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Effects of Mild Water Stress and Diurnal Changes in Temperature and Humidity on the Stable Oxygen and Hydrogen Isotopic Composition of Leaf Water in *Cornus stolonifera* L.[1]

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ABSTRACT

In this paper we make comparisons between the observed stable isotopic composition of leaf water and the predictions of the Craig-Gordon model of isotopic enrichment when plants (*Cornus stolonifera* L.) were exposed to natural, diurnal changes in temperature and humidity in a glasshouse. In addition, we determined the effects of mild water stress on the isotopic composition of leaf water. The model predicted different patterns of diurnal change for the oxygen and hydrogen isotopic composition of leaf water. The observed leaf water isotopic composition followed qualitatively similar patterns of diurnal change to those predicted by the model. At midday, however, the model always predicted a higher degree of heavy isotope enrichment than was actually observed in leaves. There was no effect of mild water stress on the hydrogen isotopic composition of leaf water. For the oxygen isotopic composition of leaf water, there was either no significant difference between control and water-stressed plants or the stressed plants had lower $\delta^{18}O$ values, despite the enriched stem water isotopic composition observed for the stressed plants.

Measurement of the carbon isotope composition of plant tissue has become an important technique for assessing the A/E[3] of plants (9, 10). The relationship between A/E and plant carbon isotopic composition occurs because both are related to the ratio of leaf intercellular CO_2 and atmospheric CO_2 concentrations (9, 10). A/E, however, is also a function of VPD. Estimates of A/E could potentially be improved if information from carbon isotope analysis was combined with long-term information concerning VPD (9, 10).

It is possible that measurements of the hydrogen and oxygen isotopic composition of leaf cellulose could be used to infer the long-term integrated value of VPD (10, 18, 21). Such a procedure may be possible because the isotopic composition of leaf cellulose is influenced by the isotopic composition of leaf water (17, 21, 22). Leaf water isotopic composition is, in turn, influenced by VPD (10, 12, 13). Cellulose should contain, therefore, long-term integrated information about the short-term changes in leaf water isotopic composition that result because of variation in VPD during leaf development (10, 13). Studies have shown that the isotopic composition of plant cellulose is strongly correlated with average daytime RH during the growing season (6, 7).

Although no fractionation of isotopes occurs during water uptake by plants, the stable isotopic composition of plant leaf water is altered during transpiration (13, 21, 25). 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 (13, 17, 21, 25). A model of isotopic fractionation, which was originally developed by Craig and Gordon (4) for processes occurring during the evaporation of water from the ocean, has been used to model leaf water isotopic composition (5, 12, 13, 21, 22). The Craig-Gordon model predicts that the isotopic composition of leaf water is a function of three factors: (a) the isotopic composition of stem water, (b) the isotopic composition of AWV, and (c) the VPD (12, 13). This model has application, therefore, in studies of the VPD.

Measurements of the isotopic composition of plant cellulose could potentially be applied, therefore, in agricultural and ecological studies of A/E and in field studies of environmental stress effects on photosynthetic gas exchange (7, 10, 18). Such an application of stable isotope techniques would reduce the need for elaborate field, micrometeorological instrumentation that is normally required to measure VPD. However, before measurements of the hydrogen and oxygen isotopic composition of leaf cellulose can be used in conjunction with the Craig-Gordon evaporative enrichment model to estimate VPD, it is necessary to (a) demonstrate quantitative correspondence between the evaporative enrichment model predictions and the isotopic composition of leaf water under a range of environmental conditions and (b) determine the exact relationship between the isotopic composition of leaf water and plant cellulose. In this paper we make comparisons between the predictions of the evaporative enrichment model

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[3] Abbreviations: A/E, water-use efficiency; VPD, the leaf-air vapor pressure difference; AWV, atmospheric water vapor; SMOW, standard mean ocean water; D/H, deuterium/hnydrogen ratio.

and the observed isotopic composition of leaf water when plants were exposed to diurnal changes in temperature and humidity in a glasshouse. In addition, we determined the effects of mild water stress on the isotopic composition of leaf water at midday under glasshouse conditions.

MATERIALS AND METHODS

Plant Material and Growing Conditions

Cornus stolonifera L. plants (1-year-old cuttings in 4-L pots) were purchased from Native Plants Inc., Lehi, UT. The plants were in a dormant state in a nursery covered with snow. The plants were removed from the nursery and placed in a glasshouse at 2°C for 14 d and then transferred to a second glasshouse where the air temperature ranged daily from a maximum of 30°C to a minimum of 17°C. RH was uncontrolled and averaged approximately 30% during the day. The plants were not watered until leaf development was initiated and then received water in increasing amounts as leaf development proceeded until they were regularly watered twice a day. Experiments were conducted with the *Cornus* plants from May 2 through May 6, 1989.

Environmental Measurements and Water Collection

Measurements of leaf and air temperature were made with fine-wire copper-constantan thermocouples connected to a solid-state data logger (CR21X, Campbell Scientific Inc., Logan, UT). Measurements of air RH were made with a capacitance humidity probe (RH and temperature probe 207, Campbell Scientific Inc.) and measurement of photon flux density with a quantum sensor (LI 190SB, Li-Cor Inc., Lincoln, NE) both connected to the data logger. Measurements of leaf temperature, air temperature, and RH were made so that the partial pressure of water vapor in the leaf intercellular air spaces and ambient air could be calculated. AWV was collected by pumping (TD-3LL Pump, Brailsford and Co. Inc., Rye, NY) air through two glass traps cooled with an ethanol-dry ice slurry. Leaf and stem samples were removed from the plant and placed in a glass tube which was sealed with a rubber stopper and wrapped with parafilm. The plant samples were then frozen until water was extracted from the tissue using a cryogenic vacuum distillation apparatus (8).

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 (3). 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 δE or δS gas isotope ratio mass spectrometer. Precision of the hydrogen isotopic analysis was determined by repeated sampling of a laboratory standard that 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 δE and ±1.0‰ for the δS mass spectrometers (±SD).

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 (23). 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 δE and ±0.12‰ for the δS mass spectrometers during the course of this study.

Isotopic compositions are expressed using δ 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 (8).

Modeling Leaf Water Isotopic Composition

A model of isotopic fractionation, originally developed by Craig and Gordon (4) for evaporation from the ocean, was used to model leaf water isotopic composition. The model can be expressed in the following form (12, 13):

$$R_l = \alpha^* \left[\alpha_k R_x\left(\frac{e_i - e_s}{e_i}\right) + \alpha_{kb} R_x\left(\frac{e_s - e_a}{e_i}\right) + R_a\left(\frac{e_a}{e_i}\right)\right] \quad (1)$$

where R is the molar ratio of the heavy to light isotope and the subscripts l, x, and a refer to leaf water, stem water, and AWV, respectively; e is the partial pressure of water vapor 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. The regression equations listed by Majoube (15) were used to calculate values for the parameter α^* at specific leaf temperatures. α_k is the kinetic fractionation factor which is determined by the relative rates of molecular diffusion of the light and heavy isotope molecules in air. The values for α_k are: H/D = 1.025 and $^{16}O/^{18}O$ = 1.0285 (16). α_{kb} is the ratio of the diffusion of light and heavy isotope molecules in a boundary layer. The values for α_{kb} are: H/D = 1.017 and $^{16}O/^{18}O$ = 1.0189 (12, 13). Ignoring the influence of boundary layer effects, Equation 1 can be modified to:

$$R_l = \alpha^* \left[\alpha_k R_x\left(\frac{e_i - e_a}{e_i}\right) + R_a\left(\frac{e_a}{e_i}\right)\right] \quad (2)$$

For both Equations 1 and 2, the model predicts the isotopic composition of leaf water at steady state (12, 13).

To compare the observed isotopic composition of leaf water with that predicted by Equations 1 and 2, we calculate a parameter f as shown below:

$$\delta_{\text{Leaf}} = \delta_{\text{Model}} \cdot f + \delta_{\text{Stem}} \cdot (1 - f) \quad (3)$$

where δ_{Leaf} is the measured isotopic composition of leaf water, δ_{Model} is the isotopic composition of leaf water predicted by either Equations 1 or 2, and δ_{Stem} is the isotopic composition

of stem water. The parameter f in Equation 3 describes how close (as a proportion) the observed leaf water is to that predicted by the evaporative enrichment model.

Diurnal Change in Leaf Water-Stable Isotopic Composition

Measurement of the diurnal course of leaf and air temperatures, RH, and photon flux density were made for plants exposed only to natural light in a glasshouse. Leaf samples were collected every 1.5 h starting from 0530 to 2200 h. Stem samples were collected at 0530, 1300, and 2200 h. AWV was collected for a 3-h period starting at 1200 h.

Effect of Mild Water Stress on Leaf Water-Stable Isotopic Composition

Eight plants of similar size and appearance were chosen for the water stress experiment. Four of the plants were randomly selected to receive reduced water applications. On May 2 the four plants undergoing stress treatment were watered to pot capacity in the morning but were not watered in the afternoon. The four plants of the control treatment received normal watering (pot capacity) in both the morning and afternoon throughout the experiment. On May 3 the plants of the stress treatment received 200 mL of water at 1530 h. On May 4, RH and air temperature were measured continuously during midday, 1230 to 1400 h. Leaf temperature measurements were made for two leaves per plant for all eight plants in the experiment, and then the leaf was collected as described above. AWV and a stem sample from each plant were also collected. Plants undergoing the water stress treatment received 50 mL of water at 1700 h on May 4. A similar procedure for environmental measurement, plant sample collection, and AWV collection was followed on May 5. Plant stem water potential was measured with a pressure chamber (PMS Instrument Co., Corvallis, OR) at 1500 h.

RESULTS

Diurnal Change in Leaf Water-Stable Isotopic Composition

The *Cornus* plants were exposed to rather large, diurnal fluctuations in environmental conditions in the glasshouse (Fig. 1). Air temperature varied approximately 13°C during the course of the day. The variation in leaf temperature was even larger, approaching a 20°C change throughout the day. The RH decreased from a high of approximately 60% in the early morning to a low of approximately 30% during midday (Fig. 1).

Measurements of air and leaf temperature and RH were used along with the isotopic composition of both plant stem water and AWV to model the isotopic composition of leaf water using Equation 2 (Fig. 2). Equation 2 predicted that leaf water isotopic composition would be enriched in heavy isotopes relative to stem water for both oxygen and hydrogen isotopes (Fig. 2). Model calculations also indicated different patterns of diurnal change for oxygen and hydrogen isotopes (Fig. 2). For oxygen isotopes, the model predicted a diurnal increase of approximately 15‰ for the isotopic composition

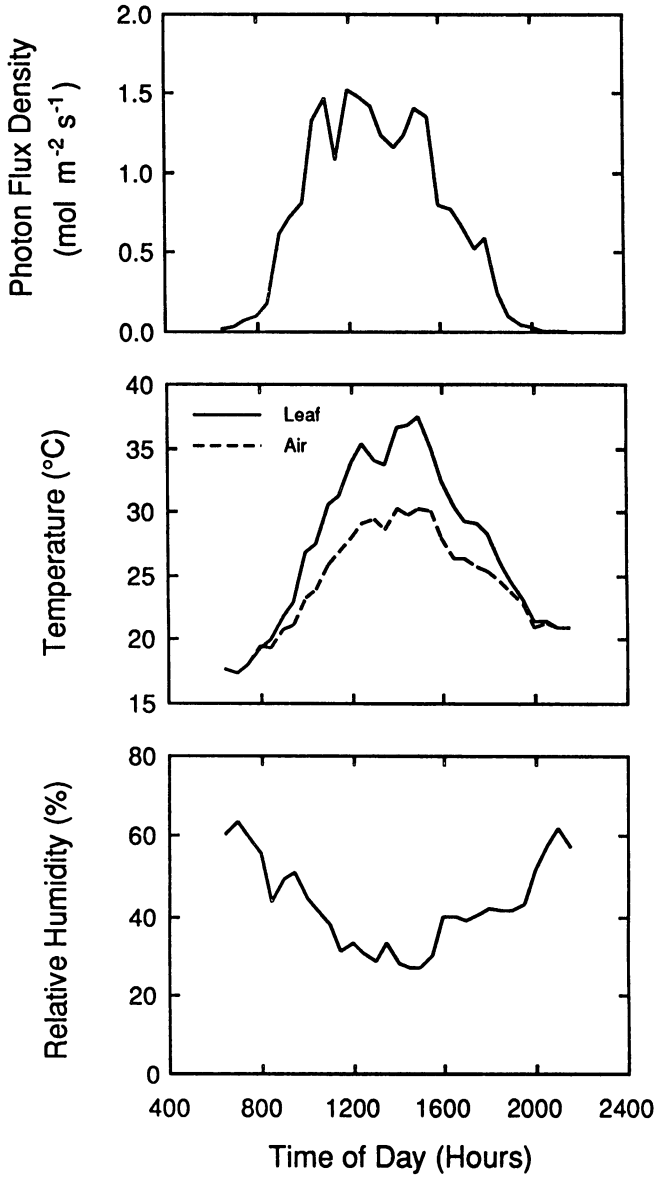

Figure 1. Diurnal changes in environmental parameters and leaf temperature of *C. stolonifera* under glasshouse conditions at the University of Utah on May 6, 1989. The partial pressure of water vapor in the air (e_a) remained relatively constant during the course of the day, averaging approximately 1.25 kPa. The VPD changed, however, in parallel with the large diurnal change in leaf temperature.

of leaf water (Fig. 2). In contrast, the model predicted a relatively constant hydrogen isotopic composition for leaf water (Fig. 2).

The observed leaf water isotopic composition for both oxygen and hydrogen isotopes was enriched above that of stem water, but the extent of isotopic enrichment was less than that predicted by the model at midday (Fig. 2). There were, however, qualitatively similar patterns of diurnal change in the observed and modeled leaf water isotopic compositions for both oxygen and hydrogen isotopes. The observed oxygen isotopic composition of leaf water increased approximately

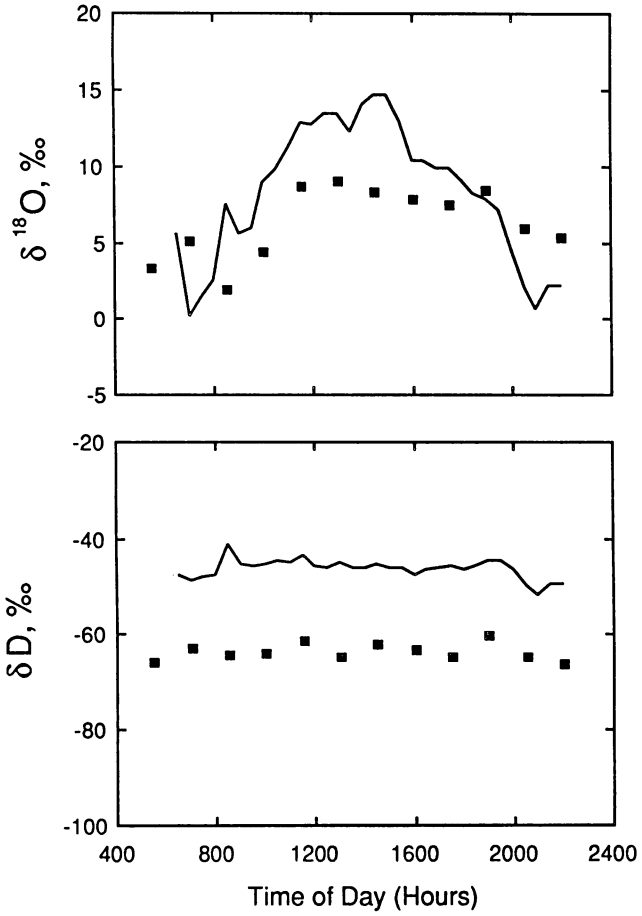

Figure 2. Diurnal pattern of change in the stable isotopic composition of leaf water in *C. stolonifera* under glasshouse conditions at the University of Utah on May 6, 1989. The solid line represents the modeled leaf water isotopic composition calculated with Equation 2. The solid symbols represent the observed isotopic composition of water extracted from *C. stolonifera* leaves. Stem water isotopic composition (mean ± SD, n = 3): $\delta^{18}O$ = −15.2‰ ± 0.2, δD = −120‰ ± 1. Isotopic composition of atmospheric water vapor: $\delta^{18}O$ = −22.6‰, δD = −144‰.

8‰ during the day in a pattern similar to that indicated by the model (Fig. 2). The observed hydrogen isotopic composition of leaf water remained relatively constant throughout the day in agreement with model calculations (Fig. 2).

Effect of Mild Water Stress on Leaf Water-Stable Isotopic Composition

Reduced water applications to the plants undergoing the stress treatment resulted in an increase in leaf temperature and a decrease in midday stem water potential (Tables I and II). The reduced water applications had no obvious effects on the physical appearance of the plants in the water stress treatment.

Both the hydrogen and oxygen isotopic composition of stem water was enriched in the plants of the stress treatment after 3 d of reduced water applications (Tables I and II). The enriched stem water values were likely a result of evaporation of water from the pots of the stressed plants.

There was no significant difference in the hydrogen isotopic composition of leaf water in the control and stress treatments after 2 d of stress (Table I). The δD value of leaf water in the stressed plants was 5‰ higher than that of the control plants after 3 d of stress (Table II). The difference in leaf water values between treatments, however, can be explained by the enriched stem water values of the stressed plants (Table II).

For the oxygen isotopic composition of leaf water, there was a significant difference between treatments after 2 d of stress, with the control treatment having the more enriched isotopic composition (Table I). This pattern occurred even though the $\delta^{18}O$ value of stem water was significantly higher in the stressed plants (Table I). No significant difference occurred between treatments, however, after 3 d of stress for the oxygen isotopic composition of leaf water, despite the fact that stem water isotopic composition was enriched in the stressed plants (Table II).

For both hydrogen and oxygen isotopic ratios, Equation 2 predicts a higher degree of leaf water isotopic enrichment than was actually observed in leaves (Tables I and II; Fig. 3). Calculations of f indicated that the hydrogen isotopic composition of leaf water ranged between 68 and 76% of the values predicted by Equation 2 (Tables I and II). The observed f values for oxygen isotopic compositions in the control plants were similar to that observed for hydrogen isotopes (Tables I and II). There was, however, a significant difference between control and stressed plants, with the stressed plants showing a greater difference between the modeled and observed oxygen isotopic composition of leaf water (Tables I and II).

For both control and water-stressed plants, there was a strong linear relationship between the oxygen and hydrogen isotopic composition for stem water, observed leaf water, and modeled leaf water (Fig. 3). For the control plants on both May 4 and 5, the observed leaf water isotopic compositions fall on or close to a line connecting the stem water and modeled leaf water isotopic compositions (Fig. 3, a and b). The data for the water-stressed plants were more variable, and the observed leaf water isotopic compositions do not fall directly on a line connecting the stem water and modeled leaf water isotopic compositions (Fig. 3, c and d).

DISCUSSION

Diurnal Change in Leaf Water-Stable Isotopic Composition

The evaporative enrichment model predicted different patterns of diurnal change for the oxygen and hydrogen isotopic composition of leaf water (Fig. 2). These different patterns result because of differences in the magnitude of the kinetic and equilibrium fractionation factors for hydrogen and oxygen isotopes (15, 16, 22). 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 for oxygen, for which the kinetic fractionation factor is quantitatively most important ($^{16}O/^{18}O$, α_k = 1.0285, α^* = 1.0094 at 25°C). During the day, as leaf temperature increases and RH decreases, the VPD in-

Table I. *Effect of 2 d of Mild Water Stress on Physiological Characteristics and Leaf and Stem Water Isotopic Composition in C. stolonifera at Midday under Glasshouse Conditions*

Average environmental conditions were: air temperature 26.1°C; RH 28.2%; photon flux density 1600 μmol m^{-2} s^{-1}; atmospheric water vapor $\delta D = -154‰$, $\delta^{18}O = -23.0‰$. Modeled leaf water isotopic compositions were calculated with Equation 2. Values are means ± SD. For all values, $n = 8$, except for stem water isotopic composition, for which $n = 4$. Significance tests were based on the result of a Kruskal-Wallis analysis of variance.

	Control	Stress	Significance
Leaf temperature (°C)	30.7 ± 2.2	35.5 ± 1.9	[a]
Stem water isotopic composition			
δD (‰)	−122 ± 1	−119 ± 2	NS
$\delta^{18}O$ (‰)	−15.2 ± 0.2	−13.4 ± 0.4	[a]
Leaf water isotopic composition			
Observed δD (‰)	−71 ± 2	−69 ± 3	NS
Modeled δD (‰)	−46 ± 2	−45 ± 1	NS
D/H *f* value	0.68 ± 0.03	0.68 ± 0.02	NS
Observed $\delta^{18}O$ (‰)	8.2 ± 0.4	5.8 ± 2.0	[a]
Modeled $\delta^{18}O$ (‰)	18.2 ± 1.0	20.8 ± 0.6	[a]
$^{18}O/^{16}O$ *f* value	0.70 ± 0.03	0.56 ± 0.06	[a]

[a] $P < 0.05$.

creases and, therefore, the evaporative enrichment model tends to predict a higher heavy isotope content of leaf water. However, the equilibrium fractionation factor is temperature dependent, *i.e.* the higher the temperature the lower the value of the equilibrium fractionation factor (15). For hydrogen isotopes, for which the equilibrium fractionation factor is dominant, the tendency for increased evaporative isotopic enrichment caused by an increased VPD is offset by the decrease in the equilibrium fractionation factor as leaf temperature increases during the day. In contrast, the kinetic fractionation factor is dominant for oxygen isotopes so that the decline in the equilibrium fractionation factor with the daily increase in leaf temperature is not large enough to offset the increase in the VPD driving isotopic enrichment via kinetic fractionation.

The observed isotopic composition of leaf water followed qualitatively similar patterns of diurnal change as that predicted by the evaporative enrichment model for oxygen and hydrogen isotopes. Complete quantitative agreement between the modeled and observed isotopic composition of leaf water was not obtained, however. At midday, the model always predicted a higher degree of heavy isotope enrichment than was actually observed in leaves (Fig. 2). In several previous studies, the Craig-Gordon evaporative enrichment model was observed to overestimate the degree of isotopic enrichment observed in leaves (1, 2, 14, 19, 20, 22, 24, 26). There are at

Table II. *Effect of 3 d of Mild Water Stress on Physiological Characteristics and Leaf and Stem Water Isotopic Composition in C. stolonifera at Midday under Glasshouse Conditions*

Average environmental conditions were: air temperature 27.7°C; RH 21.5%; photon flux density 1270 μmol m^{-2} s^{-1}; atmospheric water vapor $\delta D = -149‰$, $\delta^{18}O = -21.9‰$. Modeled leaf water isotopic compositions were calculated with Equation 2. Values are means ± SD. For all values, $n = 8$, except for stem water potential and stem water isotopic composition, for which $n = 4$. Significance tests were based on the result of a Kruskal-Wallis analysis of variance.

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

[a] $P < 0.05$.

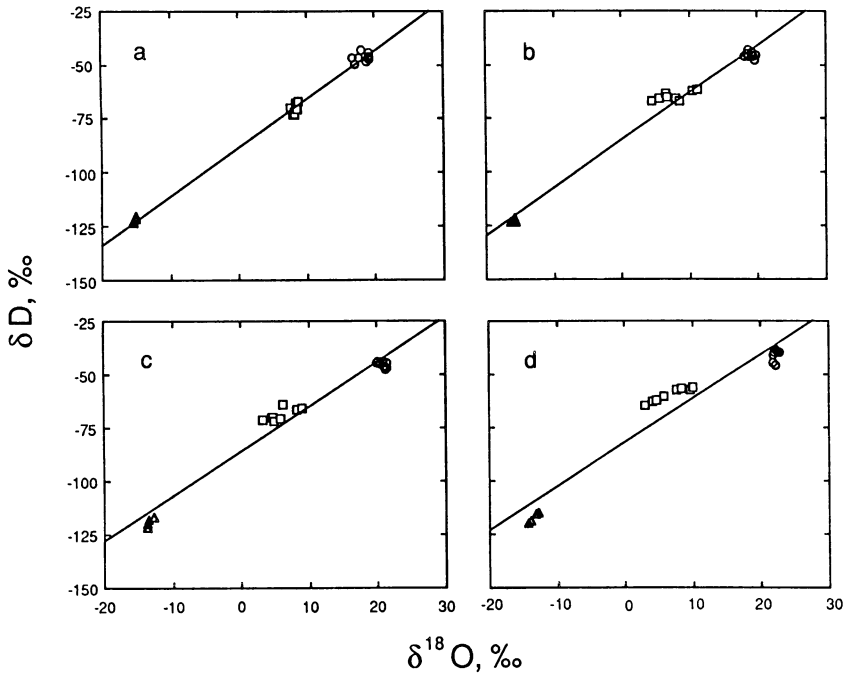

Figure 3. Relationship between the oxygen and hydrogen isotopic compositions of stem water (△), leaf water (□), and modeled leaf water (○) at midday under glasshouse conditions. Modeled values were calculated with Equation 2. a, Data from control plants on May 4; $\delta D = 2.28\ \delta^{18}O - 88.4$, $r^2 = 0.995$. b, Data from control plants on May 5; $\delta D = 2.23\ \delta^{18}O - 85.4$, $r^2 = 0.982$. c, Data from water-stressed plants on May 4; $\delta D = 2.10\ \delta^{18}O - 86.2$, $r^2 = 0.967$. d, Data from water-stressed plants on May 5; $\delta D = 2.06\ \delta^{18}O - 81.9$, $r^2 = 0.931$. Environmental conditions are listed in Tables I and II.

least three possible factors contributing to the discrepancy between the modeled and observed leaf water isotopic composition, as will be discussed below.

First, a major assumption in the derivation of the evaporative enrichment model (Eqs. 1 and 2) is that isotopic steady state is reached (12, 13, 22). 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. If leaf water is not at isotopic steady state during midday, the observed leaf water δ values should decline below that predicted by the model. The assumption of isotopic steady state was not verified in this study. In previous experiments with *Phaseolus* plants, approximately 1 h of constant environmental conditions was required for leaf water to reach isotopic steady state (12). This time course to isotopic steady state was measured after a rather drastic change in environmental conditions from darkness to high light, however. Less time may be required to reach isotopic steady state when tracking the normal, gradual changes in environmental conditions in the glasshouse. 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 (12, 13, 21). The *Cornus* plants used in this study have relatively thin leaves with low absolute water contents which should allow for leaf water to turnover quickly. The relatively constant hydrogen isotopic composition observed for leaf water during the day suggests that isotopic steady state was reached (Fig. 2). We would routinely expect that, if isotopic steady state had been reached for hydrogen isotopes, then the oxygen isotopic composition of leaf water should be at a steady state as well. Because of the complex diurnal pattern observed for the oxygen isotopic composition of leaf water, however, it is not possible to determine whether leaf water was at isotopic steady state for oxygen isotopes from the data shown in Figure 2 alone.

Second, Equation 2, which was used to predict the leaf water isotopic composition in this study, does not include boundary layer effects on leaf water isotopic enrichment. Equation 2 predicts more isotopic enrichment than does Equation 1, which includes boundary layer effects (12, 13). The magnitude of the difference between the two equations is dependent on boundary layer conductance, leaf temperature, the VPD, and the particular element being considered (12). Air movement is restricted in the glasshouse compared to field situations, so it is expected that boundary layer conductance would be low, although it was not measured in this study. Assuming an extreme situation in which boundary layer conductance is one-half the value for stomatal conductance, the maximal difference between Equations 1 and 2 is approximately 4‰ for D/H ratios under conditions occurring during midday in the glasshouse (12). Boundary layer effects cannot completely explain, therefore, the discrepancy between the observed and modeled hydrogen isotopic composition of leaf water (Fig. 2). For oxygen isotopes, the maximal difference between Equations 1 and 2 is approximately 5‰ under conditions occurring at midday in the glasshouse, assuming that boundary layer conductance is one-half the value of stomatal conductance (12). Boundary layer effects could, therefore, explain the majority of the discrepancy between the observed and modeled oxygen isotopic composition of leaf water during midday if boundary layer conductance was only one-half the value of stomatal conductance (Fig. 2). It is unlikely, however, that such an extremely low boundary layer conductance occurred in the glasshouse. We suggest, therefore, that low boundary layer conductance was not the only factor contributing to the discrepancy between the modeled and observed leaf water isotopic compositions.

Third, in a previous investigation conducted under controlled environment conditions, in which the assumption of isotopic steady state was verified and boundary layer effects

on leaf water enrichment were accounted for, there was still a small discrepancy between the observed and modeled leaf water isotopic compositions (12). The extent of the discrepancy between the modeled and observed leaf water isotopic composition was a strong linear function of the leaf transpiration rate (12). It was proposed that the discrepancy between the modeled and observed leaf water isotopic composition results 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. A model of leaf water isotopic enrichment incorporating the above effects was originally suggested by G. D. Farquhar (as described in ref. 21). When transpiration rate is high, the bulk flow of unfractionated water into the leaf may override some of the back diffusion of heavy isotope molecules from the sites of evaporation, resulting in a significant discrepancy between the modeled and observed leaf water isotopic compositions. It is expected that the well-watered *Cornus* plants, grown under favorable conditions in the glasshouse, had high transpiration rates which may have, in part, contributed to the difference between the modeled and observed leaf water isotopic compositions.

Effect of Mild Water Stress on Leaf Water-Stable Isotopic Composition

There was no effect of mild water stress on the hydrogen isotopic composition of leaf water in *Cornus* (Tables I and II). After 3 d of water stress, the δD value of leaf water in the stressed plants was 5‰ higher than that of the control plants. The difference in leaf water values between treatments, however, can be explained by the enriched stem water δD values in the stressed plants (Tables I and II). Yakir *et al.* (24) also observed that the hydrogen isotopic composition of leaf water was enriched approximately 5‰ in *Gossypium* plants receiving reduced water applications under field conditions. The isotopic composition of stem water was unfortunately not measured in the study with *Gossypium* plants, however (24). Without stem water measurements, it is not possible to determine whether the drought effect was due to enriched stem water values or whether there was an actual effect on leaf water isotopic composition.

For the oxygen isotopic composition of leaf water, there was either no significant difference between control and stress plants or the stressed plants had lower δ^{18}O values, despite the fact that the isotopic composition of stem water in the stressed plants was enriched above that observed in the control plants (Tables I and II). This pattern not only contrasts with that observed for the hydrogen isotope data but is different from patterns observed in other studies with *Phaseolus* and *Gossypium* plants in which the oxygen isotopic composition of leaf water was enriched in the water-stressed plants (11, 24). Mild water stress would cause a reduction in the leaf transpiration rate and, therefore, could increase the time required for leaf water to reach isotopic steady state (12). If isotopic steady state had not been reached during midday, the leaf water isotopic composition should decrease below that predicted by the model, and the discrepancy between modeled and observed data should be larger in the stressed plants than in the control plants. A similar pattern of response should also occur for the hydrogen isotope data, however, which was not observed. The time course to isotopic steady state may not be the same for both hydrogen and oxygen isotopes. Perhaps, because of the different relative magnitudes of the two fractionation factors for oxygen and hydrogen isotopes, a longer time is required for the oxygen isotopic composition of leaf water to reach isotopic steady state. This would explain why the observed leaf water isotopic compositions are offset from a line connecting the stem water and modeled leaf water isotopic compositions for the water-stressed plants in Figure 3.

Implications for Use of the Model in Agricultural and Ecological Studies

For the evaporative enrichment model to be applied in a precise, quantitative manner to studies of VPD, the following three conditions must be met (12, 13): (a) leaf water must be at isotopic steady state, (b) the isotopic composition of AWV and plant stem water must be known, and (c) there must be quantitative correspondence between the evaporative enrichment model and the isotopic composition of leaf water over a range of environmental conditions. There is little information presently available that indicates how close the isotopic composition of leaf water is to isotopic steady state under natural field conditions. Measurements of the isotopic composition of AWV and plant stem water are not regularly made for any plant species or field site. Even when the above conditions are satisfied, it appears that there is not complete agreement between the evaporative enrichment model and the isotopic composition of leaf water (12). 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, however, 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 (12, 13).

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