$), which appears to be an outlier when looking at our complete set of data. Further research is needed in order to understand the origin of this exceptionally high slope. Excluding this unexpected result, the average λ_{transp} is 0.5190 ± 0.0010.

6.3. Internal leaf variations

Table 2 presents our results of leaf water $\delta^{17}O$, $\delta^{18}O$ and λ_{transp} along the long maize leaf. Whereas $\delta^{18}O$ increases sharply along the leaf, the variations of λ_{transp} remain very small and the average λ_{transp} is 0.5195 ± 0.0005.

Table 3
Diurnal variations of the transpiration slope

Time	T (°C)	RH (%)	Replicate	Bougainvillea			Coral tree		
				$\delta^{17}O$	$\delta^{18}O$	λ_{transp}	$\delta^{17}O$	$\delta^{18}O$	λ_{transp}
10:00 (Stem)	26	40	1	−2.068	−3.997		−1.840	−3.550	
			2	−2.051	−3.961		−1.820	−3.520	
6:15	21	75	1	3.182	6.211	0.5148 ± 0.0008	3.825	7.431	0.5160 ± 0.0007
			2	3.197	6.234		3.801	7.393	
7:15	22	60	1	3.759	7.288	0.5169 ± 0.0007	5.752	11.169	0.5166 ± 0.0006
			2	3.742	7.252		5.747	11.157	
8:15	26	40	1	2.439	4.684	0.5194 ± 0.0009	4.578	8.836	0.5183 ± 0.0007
			2	2.431	4.668		4.562	8.823	
10:45	26	41	1	7.819	15.118	0.5191 ± 0.0005	6.438	12.439	0.5184 ± 0.0005
			2	7.802	15.083		6.421	12.422	
13:45	31	33	1	5.256	10.169	0.5183 ± 0.0002	4.353	8.430	0.5175 ± 0.0007
			2	5.244	10.121		4.345	8.409	
16:30	27	50	1	8.321	16.172	0.5166 ± 0.0004	5.613	10.890	0.5169 ± 0.0006
			2	8.328	16.187		5.608	10.879	
18:45	23	60	1	5.182	10.043	0.5170 ± 0.0006	5.849	11.340	0.5173 ± 0.0006
			2	5.198	10.079		5.832	11.304	
Average						0.5174 ± 0.0013			0.5173 ± 0.0007

$\delta^{17}O$ and $\delta^{18}O$ are in ‰ vs. V-SMOW. The leaves were picked at 8:15 and 13:45 from a shaded part of the canopy.

Table 4
Influence of environmental conditions on the slope of transpiration

Locations	T (°C)	RH (%)		Replicate	$\delta^{17}O$	$\delta^{18}O$	λ_{transp}
Jerusalem	25	32	Leaf	1	9.293	17.971	0.5185 ± 0.0011
				2	9.277	17.961	
			Stem	1	−2.567	−4.968	
				2	−2.587	−4.997	
Bouère	18	50	Leaf	1	3.660	7.087	0.5177 ± 0.0007
				2	3.651	7.070	
			Stem	1	−2.222	−4.278	
				2	−2.231	−4.291	
Budapest	22	45	Leaf	1	5.538	10.779	0.5180 ± 0.0004
				2	5.520	10.742	
			Stem	1	−4.281	−8.188	
				2	−4.287	−8.201	
Average							0.5183 ± 0.0004

$\delta^{17}O$ and $\delta^{18}O$ are in ‰ vs. V-SMOW for different chestnut trees.

6.4. Variations during the diurnal cycle

Table 3 displays the results associated with the diurnal cycle experiment. The leaf exposition (direct sun light or shade) has no significant influence on λ_{transp}. The two trees show similar evolution of λ_{transp} and it remains roughly constant during the day with average values of 0.5174 ± 0.0013 and 0.5173 ± 0.0007. However, the slope associated with samples taken early in the morning or late in the afternoon, with higher humidity, appears to be lower than at midday with lower humidity.

6.5. Same species at different geographic locations

In Table 4, we give the $\delta^{17}O$, $\delta^{18}O$ and λ_{transp} of stem and leaf water of chestnut trees sampled at three different

sites. As in the previous studies, the slope is very similar in the three sites despite the large variations in leaf $\delta^{18}O$. The average λ_{transp} is 0.5183 ± 0.0004.

6.6. Different species and different geographic locations

We measured $\delta^{17}O$ and $\delta^{18}O$ in leaf and stem water on a large European survey encompassing different plant species, environmental and climatic conditions (Table 5). Mostly, these data were associated with high humidity and enlarged our previous studies focused on dry climate. As can be seen from Table 5, there is a general tendency of lower λ_{transp} when humidity increases. In some cases, samples of stem or irrigation water were not available and only $\delta^{18}O$ of meteoric water was known. In these situations, we estimated $\delta^{17}O$ of source water from its $\delta^{18}O$

Table 5
European survey

Location	Date of sampling	RH (%)		Replicate	$\delta^{17}O$	$\delta^{18}O$	λ_{transp}
Denmark							
	14/08/02	95	Leaf	1	−0.502	−0.882	0.5111 ± 0.0013
				2	−0.493	−0.865	
			Stem	1	−3.615	−6.959	
				2	−3.600	−6.936	
Germany							
	2001–2002		Soil		−4.07	−7.80	
	07/10/01	Sunny—18:30	Leaf-1	1	0.114	0.289	0.5164 ± 0.0012
				2	0.106	0.271	
	07/10/01	Sunny—20:30	Leaf-2	1	1.992	4.010	0.5131 ± 0.0008
				2	2.008	4.032	
	30/07/02	Sunny—18:00	Leaf	1	6.509	12.650	0.5179 ± 0.0005
				2	6.500	12.632	
	12/08/01	Sunny—18:30	Leaf	1	4.380	8.590	0.5157 ± 0.0006
				2	4.381	8.593	
	19/9/02	Rainy—17:00	Leaf	1	3.102	6.105	0.5156 ± 0.0007
				2	3.118	6.138	
	29/10/02	Sunny—16:00	Leaf	1	1.052	2.064	0.5185 ± 0.0010
				2	1.067	2.097	
Italy							
	2001–2002		Soil		−3.75	−7.21	
	23/07/02	88	Leaf-1	1	2.671	5.228	0.5160 ± 0.0008
				2	2.677	5.240	
	23/07/02	88	Leaf-2	1	3.338	6.554	0.5147 ± 0.0007
				2	3.350	6.586	
	27/08/02	Rainy	Leaf	1	0.051	0.160	0.5148 ± 0.0013
				2	0.048	0.156	
	30/09/02	60	Leaf	1	2.491	4.857	0.5169 ± 0.0008
				2	2.479	4.832	
The Netherlands							
	30/07/02	74	Leaf	1	3.227	6.330	0.5165 ± 0.0029
				2	3.232	6.340	
			Stem	1	−3.871	−7.428	
				2	−3.880	−7.410	
Scotland							
	3/07/01	93	Leaf	1	1.950	3.801	0.5129 ± 0.0008
				2	1.941	3.782	
			Stem	1	−3.618	−7.039	
				2	−3.603	−7.024	
	9/07/01	88	Leaf	1	1.368	2.784	0.5135 ± 0.0009
				2	1.351	2.757	
	2001		Soil	1	−3.417	−6.524	
				2	−3.404	−6.498	
	13/08/01	97	Leaf	1	0.220	0.522	0.5156 ± 0.0011
				2	0.211	0.501	
	2001		Soil	1	−3.417	−6.524	
				2	−3.404	−6.498	
	10/09/01	90		1	0.300	0.680	0.5153 ± 0.0011
				2	0.284	0.648	
	2001		Soil	1	−3.417	−6.524	
				2	−3.404	−6.498	
Portugal							
	26/09/02	40	Leaf	1	6.619	12.733	0.5185 ± 0.0006
				2	6.612	12.729	
			Stem	1	−0.920	−1.841	
				2	−0.931	−1.860	

$\delta^{17}O$ and $\delta^{18}O$ are in ‰ vs. V-SMOW. When the isotopic composition of stem water or source (soil) water could not be measured, their $\delta^{17}O$ were estimated from $\delta^{18}O$ (based on published data) and the slope of the meteoric water line (see text). When humidity was not measured, we indicate the regional weather of the day and the sampling time if it was known.
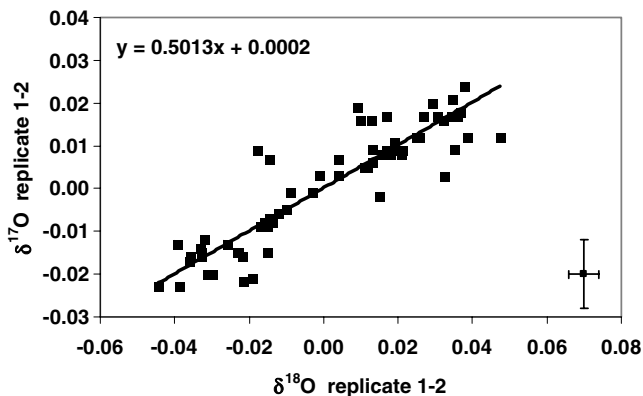
Fig. 1. A plot showing the dependence of errors due sample preparation in $\delta^{17}O$ on those in $\delta^{18}O$. The internal errors due to mass spectrometer measurements (0.004‰ for $\delta^{18}O$ and 0.008‰ for $\delta^{17}O$) are indicated in the corner. The slope of about 0.5 demonstrates that the errors result from mass-dependent fractionation during sample handling and preparation.

using the slope of the meteoric water line (0.528, Meijer and Li, 1998).

6.7. Correlation between relative humidity and λ_{transp}

Our results show that λ_{transp} remains very stable within a leaf and among different species and environments. However, variations in the ambient humidity seem to influence λ_{transp}. Fig. 2 presents the empirical relationship between λ_{transp} and humidity from our complete set of data. Over the humidity, h, between 32% and 100% λ_{transp} decreases linearly as h increases:

$$\lambda_{\text{transp}} = (-0.0078 \pm 0.0026) \times h + 0.5216 \pm 0.0008 \quad (10)$$

Eq. 10 was obtained by using Isoplot 3.00 (Ludwig, 2003), which takes into account uncertainties in both relative humidity and λ_{transp}.
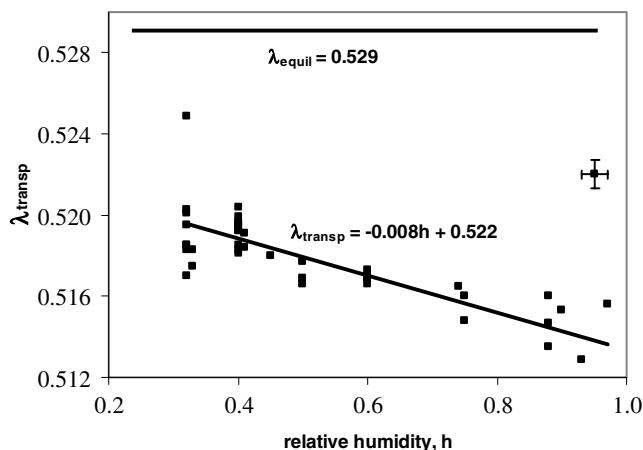
In summary, all the directly derived values of λ_{transp} are significantly larger than the crude estimate 0.512 of Angert et al. (2004), but are clearly smaller than the equilibrium slope between liquid water and vapor (0.529, Barkan and Luz, 2005). This important difference was first pointed out by Angert et al. (2004) who also discussed the potential of the $\delta^{17}O$ and $\delta^{18}O$ pair as a new tracer in hydrology.

7. Discussion

7.1. The dependence of λ_{transp} on relative humidity

The influence of humidity on λ_{transp} can be tentatively explained based on Section 5 above, and the assumption that the relationship between $\delta^{17}O$ and $\delta^{18}O$ in bulk leaf water can be described by a simple expression of isotopic enrichment in leaf water (Eqs. 5 and 8). As follows from Eq. 5, the model includes a combination of equilibrium liquid–vapor fractionation, and water vapor diffusion fractionation in air. These processes are associated with water vapor flux in both directions: from leaf to atmosphere and *vice versa*. This results in an effective isotopic exchange between leaf water and atmospheric water vapor, which is weighted by relative humidity.

The interplay among these processes is shown conceptually in Fig. 3. First, the effect of equilibrium fractionation is to drive the isotopic composition of the remaining water in the leaf from stem water (A) to point B along the equilibrium line (slope 0.529). Second, for a relative humidity less than 100%, diffusion transport fractionation modifies this water isotopic composition: it goes along a line (BC) of slope 0.518. The kinetic enrichment due to diffusion transport fractionation is greater under low humidity. As a consequence, the intermediate isotopic composition of the

Fig. 2. Variation of λ_{transp} with respect to relative humidity h. The uncertainty in h is ±0.02 and the average uncertainty in λ_{transp} is ±0.0007 (both uncertainties are indicated on the right of the figure). The outlier point ($\lambda_{\text{transp}} = 0.5249$ for $h = 0.32$) was not considered in the linear regression. We display the equilibrium slope (λ_{equil}) for comparison with λ_{transp}.
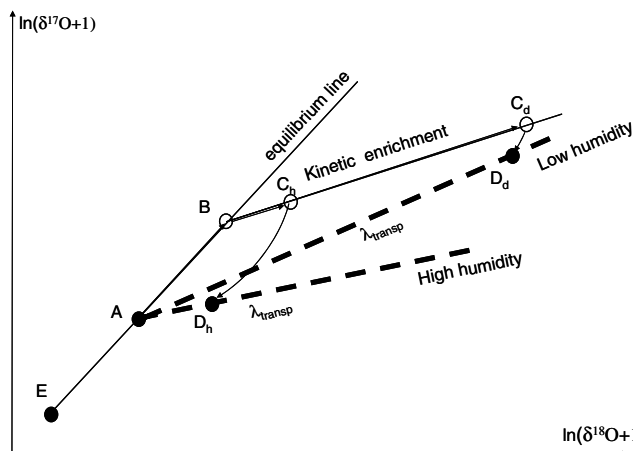

Fig. 3. Schematic representation of fractionation during transpiration from stem water (A) to leaf water (D) for high and low air humidity of isotopic composition E. The empty circles stand for conceptual intermediate states during the transpiration process. (A–B) Fractionation due to equilibrium. (B–C) Fractionation due to diffusion transport. (C–D) Isotopic exchange with air moisture (along a slightly curved line, see text). (A–D) Resulting transpiration line.

remaining water in the leaf is C_h for high humidity and C_d for low humidity. Finally, the effect of isotopic exchange with the surrounding atmospheric vapor is presented through the arrows $(C_h D_h)$ and $(C_d D_d)$ for high and low humidity, respectively. Arrows CD represent mixing between leaf water, that would have been generated at $h = 0$, and atmospheric moisture. Note, that in a $\ln(\delta^{17}O + 1) - \ln(\delta^{18}O + 1)$ plot a mixing line between two reservoirs is slightly curved (for details see Luz and Barkan, 2005). The length of the arrows increases with relative humidity ($C_h D_h$ is longer than $C_d D_d$) and with the difference in isotopic composition between atmospheric moisture (E) and stem water. In cases where this difference is large enough, the situation is the one depicted on Fig. 3: point D_h, i.e., the final leaf water isotopic composition for high humidity, lies below point D_d, i.e., the final leaf water isotopic composition for low humidity. As a result, λ_{transp} shows a decrease with increasing humidity.

7.2. Comparison with the model of isotopic enrichment in leaf water

The derivation of the Craig and Gordon model (Eq. 5) has been extensively used for the modeling of leaf water isotopic composition ($\delta^{18}O$, δD). An interesting perspective is hence to study how the model outputs can be quantitatively compared with our experimental determination of the relationship between $\delta^{17}O$ and $\delta^{18}O$ in leaf water, namely λ_{transp}.

Humidity, temperature and source water isotopic composition were measured during the sampling. For the water vapor isotopic composition, long-term measurements (Jacob and Sonntag, 1991) show that the difference between the mean annual $\delta^{18}O$ of precipitation and water vapor $\delta^{18}O$ varies seasonally between 6‰ and 15‰. Fig. 4 depicts the variations of the modeled λ_{transp} with respect to the $\delta^{18}O$ difference between water vapor and source water and for different values of humidity (30% or

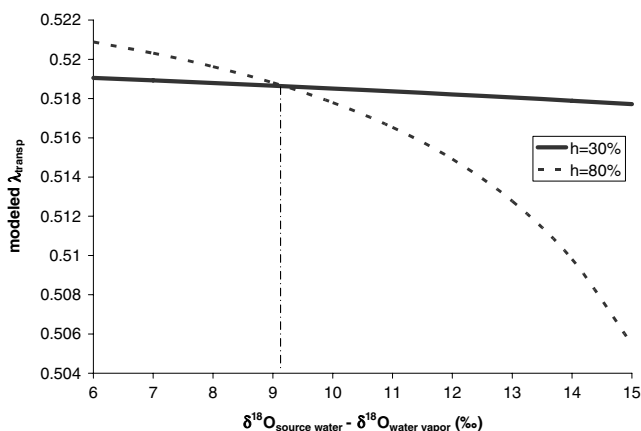Fig. 4. Evolution of the modeled λ_{transp} with respect to the difference in $\delta^{18}O$ between the source water and the water vapor using the derivation of the Craig and Gordon approach for leaf water isotopic composition (Eq. 5) for relative humidity of 30% and 80%.

80%). For low humidity, the variations of the water vapor isotopic composition do not significantly affect the modeled λ_{transp} which is equal to 0.519 ± 0.001‰. Such a result is in excellent agreement with our data. Note however, that our modeled λ_{transp} is highly dependent on the values of λ_{diff} and λ_{equil}. While λ_{equil} was precisely determined by Barkan and Luz (2005), experimental data are needed to check the theoretical estimate of λ_{diff}.

For high humidity, λ_{transp} decreases from 0.521 to 0.504 when the difference in $\delta^{18}O$ between water vapor and source water increases from 6‰ to 15‰. Such a huge variability of λ_{transp} was not observed in our data. Unfortunately, the water vapor isotopic composition was not measured during the sampling so that no clear conclusion can be reached from the comparison between our data and the model for high humidity.

Finally, while the sensitivity tests discussed here suggest that our observation of λ_{transp} at high humidity is perhaps not a universal response, in light of the scarcity of water vapor isotopic measurements, we assume that our empirical relationship presented on Fig. 2, over a large range of environmental climatic and species variations, probably represents a global perspective. It should also be emphasized that relatively large variations of λ_{transp} at high humidity are associated with low isotopic enrichment of leaf water, and therefore with a relatively small effect on the global budget of triple isotopes of oxygen in the atmosphere.

8. Conclusions

We have measured, for the first time, the relationship between $^{17}O/^{16}O$ and $^{18}O/^{16}O$ during the transpiration of leaf water (λ_{transp}). This relationship reflects the fractionation effects of liquid–water equilibrium, water vapor diffusion in air and isotopic exchange with surrounding water vapor. We have shown that because stem water is close in isotopic composition to meteoric or irrigation water, λ_{transp} can be used to estimate leaf water isotopic composition when meteoric water isotopic composition is known. It was determined that λ_{transp} does not depend on the location along a leaf, on the plant species, and on the environmental conditions in which trees grow. However, λ_{transp} slightly depends on humidity, h, as: $\lambda_{transp} = 0.522 - 0.008 \times h$.

Overall, the slopes we derived for transpiration are considerably smaller than the liquid–vapor equilibrium slope 0.529. This difference, as pointed out by Angert et al. (2004), is expected to affect the isotopic composition of meteoric waters, and thus the $\delta^{17}O$ and $\delta^{18}O$ pair should be a useful tracer of evaporation effects in hydrology.

We have also shown that λ_{transp} can be predicted with a simple model for leaf water isotopic composition for dry conditions, but further study is needed to verify the validity of this model for humid conditions and to check the theoretical determination of the slope associated with water vapor diffusion in air (λ_{diff}). Finally, our first experimental data on λ_{transp} can be used for global modeling of the triple isotope composition of atmospheric O_2.

Acknowledgments

Associate Editor: Clark M. Johnson

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