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Comparison of Modeled and Observed Environmental Influences on the Stable Oxygen and Hydrogen Isotope Composition of Leaf Water in *Phaseolus vulgaris* L.[1]

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ABSTRACT

In this paper we describe how a model of stable isotope fractionation processes, originally developed by H. Craig and L. I. Gordon ([1965] *in* E Tongiorgi, ed, Proceedings of a Conference on Stable Isotopes in Oceanographic Studies and Paleotemperature, Spoleto, Italy, pp 9–130) for evaporation of water from the ocean, can be applied to leaf transpiration. The original model was modified to account for turbulent conditions in the leaf boundary layer. Experiments were conducted to test the factors influencing the stable isotopic composition of leaf water under controlled environment conditions. At steady state, the observed leaf water isotopic composition was enriched above that of stem water with the extent of the enrichment dependent on the leaf-air vapor pressure difference (VPD) and the isotopic composition of atmospheric water vapor (AWV). The higher the VPD, the larger was the observed heavy isotope content of leaf water. At a constant VPD, leaf water was relatively depleted in heavy isotopes when exposed to AWV with a low heavy isotope composition, and leaf water was relatively enriched in heavy isotopes when exposed to AWV with a large heavy isotope composition. However, the observed heavy isotope composition of leaf water was always less than that predicted by the model. The extent of the discrepancy between the modeled and observed leaf water isotopic composition was a strong linear function of the leaf transpiration rate.

The stable isotopic composition of plant leaf water is altered during transpiration. Water vapor molecules containing the lighter isotopes of oxygen and hydrogen escape from the leaf more readily than do heavy isotope molecules, so that during transpiration, leaf water becomes enriched in heavy isotope molecules (19, 24, 28). A model of isotopic fractionation was originally developed by Craig and Gordon (6) for processes occurring during the evaporation of water from the ocean. The Craig and Gordon model has potential applications in plant physiology and ecology in studies of the leaf-air vapor pressure gradient (VPD[3]), an important parameter affecting photosynthetic gas exchange and plant water-use efficiency (10, 11, 20, 24, 28).

Previous attempts to use the Craig and Gordon model in studies of leaf water isotopic enrichment have been complicated by two factors. First, there has been uncertainty about the value to use for the kinetic fractionation factor. Values for the relative rates of diffusion of water vapor molecules containing light and heavy isotopes of oxygen and hydrogen have been measured (16). These measured values for the kinetic fractionation factor are appropriate for molecular diffusion only, however, and need to be modified for turbulent conditions in a boundary layer. In previous studies there has been uncertain and inconsistent modification of the kinetic fractionation factor to account for turbulence in the water vapor diffusion pathway (3, 7, 11, 14, 20, 22, 23, 25). Second, a major assumption in the derivation of the evaporative enrichment model is that isotopic steady state is reached. When leaf water is at isotopic steady state, the isotopic composition of transpiration water is the same as the source or stem water isotopic composition (24). The assumption of isotopic steady state has not been verified in most studies attempting to test the ability of the Craig and Gordon model to predict leaf water isotopic enrichment (1, 3, 7, 29).

In this paper we outline how the Craig and Gordon model can be applied to studies of the VPD. We also demonstrate how the original model can be modified to account for turbulence in the water vapor diffusion pathway. Experiments were conducted to test the time required to reach isotopic steady state under controlled environment conditions. Comparisons were then made between the observed, steady-state leaf water isotopic composition and that predicted by the model when changes were made in the VPD and the isotopic composition of AWV.

THEORY

Isotope Effects During Transpiration

In the model developed by Craig and Gordon (6) there are two isotope effects during evaporation (a) an equilibrium effect resulting from the phase change from liquid water to water vapor and (b) a kinetic effect caused by the different diffusion rates of the light and heavy isotopes of water vapor in air.

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

$$\alpha^* = R_l/R_v \quad (1)$$

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[3] Abbreviations: AWV, atmospheric water vapor; VPD, leaf-air vapor pressure difference; SMOW, standard mean ocean water.

where R is the molar ratio of the heavy and light isotope (*i.e.* D/H or $^{18}O/^{16}O$) and the subscripts l and v refer to liquid water and water vapor, respectively. In the above definition it is assumed that the air is saturated with water vapor and that the liquid and vapor are at the same temperature. These conditions are satisfied for a leaf where water vapor in the intercellular air spaces is in equilibrium with leaf cell water. The equilibrium fractionation factors have been measured for D/H and $^{18}O/^{16}O$ over a range of temperatures by Majoube (15). The regression equations, listed by Majoube (15), were used to calculate values for the parameter α^*.

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

$$\alpha_k = g/g' \tag{2}$$

where g and g' refer to the stomatal conductance to water vapor molecules containing the light and heavy isotopes, respectively. Merlivat (16) has measured the relative rates of diffusion in air of water vapor molecules containing the light and heavy isotopes of hydrogen and oxygen. The values of α_k for molecular diffusion are: H/D = 1.025 and $^{16}O/^{18}O$ = 1.0285 (16). It is appropriate to use these values for diffusion through the stomatal pore which is a molecular process (18). The value for the kinetic fractionation factor should be modified, however, for turbulence in the boundary layer outside the stomatal pore. Based on Pohlhausen analysis, the kinetic fractionation factor in a boundary layer (α_{kb}) is calculated by taking the value of the kinetic factor for molecular diffusion and raising it to the two-thirds power (13). The values for α_{kb} are: H/D = 1.017 and $^{16}O/^{18}O$ = 1.0189 (12).

The equilibrium and kinetic fractionation factors can be incorporated into a model of isotopic enrichment during evaporation as originally described by Craig and Gordon (6). In addition to the two fractionation factors, the model also includes the influence of isotopic exchange between water vapor in the atmosphere and leaf water. In the derivation described below, we follow a procedure similar to that of Dongmann *et al.* (7) and White (25) in applying the Craig and Gordon (6) evaporative enrichment model to leaf transpiration.

The transpiration rate of a leaf can be described as:

$$E = g[(e_i - e_a)/P] \tag{3}$$

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

$$E' = g'\frac{(R_s e_i - R_a e_a)}{P} \tag{4}$$

where e_i is multiplied by R_s, the molar ratio of the heavy and light isotopes of water vapor in the intercellular air spaces, and e_a is multiplied by R_a, the molar ratio of the heavy and light isotopes of water vapor in the air outside the leaf. The ratio of the fluxes of the heavy (E') and the light (E'') isotopes will describe the isotopic composition of water transpired by a leaf (R_t). Heavy isotope molecules represent a very small fraction of the total water vapor transpired (9). The molar ratio of heavy to light isotopes in the transpiration stream (E'/E'') can be approximated, therefore, by the molar ratio of heavy isotopes to total water vapor transpired (E'/E) to simplify the following derivation. Inserting the above defined fractionation factors, the equation for the isotopic ratio of transpired water is:

$$\frac{E'}{E''} = R_t \approx \frac{E'}{E} = \frac{1}{\alpha_k}\frac{\left(\frac{R_l}{\alpha^*}e_i - R_a e_a\right)}{(e_i - e_a).} \tag{5}$$

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

$$R_s = \frac{1}{\alpha_k}\frac{\left(\frac{R_l}{\alpha^*}e_i - R_a e_a\right)}{(e_i - e_a).} \tag{6}$$

Rearranging Equation 6 to solve for R_l one obtains the following:

$$R_l = \alpha^*\left[\alpha_k R_s\left(\frac{e_i - e_a}{e_i}\right) + R_a\left(\frac{e_a}{e_i}\right).\right] \tag{7}$$

Similar equations have been developed for isotopic enrichment during leaf transpiration by Dongmann *et al.* (7) and White (25). Equation 7 assumes that there are no significant boundary layer effects and, therefore, the partial pressure of water vapor at the leaf surface (e_s) is the same as e_a. Equations describing the influence of boundary layer effects are developed below.

Because water vapor diffusion through the stomata and the boundary layer represent transport paths connected in series, at steady state both the flux rate and the isotopic composition of water vapor transported across each path must be equal. It is possible, therefore, to write two equations (Eqs. 8 and 9) which are analogous to Equation 6. Equations 8 and 9, however, represent water vapor transport through the stomata and boundary layer separately.

$$R_s = \frac{1}{\alpha_k}\frac{\left(\frac{R_l}{\alpha^*}e_i - R_s e_s\right)}{(e_i - e_s)} \tag{8}$$

$$R_s = \frac{1}{\alpha_{kb}}\frac{(R_s e_s - R_a e_a)}{(e_s - e_a)} \tag{9}$$

where R_s is the molar ratio of the heavy and light isotopes of water vapor in the air at the leaf surface, and α_{kb} is the ratio of the conductance of the light and heavy isotopes in a boundary layer.

To obtain an equation for the isotopic composition of leaf water (R_l), equation 9 is solved for R_s and then the resulting expression is inserted into Equation 8. Following substitution, Equation 8 is rearranged to solve for R_l:

$$R_l = \alpha^*\left[\alpha_k R_s\left(\frac{e_i - e_s}{e_i}\right) + \alpha_{kb} R_s\left(\frac{e_s - e_a}{e_i}\right) + R_a\left(\frac{e_a}{e_i}\right)\right]. \tag{10}$$

Equation 10 was used to predict the isotopic composition of

leaf water in the experiments described in the following sections. An alternative method to account for boundary layer effects is described by Farquhar *et al.* (11).

MATERIALS AND METHODS

Plant Material and Growing Conditions

Phaseolus vulgaris L. (cv UNS 117) plants were germinated in 12 L pots that were made from polyvinylchloride pipe and contained a soil mixed from one part vermiculite to two parts Utah soil mix that has been previously described (5). Plants were grown in a glasshouse with supplemental lighting provided by an alternating bank of metal halide and sodium vapor lamps. The maximum photosynthetic photon flux density during the day was 1.8 mmol m^{-2} s^{-1}. The air temperature in the glasshouse ranged daily from a maximum of 33°C to a minimum of 20°C. Relative humidity was uncontrolled and averaged approximately 20%. Plants were watered twice daily and fertilized weekly. The plants used in the present experiments were approximately 6 to 7 weeks old.

Gas Exchange Measurements and Water Collection

The measurements of CO_2 and water vapor exchange were made using an open gas exchange system previously described (8). Mature leaflets were sealed into the leaf chamber and maintained under conditions of controlled temperature, humidity, light and CO_2 concentration. Measurements of leaf temperature, air temperature and chamber humidity were made so that the partial pressure of water vapor in the leaf intercellular air spaces (e_i), leaf surface (e_s), and ambient chamber air (e_a) could be calculated. All gas exchange calculations were done using the equations of Ball (2) and von Caemmerer and Farquhar (21). Boundary layer conductance to water vapor in the leaf chamber was 2 mol m^{-2} s^{-1}.

The water vapor in the air of the leaf chamber was collected by passing a portion of the air exiting the chamber through two ethanol-dry ice traps. The leaf was removed from the chamber, immediately after gas exchange measurements were complete, and placed in a glass tube which was sealed with a rubber stopper and wrapped with Parafilm. A stem or petiole sample was also collected at the same time and sealed in a glass tube. The plant samples were then frozen until water was extracted from the tissue using a cryogenic vacuum distillation apparatus (9).

Isotopic Analysis

Water samples were prepared for measurements of the hydrogen isotopic composition by reacting approximately 5 μL of water with zinc in an evacuated tube at 500°C (4). The zinc was obtained from the laboratory of J. M. Hayes, Departments of Chemistry and Geology, Indiana University. The resulting hydrogen gas was analyzed for isotopic composition on either a Finnigan-MAT delta E or delta S gas isotope ratio mass spectrometer. Precision of the hydrogen isotopic analysis was determined by repeated sampling of a laboratory standard which had been calibrated in relation to SMOW obtained from the U.S. National Bureau of Standards and had also been analyzed by two independent stable isotope laboratories. The precision of measurements of the laboratory standard was ± 1.4‰ for the delta E and ± 1.0‰ for the delta S mass spectrometers (± standard deviation).

Water samples were prepared for measurements of the oxygen isotopic composition by converting the oxygen in water to CO_2 using the guanidine hydrochloride method (26). The CO_2 gas was then analyzed for oxygen isotopic composition on the mass spectrometers described above. Precision of the method was checked by making repeated measurements of SMOW. Precision was found to be ±0.31‰ for the delta E and ±0.12‰ for the delta S mass spectrometers during the course of this study.

Isotopic compositions are expressed using delta notation in parts per thousand (‰) as shown below:

$$\delta = \left[\frac{R_{\text{Sample}}}{R_{\text{Standard}}} - 1\right] \times 1000$$

where R is the molar ratio of the heavy to light isotope (D/H or $^{18}O/^{16}O$). All sample isotopic compositions were expressed relative to the standard SMOW. The absolute ratios for SMOW used in the calculations were D/H = 0.00015576 and $^{18}O/^{16}O$ = 0.0020052 (9).

Experiment 1

The purpose of the first experiment was to test the time required for leaf water to reach isotopic steady state. In this experiment single leaflets were maintained under constant environmental conditions for approximately six hours. The air line entering the leaf chamber contained completely dry air, so that humidification of the leaf chamber was a result of leaf transpiration only. After the leaf was sealed in the chamber, the light source was turned on. When light intensity reached 1.3 mmol m^{-2} s^{-1} (approximately 5 min), the collection of water vapor leaving the chamber was begun. Water vapor was collected for 30 min during the first collection interval. In subsequent intervals, water vapor was collected for approximately 45 min to 1 h. Water vapor in the air line exiting the chamber was trapped for five successive intervals. The isotopic composition of water transpired by a leaf was compared to that of water extracted from the petiole or stem of the plant. When leaf water is at isotopic steady state, the isotopic compositions of transpiration water and stem water should be the same.

Experiment 2

The second experiment had two objectives. The first objective was to simultaneously test that leaf water was at isotopic steady state and to compare the observed leaf water enrichment with that predicted by Equation 10. The second objective was to test if the initial isotopic composition of leaf water strongly influenced the isotopic composition of leaf water after an extended period of time under constant environmental conditions.

To accomplish the first objective, the air line entering the leaf chamber contained completely dry air so that humidification of the leaf chamber was a result of transpiration only. In this situation the isotopic composition of transpiration water could be compared to the isotopic composition of stem

water to test for isotopic steady state in a manner similar to experiment 1. After different amounts of time, the leaf was removed from the chamber, water was extracted and the isotopic composition of the leaf water was compared to that predicted by Equation 10.

The *Phaseolus* plants used in the present experiments do not completely close their stomata during the night so that some night time transpiration occurs. It is possible, therefore, to increase the humidity around a plant by placing a bag overtop of the plant at night. This was done for two sets of plants, with one set covered by plastic bags (high humidity) and a second set covered by paper bags (low humidity). The plants were kept covered with the bags until they were used in the gas exchange experiments the following day. Before a leaflet was sealed into the leaf chamber, a different but similar sized leaflet was removed from the plant and sealed in a glass tube. The isotopic composition of water extracted from the "dark" leaf was used as an indication of the initial starting point for leaf water. Plants covered by plastic bags had different initial leaf water isotopic compositions than plants covered with paper bags. Plants from these two treatment groups could be used, therefore, to test if the initial leaf water isotopic composition influenced the final isotopic composition of leaf water after an extended period of time under constant environmental conditions.

Experiment 3

The purpose of the third experiment was to compare the isotopic composition of leaf water predicted by Equation 10 with that actually observed in leaves that were exposed to different VPD and different AWV isotopic compositions. Different leaves were exposed to vapor pressure gradients of 1.0, 2.0, 2.6, and 3.0 kPa.Because the air in the leaf chamber was well mixed, the isotopic composition of chamber air (R_a) was taken to be that of water vapor in the air line leaving the chamber. The isotopic composition of water vapor in the chamber air was modified by using water with different isotopic compositions in the device that humidified the air line entering the leaf chamber. The isotopic composition of water in the humidifying device of the gas exchange system varied from: $\delta^{18}O$, −18.9‰ to −5.5‰ and δD, −134‰ to −56‰. The isotopic composition of AWV in the leaf chamber was dependent on three factors: (a) the isotopic composition of water in the gas exchange humidifying device; (b) the isotopic composition of transpiration water; and (c) the transpiration rate of the leaf.

RESULTS

Experiment 1

The isotopic composition of water transpired by a leaf remained steady after a leaf had been exposed to constant environmental conditions for approximately 2 h (Fig. 1). The isotopic composition of transpiration water, collected after 2 h, was similar to that of the water extracted from a petiole, indicating that leaf water was at isotopic steady state. Because 1 h was required to trap water vapor exiting the leaf chamber and the points in Figure 1 represent the time when a particular water vapor collection was stopped, approximately 1 h of

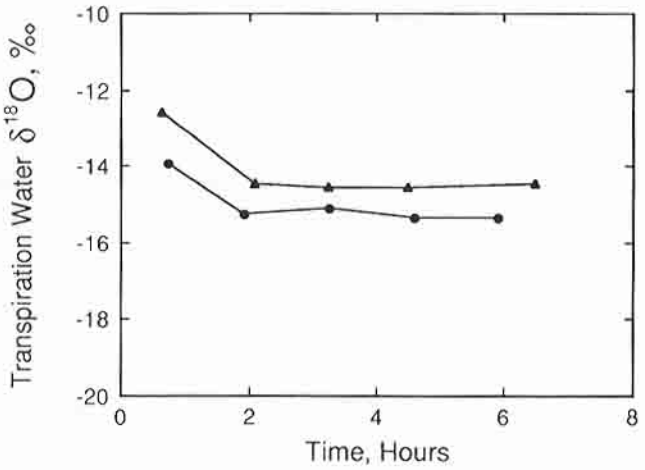

Figure 1. The oxygen isotopic composition of water transpired by two different plants of *P. vulgaris* L. maintained under constant environmental conditions in a controlled environment gas exchange chamber. Environmental conditions were: leaf temperature 30°C, VPD 2.0 kPa, light intensity 1.3 mmol m^{-2} s^{-1} (400–700 nm), boundary layer conductance to water loss 2 mol m^{-2} s^{-1}. The isotopic compositions ($\delta^{18}O$, ‰) of stem water were: triangles (▲), −14.8; circles (●), −14.2.

constant environmental conditions was required to reach isotopic steady state.

Experiment 2

The isotopic composition of transpiration water and stem water were essentially equal, within the range of measurement precision, for all leaves held under constant environmental conditions for at least 1 h (Tables I, II), indicating that leaf water was at isotopic steady state. Despite the fact that leaf water was at isotopic steady state, the observed oxygen and hydrogen isotopic compositions of leaf water were lower than that predicted by Equation 10.

The initial leaf water isotopic composition, of plants kept under the plastic bags, was less enriched in heavy isotopes than leaves of the plants kept overnight under the paper bags (Tables I, II). Approximately the same degree of leaf water enrichment was observed in both treatments, however, after leaves were exposed to the same constant environmental conditions in the leaf chamber (Tables I, II). There appeared to be no effect, therefore, of the initial leaf water isotopic composition on the final isotopic composition observed after an extended period of time under constant environmental conditions.

Experiment 3

Based on the results of Experiment 1 and 2, collection of water vapor exiting the leaf chamber in experiment 3 was not started until the leaf had been exposed to constant environmental conditions for at least 1 h. Usually leaf gas exchange parameters had been constant for at least 45–60 min before water vapor collections were begun.

Leaf water isotopic composition was affected by both VPD

Table I. *Oxygen Isotopic Compositions ($\delta^{18}O$, ‰) of Stem Water, Transpiration Water, and Leaf Water in P. vulgaris Plants*

The leaf water isotopic composition was measured on plants kept overnight in the dark (initial) and after leaves were exposed to constant environmental conditions in a leaf gas exchange chamber for the indicated period of time (observed). Average environmental conditions were: light intensity 1.3 mmol m^{-2} s^{-1}, leaf temperature 30°C, VPD 2.0 kPa. Plants of the humid treatment were kept covered by plastic bags overnight. The plants of the dry treatment were covered with paper bags overnight. The modeled leaf water values were calculated with Equation 10.

Treatment	Leaf Water Initial	Time	Leaf Water			
			Stem	Transpired	Observed	Model
	‰	*h*		‰		
Humid	−2.3	1	−14.8	−15.1	−0.8	4.3
Humid	−3.9	2	−14.6	−14.5	0.8	5.1
Humid	−5.8	3	−15.2	−15.1	0.0	4.7
Dry	6.3	1	−14.3	−12.8	0.4	5.7
Dry	6.8	2	−14.6	−15.4	−0.4	4.1
Dry	6.3	3	−14.3	−15.2	0.0	4.7
Dry	6.0	6	−14.2	−15.4	0.0	4.6
Dry	6.8	7	−14.8	−14.5	0.5	4.6

and the isotopic composition of AWV. The larger the VPD that a leaf was exposed to, the higher was the heavy isotope composition of leaf water (Figs. 2, 3). At a constant VPD, the observed leaf water isotopic composition was relatively depleted in heavy isotopes when exposed to AWV that had a low heavy isotope content and leaf water was relatively enriched in heavy isotopes when exposed to AWV that had a large heavy isotope content (Figs. 2, 3). There was, therefore, good qualitative agreement between the leaf water isotopic enrichment predicted by the model and that observed in leaves. However, the observed δ values for leaf water were always less than that predicted by the evaporative enrichment model (Figs. 2, 3). The extent of the discrepancy between the modeled and observed leaf water isotopic composition was a strong linear function of the leaf transpiration rate (Fig. 4). The higher the transpiration rate, the larger the discrepancy between the leaf water isotopic composition predicted by the model and that actually observed in leaves (Fig. 4).

DISCUSSION

In our experiments exposure to constant environmental conditions for approximately 1 h was required for leaf water to reach isotopic steady state (Fig. 1; Tables I, II). The water volume of a leaf and the leaf transpiration rate are characteristics that will influence the turnover time of leaf water and, therefore, affect the time required to reach isotopic steady state. The *Phaseolus* plants used in our experiments have relatively thin leaves, low absolute water contents, and high transpiration rates, all factors which indicate that leaf water should turnover quickly. In other plant species that have thick, succulent leaves and low transpiration rates, longer

Table II. *Hydrogen Isotopic Compositions (δD, ‰) of Stem Water, Transpiration Water, and Leaf Water in P. vulgaris Plants*

The leaf water isotopic composition was measured on plants kept overnight in the dark (initial) and after leaves were exposed to constant environmental conditions in a leaf gas exchange chamber for the indicated period of time (observed). Average environmental conditions were: light intensity 1.3 mmol m^{-2} s^{-1}, leaf temperature 30°C, VPD 2.0 kPa. Plants of the humid treatment were kept covered by plastic bags overnight. The plants of the dry treatment were covered with paper bags overnight. The modeled leaf water values were calculated with Equation 10.

Treatment	Leaf Water Initial	Time	Leaf Water			
			Stem	Transpired	Observed	Model
	‰	*h*		‰		
Humid	−63	1	−118	−117	−58	−43
Humid	−65	2	−119	−116	−56	−43
Humid	−79	3	−121	−117	−60	−44
Dry	−52	1	−118	−117	−56	−43
Dry	−53	2	−121	−118	−62	−45
Dry	−48	3	−121	−117	−61	−44
Dry	−56	6	−121	−121	−61	−47
Dry	−53	7	−121	−116	−59	−44

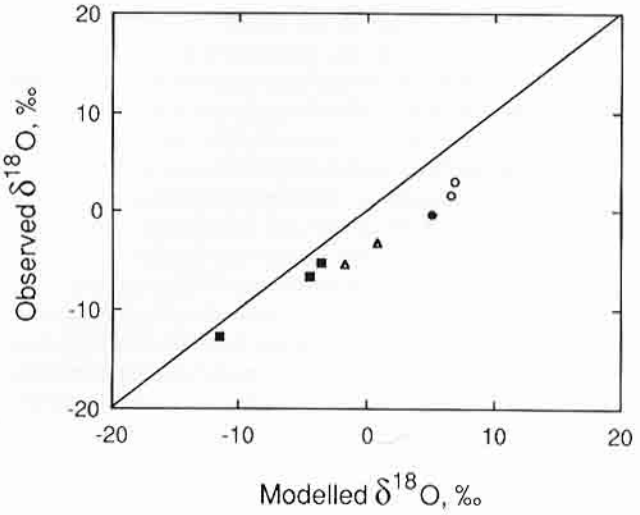

Figure 2. Comparison of the modeled and observed oxygen isotope composition of water in *P. vulgaris* leaves. Environmental conditions were: leaf temperature 30°C, light intensity 1.3 mmol m^{-2} s^{-1} (400–700 nm), boundary layer conductance to water loss 2 mol m^{-2} s^{-1}. The stem $\delta^{18}O$ values varied from −15.4‰ to −16.1‰. Modeled values were calculated using Equation 10. The points indicated by solid squares (■) were for leaves exposed to a VPD of 1.0 kPa, with $\delta^{18}O$ values of AWV of −27.6‰, −18.9‰, and −17.4‰. The open triangles (△) were points for a VPD of 2.0 kPa, with $\delta^{18}O$ values of AWV of −24.7‰ and −19.8‰. The solid circle (●) was for a VPD of 2.6 kPa, with a $\delta^{18}O$ value of AWV of −19.0‰. The open circles (○) were points for a VPD of 3.0 kPa, with $\delta^{18}O$ values of AWV of −22.6‰ and −20.6‰. Isotopic compositions are expressed relative to SMOW.

time periods will be required for leaf water to reach isotopic steady state. It should be noted, however, that the time course to leaf water isotopic steady state was measured after a rather drastic change in environmental conditions from darkness to high light (1.3 mmol m^{-2} s^{-1}, 400–700 nm). In the natural environment, environmental conditions can change rapidly, but normally light and VPD follow a rather gradual pattern of change over the course of a day. Less time may be required to reach isotopic state when tracking normal daily changes in environmental conditions in the field.

There was good qualitative agreement between the leaf water isotopic enrichment predicted by the model (Eq. 10) and that observed in leaves. The higher the VPD that a leaf was exposed to, the higher was the heavy isotope composition of leaf water (Figs. 2, 3). At a constant VPD, leaf water was relatively depleted in heavy isotopes when exposed to AWV with a low heavy isotope composition, and leaf water was relatively enriched in heavy isotopes when exposed to AWV with a large heavy isotope composition (Figs. 2, 3). Such patterns in the change in leaf water are expected based on the evaporative enrichment model described by Equation 10.

Complete quantitative agreement between the evaporative enrichment model and the observed isotopic composition for leaf water was not obtained, however. The model always predicted a higher degree of heavy isotope enrichment than was actually observed in leaves (Tables I, II; Figs. 2, 3). Possible reasons for the discrepancy between the modeled and observed leaf water isotopic composition are discussed below.

Several previous studies have also observed that the evaporative enrichment model developed by Craig and Gordon (6) predicts a higher heavy isotope composition than is actually observed in leaves (1, 3, 5, 12, 14, 22, 23, 25, 27). In a number of these studies it was suggested that the discrepancy might result from unfractionated water in leaf vein tissue (1, 14, 23, 25). Attempts were made to model the observed isotopic composition of leaf water as a mixture of two pools, one that is described by the evaporative enrichment model (Eq. 10), and one that is unfractionated and has the same isotopic composition as stem water. The two pool model used in in previous studies is described in Equation 11:

$$\delta_{Leaf} = \delta_{SS} \cdot f + \delta_{Stem} \cdot (1 - f) \qquad (11)$$

where δ_{Leaf} is the measured isotopic composition of a leaf, δ_{SS} is the modeled isotopic composition of leaf water at steady state, δ_{Stem} is the isotopic composition of stem water. The portion that represents the unfractionated pool is often calculated to be 20 to 30% of the total water volume of a leaf (1, 14, 23). Similar calculations with our data indicate that the unfractionated portion varies from 13 to 33% depending on leaf transpiration rate. These calculated fractions are too large to represent leaf vein water, which should only comprise approximately 5% of the total water volume of a leaf (17). In addition, if the discrepancy between the observed and modeled isotopic compositions is related only to an unfractionated

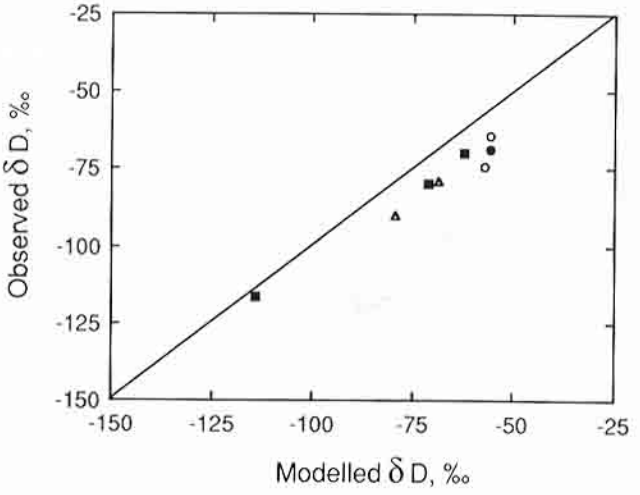

Figure 3. Comparison of the modeled and observed hydrogen isotope composition of water in *P. vulgaris* leaves. Environmental conditions were: leaf temperature 30°C, light intensity 1.3 mmol m^{-2} s^{-1} (400–700 nm), boundary layer conductance to water loss 2 mol m^{-2} s^{-1}. The stem δD values varied from = −119‰ to −123‰. Modeled values were calculated using Equation 10. The points indicated by solid squares (■) were for leaves exposed to a VPD of 1.0 kPa, with δD values of AWV of −195‰, −134‰, and −143‰. The open triangles (△) were points for a VPD of 2.0 kPa, with δD values of AWV of −174‰ and −154‰. The solid circle (●) was for a VPD of 2.6 kPa, with a δD value of AWV of −143‰. The open circles (○) were points for a VPD of 3.0 kPa, with δD values of AWV of −161‰ and −150‰. Isotopic compositions are expressed relative to SMOW.

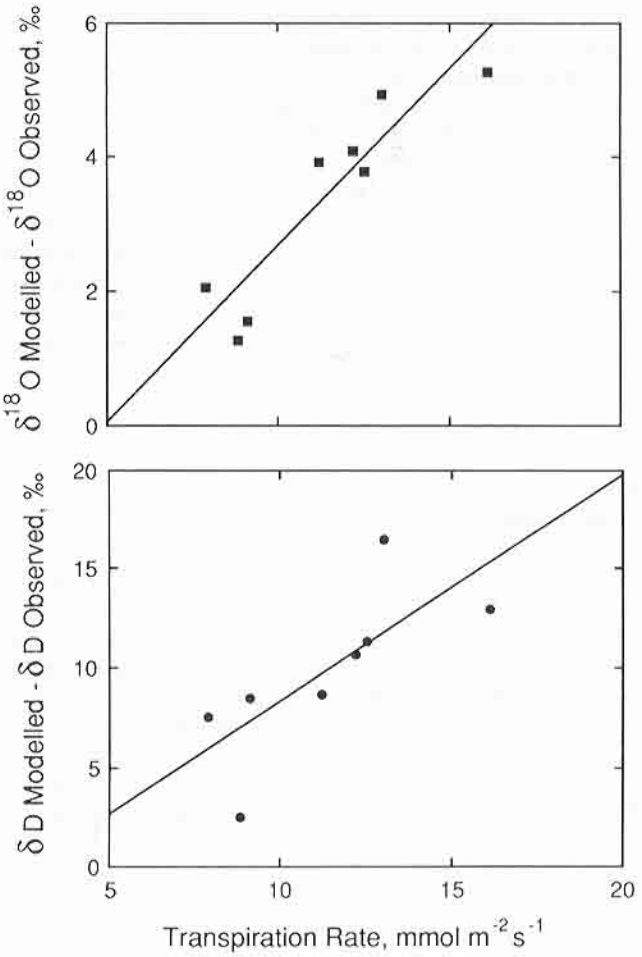

Figure 4. The influence of transpiration rate on the discrepancy between the modeled and observed leaf water isotopic compositions in *P. vulgaris*. Modeled values were calculated with Equation 10. In the top panel, the equation for the regression line shown is: $y = 0.526x - 2.61$, $P < 0.001$, $r = 0.92$. In the bottom panel, the equation for the regression line shown is: $y = 1.148x - 3.2$, $P < 0.034$, $r = 0.75$.

pool associated with some anatomical component of leaf tissue, the size of the fraction should not change when transpiration rate is varied. The data shown in Figure 4 clearly indicate that the magnitude of the discrepancy between the modeled and observed leaf water isotopic composition is strongly related to leaf transpiration rate. Walker *et al.* (23) also found that the discrepancy between the observed leaf water isotopic composition and that predicted by the Craig and Gordon (6) model increased with transpiration rate.

As an alternative, the discrepancy between the modeled and observed leaf water isotopic composition may result from a shifting balance between the bulk flow of unfractionated water into the leaf and the back diffusion of heavy isotope molecules from sites of evaporative enrichment within the leaf. When transpiration rate is low, the bulk flow of unfractionated water into the leaf tissue is low which provides opportunity for a large component of back diffusion of heavy isotope molecules from the evaporation sites. When transpiration is increased, however, the higher rate of bulk flow of unfractionated water into the leaf tissue overrides some of the back diffusion of heavy isotope molecules so that the discrepancy between the modeled and observed enrichment is increased. It is expected that the discrepancy between the modeled and observed leaf water isotopic composition would vary among species because of different path lengths for water movement between the veins and evaporative sites within leaves. The longer the path length, the larger the expected discrepancy between the modeled and observed leaf water isotopic compositions. White (24) provides a description of a model developed by G. D. Farquhar that considers the effect of transpiration rate on the isotopic composition of leaf water.

A second potential factor contributing to the increased discrepancy seen at high transpiration rates, is that nonuniform stomatal closure may occur when the leaf is exposed to low humidity. Recent studies have shown that individual stomata can vary in their response to closing stimuli such as low humidity (10, 11). If patches of closed stomata occur across the leaf surface, water in the cells adjacent to the closed stomata may be less enriched than water in cells next to the open stomata. Such a patchy pattern of stomatal closure could contribute to the observed leaf water isotopic composition being lower than that predicted by the model.

No previous study has considered the influence of leaf boundary layer conditions on the isotopic composition of leaf water. The influence of boundary layer conditions on leaf water isotopic enrichment can be calculated by comparing values predicted by Equations 7 and 10. Equation 7, which

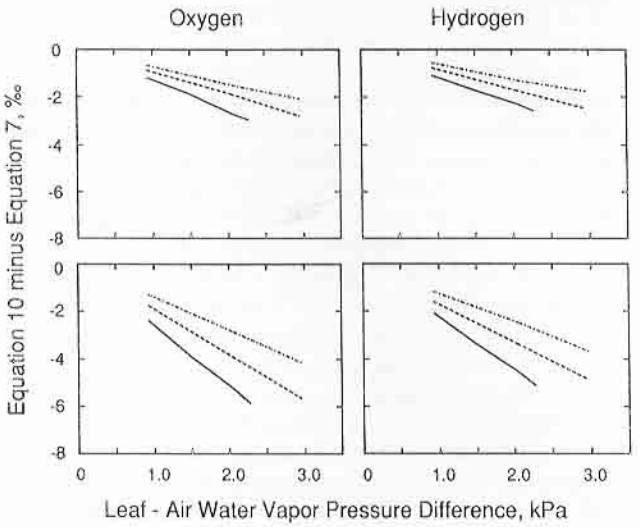

Figure 5. The influence of boundary layer conditions on leaf water isotopic enrichment. The ordinate shows the difference between leaf water isotopic compositions predicted by Equations 10 and 7. The different style of lines represent calculations done for different leaf temperatures: solid (———) 20°C, dashed (- - - -) 25°C, dot-dash (-·-·-) 30°C. For the two panels in the column labeled oxygen, the top panel shows calculations done with boundary layer conductance two times the value of stomatal conductance and the bottom panel shows calculations done with boundary layer conductance 0.5 times the value for stomatal conductance. A similar format is followed for the two panels in the column labeled hydrogen.

does not include boundary layer effects, predicts more isotopic enrichment than does Equation 10. The magnitude of the difference between the predictions of the two equations depends on leaf temperature, VPD, and the particular element being considered (Fig. 5).

For D/H ratios, the largest difference between Equations 7 and 10 is approximately equal to the precision of measurement techniques (±2‰) when boundary layer conductance is twice as large as stomatal conductance (Fig. 5). The maximal difference between the two equations increases to approximately 5‰, however, when boundary layer conductance is reduced to one-half the value of stomatal conductance. Such a low boundary layer conductance would be uncommon under natural 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 boundary layer conductance is twice the value of stomatal conductance, there may be approximately a 3‰ difference between the values predicted by Equations 7 and 10, when the VPD is large (Fig. 5). The maximal difference between the two equations increases to approximately 6‰ when boundary layer conductance is reduced to one-half of the value for stomatal conductance. Part of the discrepancy observed between the modeled and observed leaf water isotopic compositions in previous field studies may be related to the influence of boundary layer conditions, particularly for oxygen isotope studies.

For the evaporative enrichment model to be applied in a precise, quantitative manner to field studies of VPD, the following three conditions must be met (12): (a) leaf water must be at isotopic steady state, (b) the isotopic composition of AWV and plant stem water must be known, and (c) the magnitude of the discrepancy between the modeled and observed leaf water isotopic compositions must be known for the particular plant species of interest. Because of these complications, it is unlikely that the evaporative enrichment model can be applied routinely to obtain quantitative information about VPD. It still may be possible to obtain some qualitative information from measurements of the isotopic composition of leaf water if comparisons are made among leaves from the same plant species and it is assumed that leaf water is at isotopic steady state.

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