ENVIRONMENTAL INFLUENCES ON THE STABLE HYDROGEN AND OXYGEN ISOTOPE COMPOSITION OF PLANT LEAF WATER

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Abstract

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A model of isotopic enrichment is applied to leaf transpiration. The authors describe the conditions that are necessary for the model to be used to obtain estimates of the leaf–air water vapour pressure gradient. Experiments have been conducted to test the factors influencing the stable isotopic composition of leaf water under field and controlled environment conditions. The data indicate that there was close agreement between the isotopic enrichment predicted by the model and that measured in leaves exposed to a range of water vapour pressure gradients in a controlled environment leaf chamber. The model, however, slightly overestimates the degree of isotopic enrichment observed in *Phaseolus vulgaris* leaves. This discrepancy between the modelled and observed data may in part be related to the presence of unfractionated xylem water in leaf vein tissue. The data from field experiments suggest that leaf water is not at isotopic steady state under natural conditions. If leaf water isotopic composition does not normally reach steady state under field conditions, it may be inaccurate to apply the evaporative enrichment model to studies which use the δD and $\delta^{18}O$ values of plant cellulose to infer long term changes in the leaf–air water vapour pressure gradient.

1. INTRODUCTION

Measurement of the carbon isotopic composition of plant tissue has become an important technique for assessing the water use efficiency of plants [1]. A major assumption, however, in using the carbon isotopic composition of leaf tissue to estimate water use efficiency is that the leaf–air water vapour pressure gradient remains constant [1]. Estimates of water use efficiency based on $\delta^{13}C$ values could be improved if long term information about the leaf–air water vapour pressure gradient were also available.

It has been suggested that measurements of the hydrogen and oxygen isotopic composition of leaf cellulose could be used to infer the long term integrated value

of the leaf–air water vapour pressure gradient [1, 2]. The rationale for this suggestion is as follows. While there is no fractionation of isotopes during water uptake by plants, fractionation does occur during transpiration. The lighter isotopes of water escape from the leaf more rapidly during transpiration so that leaf water becomes enriched in heavy isotopes. The extent of the enrichment of heavy isotopes in leaf water depends on the leaf–air water vapour pressure gradient, as will be shown below. The isotopic composition of hydrogen and oxygen in cellulose synthesized by a leaf is influenced by the isotopic composition of leaf water [3]. Leaf cellulose should contain, therefore, long term integrated information about the short term changes in leaf water enrichment that result because of variation in the leaf–air vapour pressure gradient.

Several previous studies have examined the effect of environmental changes on the enrichment of stable isotopes in leaf water [2, 3]. A number of uncertainties remain, however, about the application of leaf water isotopic enrichment to studies of the leaf–air water vapour pressure gradient [3, 4]. In this paper we outline the theory for isotope effects during transpiration and show how leaf water isotopic enrichment is related to the leaf–air vapour pressure gradient. In addition, we describe experiments we have done to test a model of the factors influencing leaf water isotopic composition under field and controlled environment conditions.

2. ISOTOPE EFFECTS DURING TRANSPIRATION

There are two isotope effects during transpiration: (a) an equilibrium effect resulting from the phase change from liquid water to water vapour, and (b) a kinetic effect caused by the different diffusion rates of the light and heavy isotopes. A model of isotopic fractionation during evaporation was originally developed by Craig and Gordon [5]. Applied to leaf water isotopic composition the model can be expressed in the following form [2]:

$$R_L = \alpha_* \left[\alpha_k R_S \left(\frac{e_i - e_s}{e_i} \right) + \alpha_{kb} R_S \left(\frac{e_s - e_a}{e_i} \right) + R_A \frac{e_a}{e_i} \right] \quad (1)$$

where R is the molar ratio of the heavy and light isotopes (i.e. D/H or $^{18}O/^{16}O$) and the subscripts L, S and A refer to leaf water, stem water and atmospheric water vapour respectively; e is the partial pressure of water vapour and the subscripts i, s and a refer to the leaf intercellular air spaces, the leaf surface and the ambient air respectively; α_* is the equilibrium fractionation factor, which has been measured by Majoube [6]; α_k is the kinetic fractionation factor, which is determined by the relative rates of diffusion of the light and heavy isotopes in air (the values for α_k are: H/D = 1.025 and $^{16}O/^{18}O$ = 1.028 [2, 7]); α_{kb} is the ratio of the diffusion rates of the light and heavy isotopes in a boundary layer (the values for α_{kb} are:

H/D = 1.017 and $^{16}O/^{18}O$ = 1.019 [2]). Ignoring the influence of boundary layer effects, Eq. (1) can be modified to:

$$R_L = \alpha_* \left[\alpha_k R_s \left(\frac{e_i - e_a}{e_i} \right) + R_A \frac{e_a}{e_i} \right] \quad (2)$$

Previous studies have attempted to include boundary layer effects by adjusting the value of α_k in Eq. (2) [2, 3]. Such a procedure is incorrect since diffusion through the stomatal pore is a molecular process [8] and turbulence is only encountered outside the stomatal pore. For both (1) and (2), the model predicts the isotopic composition of leaf water at steady state.

3. BOUNDARY LAYER EFFECTS ON LEAF WATER ENRICHMENT

The influence of boundary conditions on leaf water isotopic enrichment can be calculated by comparing values predicted by Eqs (1) and (2). Equation (2), which does not include boundary layer effects, predicts more isotopic enrichment than does Eq. (1). The magnitude of the difference between the predictions of the two equations depends on leaf temperature, the leaf–air vapour pressure gradient and the particular element being considered (Fig. 1).

For D/H ratios, the largest difference between Eqs (1) and (2) is approximately equal to the precision of the measurement techniques (±2‰) when the boundary layer conductance is twice as large as the stomatal conductance (Fig. 1). The maximal difference between the two equations increases to approximately 5‰, however, when the boundary layer conductance is reduced to half the stomatal conductance. Such a low boundary layer conductance would be uncommon under field conditions. The influence of boundary layer conductance on deuterium enrichment in leaf water appears to be negligible, therefore.

Boundary layer effects are quantitatively more important when considering $^{18}O/^{16}O$ ratios. Even when the boundary layer conductance is twice the stomatal conductance, there may be approximately a 3‰ difference between the values predicted by Eqs (1) and (2), when the leaf–air vapour pressure difference is large (Fig. 1). The maximal difference between the two equations increases to approximately 6‰ when the boundary layer conductance is reduced to half the stomatal conductance. A slight overestimation of at least 3‰ should be expected, therefore, when using Eq. (2) to predict ^{18}O enrichment in leaf water under field conditions.

Boundary layer conditions have different effects on hydrogen and oxygen isotopic compositions because of differences in the relative magnitudes of the kinetic and equilibrium fractionation factors for the two elements. For hydrogen the equilibrium fractionation factor is quantitatively more important than the kinetic fractionation factor (H/D, α_k = 1.025, α_* = 1.079 at 25°C). The opposite is true

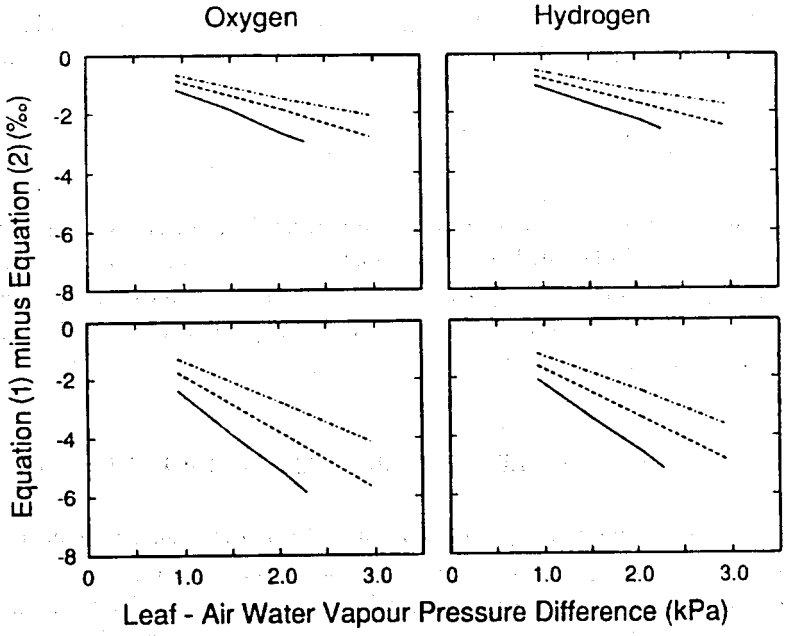

FIG. 1. The influence of boundary layer conditions on leaf water isotopic enrichment. The ordinate shows the difference between leaf water isotopic compositions predicted by Eqs (1) and (2). Calculations were done for different leaf temperatures: 20°C (——), 25°C (– – –) and 30°C (–·–·–). For both oxygen and hydrogen, the top panel shows calculations done with a boundary layer conductance that is twice the stomatal conductance and the bottom panel shows calculations done with a boundary layer conductance that is half the stomatal conductance.

for oxygen, where the kinetic fractionation factor is quantitatively more important ($^{16}O/^{18}O$, $\alpha_k = 1.028$, $\alpha_* = 1.0136$ at 25°C). Since the influence of boundary layer effects is governed by the magnitude of the kinetic fractionation factor, the oxygen isotopic composition of leaf water should be more sensitive to boundary layer conditions.

4. TESTS OF THE MODEL OF LEAF WATER ISOTOPIC ENRICHMENT

Comparisons have been made of the isotopic enrichment predicted by Eqs (1) and (2) and the measured leaf water isotopic composition. Measurements of leaf water isotopic enrichment were made under a range of conditions: (a) while the leaf environment was held constant in an environmentally controlled gas exchange chamber, and (b) at midday under natural conditions in the field.

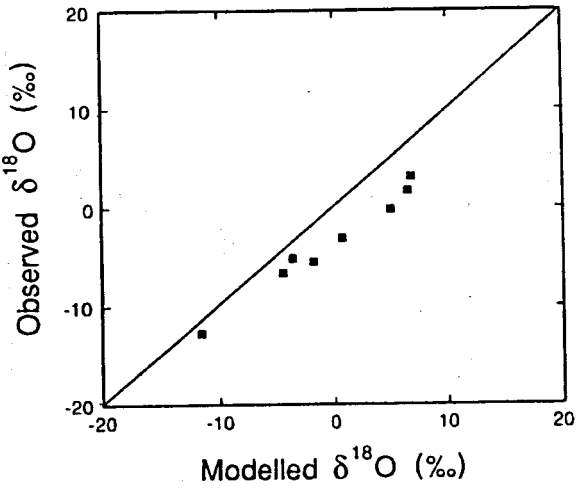

FIG. 2. Comparison of the modelled and observed oxygen isotope compositions of water from Phaseolus vulgaris *leaves maintained under a range of leaf-air water vapour pressure gradients from 1.0 to 3.0 kPa in a controlled environment gas exchange chamber. Environmental conditions were: leaf temperature 30°C, light intensity 1400 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (400–700 nm), boundary layer conductance to water loss 2 $mol \cdot m^{-2} \cdot s^{-1}$, and $\delta^{18}O_{stem} = -15.8‰$. Atmospheric water vapour was varied by using water with different oxygen isotope compositions in the humidifying device of the gas exchange system. The $\delta^{18}O$ value of atmospheric water vapour ranged from -27.6 to $-17.4‰$. Modelled values were calculated using Eq. (1). Isotopic compositions are expressed relative to SMOW. (Data of L.B. Flanagan, J.P. Comstock and J.R. Ehleringer, unpublished.)*

There is close agreement between the enrichment predicted by Eq. (1) and that measured in leaves exposed to a range of water vapour pressure gradients in a gas exchange chamber (Fig. 2). The model, however, slightly overestimates the degree of isotopic enrichment observed in bean leaves. There are two possible reasons for the discrepancy between the observed and modelling enrichments. The first reason is that water extracted from leaves by vacuum distillation removes water from leaf vein tissue in addition to water from leaf mesophyll cells. Vein water should be unfractionated and have the same isotopic composition as root and stem water. This would cause the observed isotopic enrichment of the vacuum extracted leaf water to fall below that predicted by Eq. (1). The extent of the discrepancy would depend on the relative amounts of vein water and mesophyll water that are present in a leaf.

A second reason for the lack of complete agreement between the modelled and measured data in Fig. 2 is that leaf water may not have reached steady state isotopic enrichment. As outlined in the theory section, Eqs (1) and (2) assume that leaf water isotopic composition has reached steady state. Subsequent experiments have shown

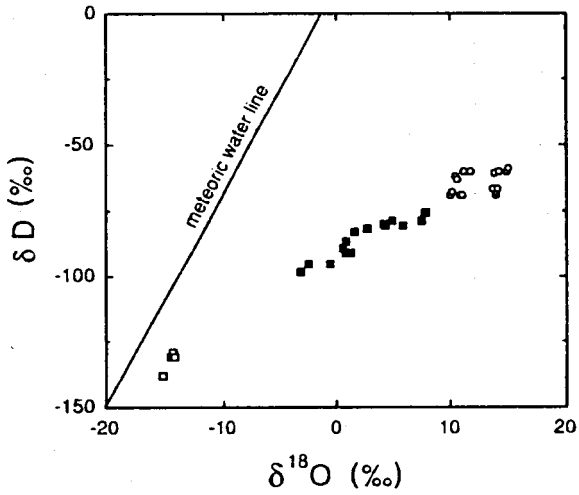

FIG. 3. The relationship between the hydrogen and oxygen stable isotope composition of leaf water at midday under field conditions. The solid squares represent measurements made on leaf water extracted from four species: Amaranthus retroflexus, Chenopodium album, Helianthus annulus *and* Kochia. *The open circles are the leaf water isotopic values modelled using Eq. (2). The open squares are measurements of the stem water isotopic composition. (Also shown is the meteoric water line.) Average environmental conditions were: leaf temperature 26°C, air temperature 25.5°C, relative humidity 25%, light intensity 1700 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (400–700 nm), atmospheric water vapour $\delta D = -202‰$, $\delta^{18}O = -26.6‰$. Isotopic compositions are expressed relative to SMOW. (Data of L.B. Flanagan, J.F. Bain and J.R. Ehleringer, unpublished.)*

that under conditions similar to those used for the experiment of Fig. 2, the isotopic composition of transpired water is equal to that of the stem water, indicating that isotopic steady state had been reached [2]. Approximately 40 min to 1 h of constant environmental conditions are required for steady state to be reached, however.

In the field, environmental conditions may change too rapidly during the course of a day for isotopic steady state to be reached. A comparison of the modelled and observed leaf water isotopic compositions for plants growing under field conditions is shown in Fig. 3. During the approach to steady state, leaf water values should fall on a line connecting the stem water isotopic composition and the modelled steady state isotopic composition, in a plot of δD and $\delta^{18}O$ [9]. Our data indicate that the observed leaf water isotopic compositions were not at steady state (Fig. 3).

During the approach to steady state, the observed isotopic composition of leaf water can be modelled using Eq. (3) [9]:

$$\delta_{leaf} = \delta_{ss} f + \delta_{stem}(1 - f) \quad (3)$$

where δ_{leaf} is the measured isotopic composition of a leaf, δ_{ss} is the isotopic composition of leaf water at steady state and δ_{stem} is the isotopic composition of stem water. The parameter f describes how close the observed leaf water is to isotopic steady state. Calculations of f for the data in Fig. 3 range between 0.50 and 0.75. Biological factors that will influence the time required to reach isotopic steady state are the transpiration rate and the total water volume of a leaf [2, 9].

5. WATER STRESS EFFECTS ON LEAF WATER ISOTOPIC ENRICHMENT

The effect of mild water stress on leaf water isotopic enrichment was studied in *Cornus stolonifera* plants that were grown in a glasshouse. Reduced water applications to one set of plants over a three day period resulted in a reduction of midday water potential and an increase in leaf temperature (Table I). Both the hydrogen and the oxygen isotopic composition of stem water were enriched in the stressed plants (Table I). The enriched stem water values were likely a result of increased evaporation of water from the pots of the stressed plants.

The δD value of leaf water in stressed plants was 5‰ higher than that of the control plants (Table I). The difference in leaf water values between treatments, however, can be explained by the enriched stem water values in the stressed plants (Table I). For the oxygen isotopic composition of leaf water, there was no significant difference between stressed and control treatments, despite the enriched stem water values for the stressed plants (Table I).

For both hydrogen and oxygen isotopic ratios, Eq. (2) predicts a higher degree of leaf water isotopic enrichment than was actually observed (Table I). Calculations of f for the water stress experiment indicate that the hydrogen isotopic composition of leaf water is approximately 75% of the steady state value, with no difference between the control and stressed plants (Table I). The observed oxygen isotopic compositions of leaf water were further from steady state than the hydrogen values (Table I). In addition there was a significant difference between control and stressed plants, with the stressed plants showing a greater difference between the modelled and the observed oxygen isotopic composition of leaf water (Table I).

Water stress would cause a reduction in the leaf transpiration rate and therefore increase the time required for leaf water to reach isotopic steady state. It is expected that the reduced transpiration rate would have a greater effect on the oxygen isotopic composition of leaf water because of the larger relative magnitude of the diffusional or kinetic fractionation factor compared with the equilibrium fractionation factor for oxygen isotopes.

TABLE I. EFFECT OF MILD WATER STRESS ON PHYSIOLOGICAL CHARACTERISTICS AND LEAF AND STEM WATER ISOTOPIC COMPOSITIONS IN *Cornus stolonifera* AT MIDDAY UNDER GLASSHOUSE CONDITIONS

(average environmental conditions: air temperature 27.7°C, relative humidity 21.5%, light intensity 1270 $\mu mol \cdot m^{-2} \cdot s^{-1}$, atmospheric water vapour $\delta D = -149‰$, $\delta^{18}O = -21.9‰$)

	Control	Stress	Significance[a]
Stem water potential (MPa)	−1.5 ± 0.3	−2.5 ± 0.3	*
Leaf temperature (°C)	31.7 ± 3.1	35.7 ± 2.5	*
Stem water isotopic composition			
δD (‰)	−123 ± 1	−118 ± 2	*
$\delta^{18}O$ (‰)	−15.8 ± 0.3	−13.5 ± 0.7	*
Leaf water isotopic composition			
Observed δD (‰)	−65 ± 2	−60 ± 3	*
Modelled δD (‰)	−45 ± 2	−41 ± 3	*
D/H *f* value	0.75 ± 0.03	0.76 ± 0.07	NS
Observed $\delta^{18}O$ (‰)	7.7 ± 2.3	6.6 ± 2.6	NS
Modelled $\delta^{18}O$ (‰)	18.9 ± 0.6	22.2 ± 0.4	*
$^{18}O/^{16}O$ *f* value	0.67 ± 0.06	0.56 ± 0.08	*

Note: Values are the mean ± standard deviation; $n = 8$ for all values except stem water potential and stem water isotopic composition, where $n = 4$.

[a] Significance tests were based on the result of a Kruskal–Wallis analysis of variance.

* $P < 0.05$.

6. CONCLUSIONS

The precise application of the evaporative enrichment model to studies of the leaf–air water vapour pressure gradient requires that the isotopic composition of leaf water be at steady state during the whole course of the day. Since it appears that under field conditions leaf water is not at isotopic steady state, it seems improbable that the leaf water enrichment model can be applied to studies of e_a/e_i in the same quantitative manner that carbon isotope data are applied to studies of c_i/c_a [1].

Qualitative information may still be obtained, however, if two leaves exposed to widely different water vapour gradients have approximately similar f values. Further research is required to determine the exact source of the discrepancy between the observed and modelled data shown in Fig. 2.

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