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# Hydrogen and oxygen isotope ratios of tree-ring cellulose for riparian trees grown long-term under hydroponically controlled environments

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**Abstract** Saplings of three riparian tree species (alder, birch and cottonwood) were grown for over 5 months in a hydroponics system that maintained the isotopic composition of source water in six treatments, ranging from –120 to +180‰ δD and –15 to +10‰ $\delta^{18}O$. The trees were grown in two greenhouses maintained at 25°C and at either 40 or 75% relative humidity, creating differences in transpiration rates and leaf water isotopic evaporative enrichment. The cellulose produced in the annual growth ring was linearly related to source water with differences in both slope and offset associated with greenhouse humidity. The slope of the isotopic composition of source water versus tree-ring cellulose was less than 1 for both δD and $\delta^{18}O$ indicating incomplete isotopic exchange of carbohydrate substrate with xylem water during cellulose synthesis. Tests using the outer portion of the tree-ring and new roots were similar and showed that the tree-ring values were representative of the cellulose laid down under the imposed environmental conditions. The fraction of H and O in carbohydrate substrate that isotopically exchange with medium water was calculated to be 0.36 and 0.42 respectively, and biochemical mechanisms for these observed fractions are discussed. A mechanistic model of the biochemical fractionation events for both δD and $\delta^{18}O$ leading to cellulose synthesis was robust over the wide range of cellulose stable isotope ratios. The experimental results indicate that both water source and humidity information are indeed recorded in tree-ring cellulose. These results help to resolve some of the disparate observations regarding the interpretation of stable isotope ratios in tree-rings found in the literature.

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## Introduction

The stable isotopes in meteoric water vary geographically and seasonally, since the condensation temperature of precipitation directly affects its isotopic composition (Dansgaard 1964). This water is taken up by plants and can be used as a tracer of plant water source (Ehleringer and Dawson 1992). The isotopic composition of tree-ring cellulose reflects, to a first approximation, precipitation inputs (Yapp and Epstein 1982; Lawrence and White 1984; White et al. 1994), and many studies have used the variations in δD and $\delta^{18}O$ in tree-rings for temperature reconstruction (Schiegl 1974; Gray and Thompson 1976; Epstein and Yapp 1977; Feng and Epstein 1994). However, the slope of the regression for the isotopic composition of tree-ring cellulose and source water is often 0.8–1.0, implying that tree-rings may record additional information related to biological processes that respond to local environmental variation. Unfortunately, there has been substantial confusion over what information, both biological and environmental, is contained in the isotopic composition of tree-ring cellulose (DeNiro and Cooper 1990; Edwards 1990). For example, some studies report that humidity information was recorded in tree-ring cellulose (Edwards and Fritz 1986; Lipp et al. 1993), while others find no evidence for a humidity signal (DeNiro and Cooper 1989; White et al. 1994; Terwilliger and DeNiro 1995).

Many studies have correlated environmental conditions (water source, humidity, temperature, precipitation) with the isotopic composition of the corresponding annual growth ring to extract environmental information recorded in cellulose (Schiegl 1974; Gray and Thompson 1976; Epstein and Yapp 1977; Burk and Stuiver 1981). Interpretation of such data is potentially biased by the assumptions made and the choice of environmental information included. Tree-rings are not a direct recorder of

the isotopic composition of precipitation, since there are many steps along the path from source water to cellulose. In addition, the assumption that mean annual precipitation represents a correct, adequate descriptor of the water used by plants has been called into question by some studies (Dawson and Ehleringer 1991; Ehleringer et al. 1991; Sternberg et al. 1991; Busch et al. 1992; Flanagan et al. 1992; Thorburn and Walker 1993; Valentini et al. 1992), because of both the biseasonal nature of precipitation in some ecosystems and differential root activities.

White et al. (1985) observed that pine trees from wet and dry microsites, which presumably receive similar precipitation inputs, exhibited differences in their cellulose δD values, suggesting that water stress and/or humidity differences may alter leaf water δD and thus tree-ring cellulose δD. The path from precipitation to tree-ring cellulose is complicated and thus correlation studies may not be sufficient to answer some of these questions regarding the information contained within the tree-ring and that experimental approaches are needed.

Unfortunately, previous experimental studies that have attempted to address the mechanisms by which hydrogen and oxygen isotopes of water are incorporated into cellulose have limited applications to tree-rings. DeNiro and Cooper (1989) using potato and Terwilliger and DeNiro (1995) using avocado have concluded that at the time of cellulose synthesis there is complete exchange with the medium water, wiping out all previous fractionation events and making the cellulose a direct measure of source water. However, the processes of cellulose synthesis in both potato and avocado are not analogous to that which takes place in the cambium of a tree. Both systems could utilize substantial amounts of stored carbohydrates which have different pathways to cellulose and potentially different fractionation events. There are also questions regarding the length of the experiment and the possibility of stem and tuber evaporative enrichment in these systems. Thus, it is still unclear whether the isotopic composition of tree rings simply reflects source water or whether other useful paleoclimatic information might be included as well.

To address some of these lingering questions regarding the nature of environmental information that is recorded in the stable isotope ratios of tree-ring cellulose, a mechanistic understanding of how source water inputs are transformed into cellulose is needed. The first step towards that goal and the objective of this study is to determine the relationships between source water and tree-ring cellulose obtained from a long-term experimental system where trees are utilized and annual rings produced under controlled environmental conditions (including differences in humidity and variation in source water input). There is reason to believe that shorter-term studies in which stored substrates contribute to stem cellulose may be inappropriate because of variations in the exchangeable H and O isotopes, depending on the extent to which carbohydrate or lipids contribute to transported sugar and the extent to which triose-phosphates are involved.

Roden et al. (1999) developed a model to describe the fractionation events involved between water uptake and cellulose synthesis. This modeling effort included two components: (1) the prediction of leaf water evaporative enrichment using a modified version of the Craig and Gordon (1965) model developed by Flanagan et al. (1991) and environmental inputs; and (2) the prediction of cellulose isotopic composition based on biochemical fractionation factors and isotopic exchange with medium water. Another objective of this study was to test the predictive power of these models using the environmental information collected and the cellulose produced under controlled experimental conditions.

## Materials and methods

### Plant material and growth conditions

Two- to three-year-old saplings of three tree species commonly found in riparian zones, alder *(Alnus incana* L. Moench), water birch *(Betula occidentalis* Hook) and cottonwood *(Populus fremontii* Wats) were obtained from local nurseries. All saplings were dormant or just beginning the earliest stages of spring flush prior to the start of the experiment. The plants were grown for over 5 months (156 days) to ensure the production of a new tree-ring.

Plants were grown in two greenhouses, each maintained at 25°C. One greenhouse was operated at low (ambient humidity, approximately 40%) and the second at high relative humidity (greenhouse humidified to approximately 75%) by adding water vapor with a misting humidifier (Humidifan, Jaybird Manufacturing, State College, Pa., USA). A Vaisala humidity sensor and thermistor (model HMP-35C, Campbell Scientific, Logan, Utah, USA) measured relative humidity and air temperature every 5 min in each greenhouse and a CR21x data logger (Campbell Scientific, Logan, Utah, USA) recorded these data as hourly averages over the entire experiment. Humidity and temperature gradients naturally occur with height. The humidity measurements were corrected using empirical relationships, derived from measured humidity gradients, to represent actual humidity and temperature at plant height. Periodically over the course of the growing season, stomatal conductance and transpiration rates were measured using a diffusion porometer (Li Cor 1600, Lincoln, Neb., USA) on leaves sampled from all water source treatments in both greenhouses.

### Hydroponics setup

The trees were grown hydroponically in 190-l tanks (stock tanks, Rubbermaid Inc., Wooster, Ohio, USA) with aquarium pumps and airstones providing oxygen to the roots. Each tank contained nine plants (three of each species) and were fixed in position by 5.5-cm-thick closed cell foam attached to a wooden frame that allowed the lifting of all nine trees and the foam for easy access to the tank. The foam was shaped to fit tightly on the tank preventing evaporation. Nutrients were provided to the roots as a 1/10 Hoagland's solution. The trees were grown in one of six source water treatments: –120/–15‰ ($\delta D/\delta^{18}O$), –60/–10, 0/–5, 60/0, 120/5 and 180/10. Each treatment was replicated twice in each greenhouse for a total of 24 tanks and 216 trees (3 individuals×3 species×6 source water treatments×2 replicate tanks×2 greenhouse humidities). A 600-l tank was used to mix mineral nutrients with Salt Lake City municipal water (–120/–15‰, $\delta D/\delta^{18}O$) along with $D_2O$ and 10 atom% $^{18}O$ enriched water (Europa Scientific, Crewe, UK). The four tanks (2 replicates×2 humidities) from each treatment were then filled from this larger volume to ensure that they all contained the same source water. Due to the high expense of $^{18}O$-enriched water, desalinized sea water (–3/–0.5‰, $\delta D/\delta^{18}O$) using a reverse osmosis water purification unit and seawater taken

from Port Hueneme, California, was mixed with Salt Lake City water to reduce the amount of $^{18}O$-enriched water required. The water depletion by transpiration was measured biweekly, and on average 19–38 l of water had to be added to each tank. Nutrients and enriched water were added to a 190-l mixing tank and used to top off the four tanks of each water source treatment. Every 8 weeks the water in each tank was completely changed. At the start of the experiment the tanks were randomized within each greenhouse. Thereafter the tanks were moved systematically to a new greenhouse position three times a week. Every 2 weeks the tanks were rerandomized at the time of water additions and the moving process began again. This procedure limited the time any tank spent at any one position in the greenhouse, reducing potential problems associated with pseudo-replication.

### Isotope sampling

Approximately 5 ml of water was sampled from each tank both prior to and after water additions for analysis of δD and $\delta^{18}O$. Leaf material, with the mid-vein removed, was placed into a glass vial, sealed with parafilm and placed into a freezer (–5°C) until the water could be extracted for isotopic analysis. At the same time as leaf collection (four sampling periods), the atmospheric water vapor was sampled from both greenhouses using a pump to draw air through a glass trap submerged in a mixture of ethanol and dry ice (–78°C).

At the end of the growing season, the main stems were dried and the annual growth ring produced during the experiment was sampled. The bark was removed and approximately the outer 2/3 of the tree-ring was sampled to avoid potential contamination from previous year's cellulose. The wood was then ground to pass a 40-mesh screen using a Wiley mill (Arthur H. Thomas Co. Philadelphia, Pa., USA). To test for contamination from previous year's carbon, the tree-ring from the most enriched treatment was sampled a second time by cutting only the outer one-third of the tree-ring. New roots were sampled from cottonwood saplings (since they produced long hair-like white roots that were easy to distinguish from old roots and easier to sample than those of the other species) as an additional test of the cellulose model.

### Sample preparation and analysis

Leaf water was obtained by cryogenic extraction as described by Ehleringer and Osmond (1989). The sample was frozen in liquid nitrogen (–l90°C) and once evacuated, the system was then isolated from the vacuum pump and immersed in boiling water. The water from the leaf was then collected in a tube immersed in liquid nitrogen until all water was extracted. The δD of water samples from the tanks, leaves and atmospheric vapor were obtained by reducing the H in 2 µl of $H_2O$ to $H_2$ using 100 mg of a Zn catalyst (J. Hayes, Indiana University) in a 500°C oven (modification of Coleman et al. 1982). The $\delta^{18}O$ of water samples were obtained by equilibrating 0.5 ml of water with approximately 16 kPa of $CO_2$ in a 25°C water bath for 48 h. The $CO_2$ was extracted cryogenically using liquid nitrogen and dry-ice/ethanol traps. Both the $H_2$ and $CO_2$ were analyzed on a Finnagan MAT delta S isotope ratio mass spectrometer (San Jose, Calif., USA) with a precision of ±1‰ for δD and ±0.2‰ for $\delta^{18}O$.

The $\delta^{18}O$ recorded in tree-rings (or roots) are obtained from α-cellulose (Leavitt and Danzer 1992) which involves a delipification step (using toluene and ethanol), boiling in water (to remove soluble sugars), bleaching with sodium chlorite and acetic acid (to remove lignin and proteins) and washing in a strong alkaline solution (to remove hemicellulose). Approximately 1.2 mg of α-cellulose is then placed in a silver capsule and converted to CO by pyrolysis (Saurer et al. 1998) in a hot (1100°C) quartz combustion furnace (Carlo-Erba interface) and separated from other gases in a 1-m molecular sieve gas chromatograph (GC) column connected to a Finnagan MAT delta S isotope ratio mass spectrometer. Repeated sampling was utilized to reduce memory effects and resulted in a precision of ±0.4‰.

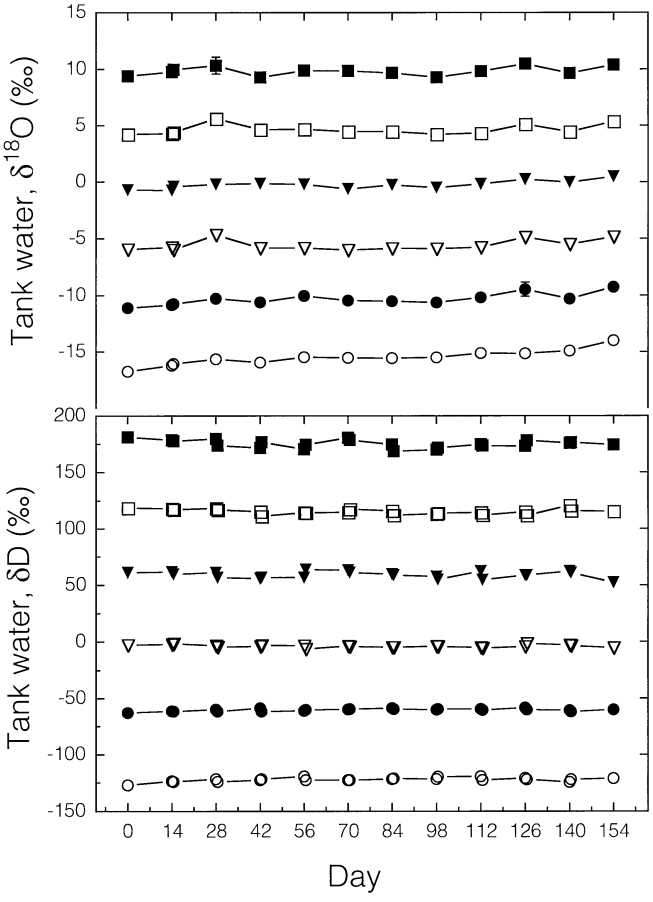


**Fig. 1** The isotopic composition of tank water over the course of the experiment. Values are means (*n*=4) and SEs (most SEs are contained within the symbol). *Two symbols* present on any day indicate sampling both prior to and after tank water additions

To obtain the δD recorded in tree-ring cellulose the α-cellulose obtained, as described above, was nitrated to remove the exchangeable hydrogens. The α-cellulose was placed in a flask with a solution of nitric acid and acetic anhydride. The material was then washed, dissolved in acetone (to obtain purified tri-nitrated cellulose) and freeze-dried for storage. Approximately 11 mg of nitrated cellulose was placed in a pyrex tube with 1 g of cupric oxide, evacuated, sealed and combusted for 3 h at 520°C. The resulting gases were separated cryogenically using liquid nitrogen and dry-ice/ethanol traps to move the water vapor to a tube containing the zinc catalyst for hydrogen reduction as described above.

## Results

Tank water isotopic composition for each treatment was well maintained throughout the experiment (Fig. 1). The δD of tank water was sampled both prior to and after any water additions to track evaporative enrichment in source water. Clearly, only minor changes occurred in source water isotopic composition between each water addition and treatment differences were maintained throughout the experiment (Fig. 1). Since no significant differences in the δD or the $\delta^{18}O$ of tank water were observed between replicates and greenhouses, the data are

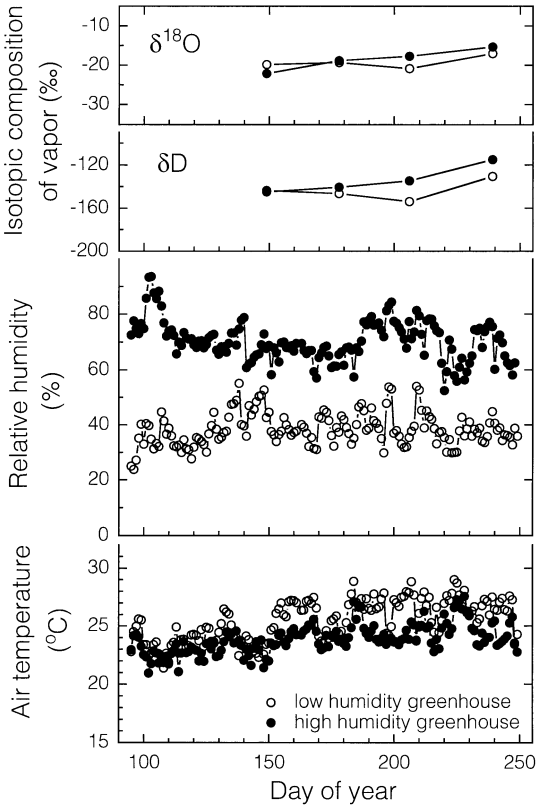


**Fig. 2** Greenhouse environmental parameters over the course of the experiment

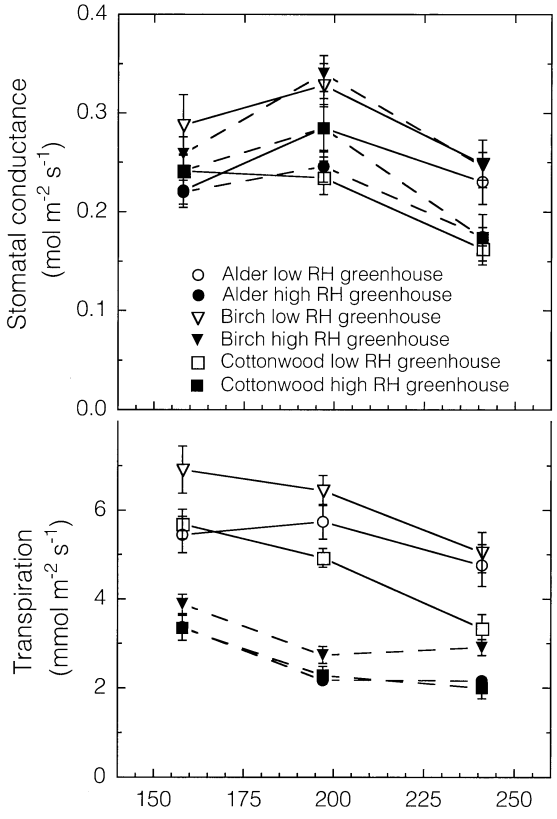


**Fig. 3** Leaf stomatal conductance and transpiration rates over the course of the experiment. *Values* are pooled means over all source water treatments and SEs

pooled means of four tanks (SE bars are generally within the symbol).

The mean daily greenhouse air temperature varied between 21 and 29°C (Fig. 2). The high humidity greenhouse had slightly lower air temperatures associated with the enhanced evaporative cooling from the humidification system. However, differences between greenhouses were rarely more than a few degrees. The relative humidity in the "high humidity" greenhouse was on average 25–30% higher than the "low humidity" greenhouse (Fig. 2). The humidity in both greenhouses varied depending on ambient humidity input from outside (evaporative cooling units). The isotopic composition of atmospheric vapor remained relatively stable (Fig. 2, δD between –115 and –155‰ and $\delta^{18}O$ between –15 and –22‰) with some slight increases in both δD and $\delta^{18}O$ later in the experiment. Although the high humidity greenhouse tended to have higher atmospheric vapor δD values later in the experiment, the trend was not repeated for $\delta^{18}O$ and no replication was performed to enable statistical analysis of these differences.

The stomatal conductance and transpiration rates of leaves were measured mid-morning on three days during the experiment (Fig. 3). No differences between water source treatments were detected so the data are pooled across all six treatments. Most of the differences in stomatal conductance between species and greenhouses were not significant. However, the leaves clearly transpired more water in the low than the high humidity greenhouse for all species (Fig. 3) which is one of the factors that created differences in leaf water evaporative enrichment between greenhouses of 5–13‰ in δD and 3–6‰ in $\delta^{18}O$ (–120/–15‰, $\delta D/\delta^{18}O$, treatment only, data not shown).

The δD of tree-ring cellulose varied linearly with source water δD (Fig. 4). Plants grown in the low humidity greenhouse showed a greater enrichment in tree-ring cellulose δD than those grown in high humidity (differences in slope and intercept were statistically significant, $P<0.05$). The $\delta^{18}O$ of tree-ring cellulose showed a similar pattern although there was less of a change in slope between the high and low humidity greenhouses (Fig. 5). The differences in tree-ring cellulose δD and $\delta^{18}O$ between the humidity treatments was consistent with the magnitude of leaf water enrichment observed for the –120/–15‰ ($\delta D/\delta^{18}O$) treatment. Birch and cottonwood had similar slopes for both δD and $\delta^{18}O$ and low and high humidity. The similarity in slope between species implies that the mechanisms of incorporation of heavy isotopes into the cellulose of a tree-ring is not species sensitive. Alder did have slightly different slopes, but it also was the species that showed the greatest variation in measured values, and was the species that did not appear as healthy as the other two in the hydroponics system. The slope of the

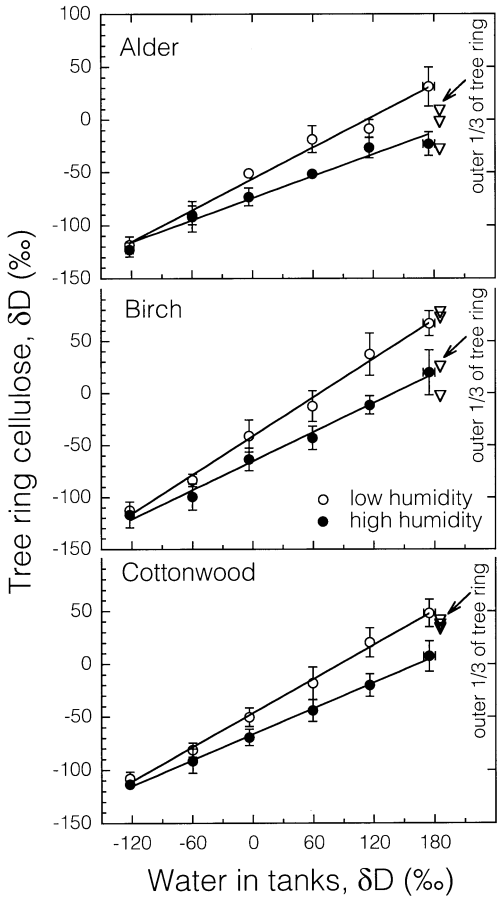


**Fig. 4** The relationship between source water and tree-ring cellulose δD for three species and two humidities. *Open triangles* indicate the values for the outer one-third of the tree-ring for both humidities (180‰ treatment only); all other points are the data from the outer two-thirds of the tree-ring. *Values* are means and SDs. The $r^2$ values for all linear regressions ranged between 0.95 and 0.99

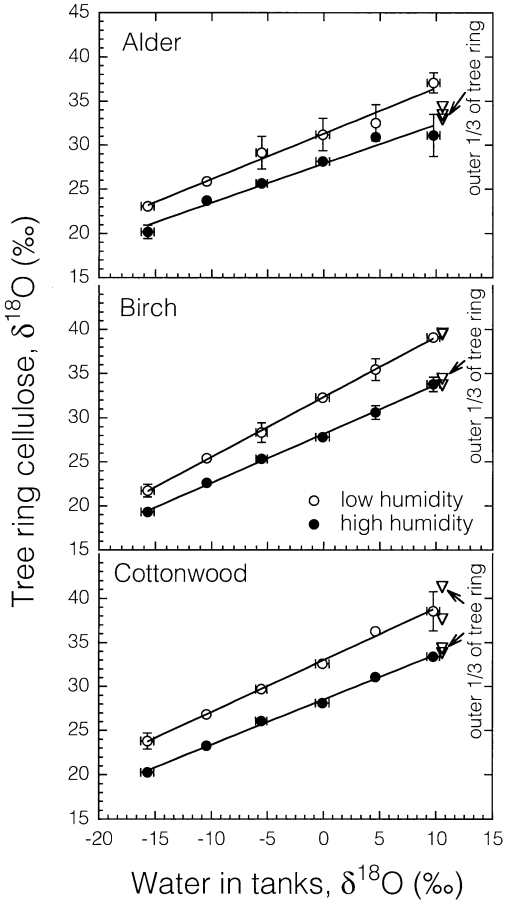


**Fig. 5** The relationship between source water and tree-ring cellulose $\delta^{18}O$ for three species and two humidities. *Open triangles* indicate the values for the outer one-third of the tree-ring for both humidities (10‰ treatment only) all other points are the data from the outer two-thirds of the tree-ring. *Values* are means and SDs. The $r^2$ values for all linear regressions ranged between 0.96 and 0.99

regression line for cellulose versus source water did not indicate a 1:1 relationship as often observed in field studies.

Two tests were performed to ensure that the tree-rings sampled were truly representative of the cellulose laid down during the experiment. The first test was to ensure that previous years' carbon did not enter into the current year's tree-ring sample. A core sample was extracted and the cellulose δD and $\delta^{18}O$ values were used for comparisons (assumed to be representative of the nursery environment the saplings were grown in prior to this experiment). When sampling the current year's tree-ring, only the outer two-thirds of the ring was sampled to eliminate possible contamination from previous years carbohydrates. However, to test this assumption further, the cellulose for the most isotopically enriched treatment (+180/+10‰, $\delta D/\delta^{18}O$) was re-sampled by cutting only the outer one-third of the tree-ring. If previous year's carbohydrates were influencing the isotopes in these tree-rings, then the most enriched treatment should have shown the largest positive effect with a reduction in the dilution. Clearly, the data for the outer one-third of the tree-ring (Figs. 4, 5) fall within the variation found in the data collected from the outer two-thirds of the tree-ring. And thus the tree-ring data presented (Figs. 4, 5) represent cellulose production under the imposed experimental conditions.

A second test used cellulose produced in new roots. Only cottonwood roots were sampled due to the ease of identifying and ensuring that only new roots were sampled. Half-way through the experiment the new roots from all cottonwood saplings were cut. At the end of the experiment the new roots were sampled again. The cellulose from these end-of-season roots should contain no isotopic signatures from previous year's carbohydrates. A plot of tree-ring cellulose versus new root cellulose (Fig. 6) demonstrates that cellulose samples collected from the outer two-thirds of the treering were not biased by carbohydrates produced in the nursery environment and that these treering data faithfully record the cellulose produced under the imposed environmental conditions.

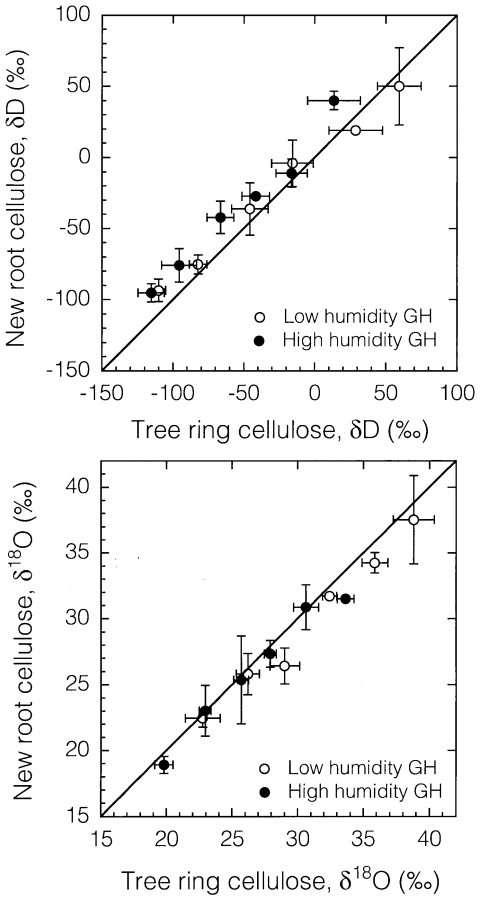


**Fig. 6** The relationship between the δD and $\delta^{18}O$ of tree-ring cellulose and new root cellulose for the cottonwood saplings only. *Values* are means and SDs. The *line* represents a 1:1 relationship

## Discussion

### Comparisons of current and previous studies on the stable isotopes in cellulose

The heavy hydrogen and oxygen isotopic composition of tree-ring cellulose did not vary with source water δD and $\delta^{18}O$ in a 1:1 fashion, which differs from the findings of other studies (Epstein and Yapp 1977; Burk and Stuiver 1981; Yapp and Epstein 1982; Lawrence and White 1984; White et al. 1994). The lack of a 1:1 relationship between source water and cellulose δD and $\delta^{18}O$ as well as the effects of humidity on the isotopic composition of cellulose (Figs. 4, 5) both imply that there was incomplete isotopic exchange with xylem water at the time of cellulose synthesis. These results differ from the conclusions of DeNiro and Cooper (1989) and Terwilliger and DeNiro (1995), but agree with the results of Luo and Sternberg (1992). A humidity influence on the isotope ratios in cellulose has also been reported by Burk and Stuiver (1981), Edwards and Fritz (1986), and Lipp et al. (1993), but not in other studies (DeNiro and Cooper 1989; White et al. 1994; Terwilliger and DeNiro 1995). Since isotopes faithfully record physical and biochemical processes, we may then ask, how can the results presented here and those of previous studies be reconciled?

### Differences in experimental design between studies

Many studies (Schiegl 1974; Gray and Thompson 1976; Epstein and Krishnamurthy 1990) utilized correlations of the measured or assumed plant water source and local weather records with the isotopic composition of cellulose in the corresponding annual growth ring to investigate the environmental information contained in tree rings. Interpretation of such data is constrained by the assumptions made and the choice of environmental information to be included in the analysis. The tortuous isotopic path from precipitation to tree-ring cellulose suggests that correlation studies may not be sufficient to answer some of these questions regarding the information contained within the tree ring and that experimental approaches are needed. Unfortunately, no single experimental study has addressed both exchange processes associated with hydrogen and oxygen isotope ratios in tree-ring cellulose.

A number of reasons allow confidence in the results presented here, including the overall experimental design. This hydroponics setup differs from other studies by (1) using tree saplings rather than non-tree species (potato, DeNiro and Cooper 1989; *Lemna*, Yakir and DeNiro 1990), (2) using material that derived its cellulose from current photosynthate rather than obtaining much of its carbon from stored reserves (potato, DeNiro and Cooper 1989, avocado Terwilliger and DeNiro 1995), (3) using suberized stems where cellulose is not synthesized in water exposed to evaporative enrichment (potato, DeNiro and Cooper 1989), and (4) utilized tree species whose roots would normally experience continuous water submersion (i.e., riparian trees). Another important factor is that these trees were grown (>5 months) under controlled conditions with little or no change in source water isotopic composition (Fig. 1) producing an actual tree-ring rather than cellulose in other tissues that is assumed to act as a tree-ring analog. This experiment also used growth environments where humidity was controlled to produce substantially different treatments (40 or 70%) which created significant differences in leaf transpiration between treatments (Figs. 2, 3).

Although the experiment was designed to eliminate problems with the interpretation of results and their application to tree-ring records used in climate reconstruction, additional tests were made to ensure that the cellulose measured was representative of that which would have been synthesized under the imposed environmental conditions. Firstly, a re-sampling of the outer one-third of the tree-ring for the most enriched treatments and secondly, a sampling of new roots both confirm (Figs. 4, 5, 6) that the tree-ring data presented provide an accurate record of the cellulose produced during the experiment. The question then becomes, can a model be developed that is general enough to predict the differences between the results presented here and those of previous studies?

## A mechanistic model for the interpretation of isotope ratios in cellulose

The hydrogen and oxygen isotopes of tree ring cellulose ultimately come from the water taken up by the roots. There is no fractionation upon uptake for either δD or $\delta^{18}O$ (White et al. 1985; Dawson 1993) and thus the δD and $\delta^{18}O$ signatures carried through the process of cellulose production should represent, to a first approximation, the integrated water uptake patterns of the root system. However, there are numerous points along the pathway to a tree-ring where the isotopic signatures of the source water may be altered. Thus a mechanistic approach needs to look at each step and determine what parameters are needed to make accurate predictions. Our model, described in detail by Roden et al. (1999), has two major components: (1) a leaf water model that uses environmental information to predict the extent of evaporative enrichment for a given isotopic input of source water and atmospheric vapor, and (2) a biochemical model that predicts cellulose isotopic composition based on autotrophic and heterotrophic fractionation factors as well as the extent of isotopic exchange between substrates and medium water in the steps leading to cellulose synthesis. Roden and Ehleringer (1999) demonstrated that the Craig and Gordon (1965) evaporative enrichment model of the hydrogen and oxygen isotopes of water, as modified by Flanagan et al. (1991), is robust in its ability to predict measured leaf water δD and $\delta^{18}O$ over a wide range of leaf waters. Since the biochemical model discussed below uses leaf water (at the site of evaporative enrichment) as a primary input, and since it was impractical to adequately sample all the leaves in the greenhouse with sufficient frequency to capture all the variability in leaf water, the Craig-Gordon model was utilized to generate estimated leaf water isotopic compositions for the different treatments. The leaf water model uses vapor pressures along with the isotopic composition of source water and atmospheric water vapor as primary inputs which were all frequently measured in these experiments as well as kinetic and equilibrium fractionation factors from Flanagan et al. (1991).

The hydrogen and oxygen isotopes incorporated into organic molecules come from the water in the leaf and may become isotopically altered due to biochemical fractionation during metabolism. Carbohydrates, primarily in the form of sucrose, are transported to the site of cellulose production in the cambium where further biochemical fractionation and exchange with xylem water might occur. A general model (Sternberg et al. 1986; Yakir and DeNiro 1990; Luo and Sternberg 1992; Roden et al. 1999) for the isotopic composition of heterotrophically produced cellulose versus the respective δD and $\delta^{18}O$ in source water has been presented as

$$\delta_c = f\cdot(\delta_w+\varepsilon) + (1-f)\cdot\delta_{\text{non-exchangeable}} \quad (1)$$

where the subscripts c, w, and non-exchangeable indicate the δD and $\delta^{18}O$ values of synthesized cellulose, medium water and non-exchangeable stable isotopes of the substrate, respectively, ε is the isotope fractionation factor for the enzyme-mediated exchange or addition of either hydrogen or oxygen, and *f* is the proportion of the carbon-bound hydrogen or oxygen that undergoes exchange with the source water.

Equation 1 is used to calculate the isotopic composition of tree ring cellulose using biochemical fractionation factors for hydrogen and oxygen along with the proportion of the carbon-bound hydrogen or oxygen that undergoes exchange with xylem water,

$$\delta D_{cx} = f_H\cdot(\delta D_{wx}+\varepsilon_{HH}) + (1-f_H)\cdot(\delta D_{wl}+\varepsilon_{HA}), \quad (2)$$

$$\delta^{18}O_{cx} = f_O\cdot(\delta^{18}O_{wx}+\varepsilon_O) + (1-f_O)\cdot(\delta^{18}O_{wl}+\varepsilon_O), \quad (3)$$

where the subscripts cx, wx and wl refer to the xylem cellulose, xylem water, and leaf water (chloroplast water at the site of sucrose synthesis rather than bulk leaf water) respectively. The isotopic composition of the non-exchangeable components to this model are derived from the substrate (generally sucrose) that contributes the hexose for cellulose synthesis and is calculated using the leaf water isotopic composition and autotrophic fractionation factors. Yakir and DeNiro (1990) calculated the autotrophic fractionation factor for hydrogen ($\varepsilon_{HA}$) to be –171‰. The autotrophic fractionation factor for oxygen ($\varepsilon_O$) is +27‰ from the carbonyl-water interaction during biosynthesis (Sternberg and DeNiro 1983). The heterotrophic fractionation factor for hydrogen ($\varepsilon_{HH}$) is +158‰ from Yakir and DeNiro (1990) which is nearly identical to the values (+144 to +166‰) reported by Luo and Sternberg (1992). Since the fractionation factor is the same for autotrophic and heterotrophic metabolism for oxygen, there is no need to distinguish between the two ($\varepsilon_O$=+27‰). The terms heterotrophic and autotrophic are simply used to distinguish fractionation events associated with carbon fixation during photosynthesis (autotrophic) and biosynthetic pathways that are dependent on carbohydrate input (heterotrophic) and not differences between autotrophic and heterotrophic organisms. Roden et al. (1999) showed that when the δD of atmospheric vapor is similar to source water δD then the opposite sign and magnitude of the heterotrophic fractionation factor ($\varepsilon_{HH}$=+158‰, cellulose formation in the cambium) and the autotrophic fractionation factor ($\varepsilon_{HA}$=–171‰, sucrose formation in the leaves) may make the cellulose δD value appear to be identical to source water, indicating complete exchange. This model also predicts that a humidity signal, acting on the isotopic composition of leaf water, should be carried through to cellulose as long as the proportion of the carbon-bound hydrogen or oxygen that undergoes exchange with xylem water ($f_H$ and $f_O$) is less than 1. Because the isotopic composition of atmospheric water vapor was not in equilibrium with source water in our system (differences between the two were as high at 300‰ in δD), leaf water isotopic signatures, and thus exported sucrose signatures, were substantially different than would be expected under equilibrium conditions, providing a clearer system for evaluating the assumptions regarding isotopic exchange.

Can the model predict both current and previous observations?

We believe that the Roden et al. (1999) cellulose model accurately predicts the isotopic composition of tree-ring cellulose observed in this study. The data for birch and cottonwood (pooled means) were fit to the model to determine the proportion of the carbon-bound hydrogen or oxygen that undergoes exchange with the medium water. The results clearly indicate (Figs. 7, 8) that the biochemical model can accurately predict measured cellulose δD and $\delta^{18}O$ values and that there is incomplete exchange with xylem water ($f_H$=0.36 and $f_O$=0.42, from the best fit using the residual sum of squares). The value for the fraction of water exchanged reported here are similar in magnitude to those reported by Yakir and DeNiro (1990) ($f_H$≈0.4 for *Lemna* under heterotrophic conditions), Yakir (1992) ($f_H$≈0.5) and Luo and Sternberg (1992) ($f_H$≈0.34) for cellulose derived from simple carbohydrates rather than lipids. Sternberg et al. (1986) estimated that $f_O$≈0.47 for carrot cell cultures grown on sucrose and Yakir (1992) indicates that $f_O$≈0.5, both similar to the value of $f_O$ reported here. The model also predicts (Figs. 7, 8) the changes in isotopic composition of tree-ring cellulose observed when trees are grown under different humidity regimes. Another model prediction that is borne out by our data (Fig. 6) is that the cellulose produced in new roots should have the same isotopic signatures as cellulose produced in tree rings since for both, the sugars exported from the leaves and the medium water for isotopic exchange are identical.

The Roden et al. (1999) model also explains the apparently conflicting observations in the literature regarding the environmental information contained in tree-ring cellulose. In field studies where the differences in the isotopic composition of source water and atmospheric vapor are limited (vapor in equilibrium with meteoric water), this model would predict the 1:1 relationship between source water and tree ring cellulose commonly observed (Epstein and Yapp 1977; Burk and Stuiver 1981; Yapp and Epstein 1982; Lawrence and White 1984; White et al. 1994). This model would also predict that humidity signals are potentially retained in the tree ring similar to the findings of other studies (Burk and Stuiver 1981; Edwards and Fritz 1986; Lipp et al. 1993). However, the lack of a tree-ring humidity signal (White et al. 1994) is also possible when the range of humidity differences are limited or if an inappropriate portion of the tree ring is sampled. The hydrogen and oxygen isotope ratios of the cellulose in early-season xylem should be interpreted with caution, since both the medium water (water remaining in the stem overwinter can be isotopically enriched from current meteoric water, Phillips and Ehleringer 1995) and the substrate (from previous seasons stored carbohydrates) may be unrelated to either current source water or humidity conditions (Roden et al. 1999).

Although this model does clarify some of the disparate observations in the literature, it does not agree with the claim of complete isotopic exchange with medium water for either hydrogen or oxygen (DeNiro and Cooper 1989; Terwilliger and DeNiro 1995). Our model does agree with the predictions of Luo and Sternberg (1992) that $f_H \approx f_O$ and that $f_H$≈0.34 for cellulose derived from

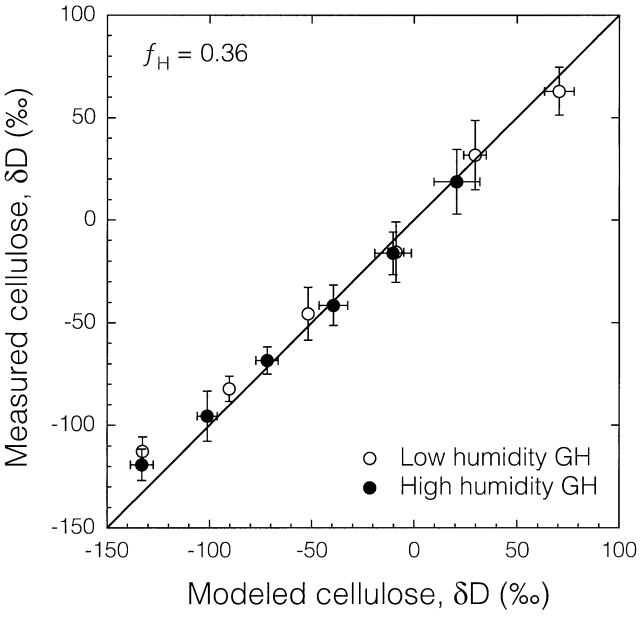


**Fig. 7** The relationship between the δD of modeled and measured tree-ring cellulose. Variations in tree-ring cellulose were generated by altering source water δD in a hydroponic system in a controlled greenhouse environment at either high or low relative humidity. *Values* are means and SDs. The *line* represents a 1:1 relationship

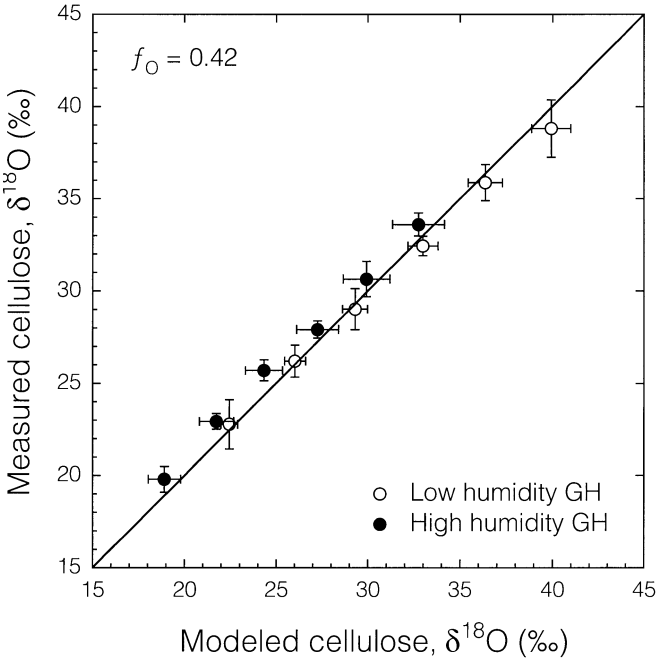


**Fig. 8** The relationship between the $\delta^{18}O$ of modeled and measured tree-ring cellulose. Variations in tree-ring cellulose were generated by altering source water $\delta^{18}O$ in a hydroponic system in a controlled greenhouse environment at either high or low relative humidity. *Values* are means and SDs. The *line* represents a 1:1 relationshi

carbohydrates rather than lipids as well as the leaf cellulose studies of *Lemna* by Yakir and DeNiro (1990). We also must disagree with the conclusion of Terwilliger and DeNiro (1995) that no universal formula exists that links source water and cellulose δD. From this study and those in the literature in which very different species were utilized some similar parameters are beginning to arise. The Craig-Gordon evaporative enrichment model has been shown to be robust over a wide range of leaf waters and imposed environmental conditions (Roden and Ehleringer 1999; Roden et al. 1999). As seen above, the heterotrophic and autotrophic fractionation factors for both hydrogen and oxygen have been shown to be valid for a variety of species. And evidence is growing that the proportion of the carbon-bound hydrogen or oxygen that undergoes exchange with xylem water may be within a fairly narrow range and may be constrained by biosynthetic pathways.

### Triose phosphate cycling and the basis for $f_H$ and $f_O$ values

The biochemical basis for the values of $f_H$ and $f_O$ can be found in the metabolic pathways leading to cellulose synthesis. Farquhar et al. (1998) has theorized that two out of the ten oxygen atoms in each cellobiose unit of cellulose undergo exchange with medium water during synthesis from sucrose. However, if some of the hexose phosphates go through the triose phosphate pathway then six out of ten are exchangeable. A simple calculation (Farquhar et al. 1998) using the percentage of hexose phosphates that are broken down into triose phosphates before being incorporated into cellulose ($y$) allows an independent determination of $f_O$.

$$f_O = 0.6y + 0.2 \quad (4)$$

Our data indicate that $y$=38%, which is similar in range to estimates for potato tubers (22–39%, Viola et al. 1991), developing wheat grain (30–40%, Keeling et al. 1988), and oak stem tissue (40–50%, Hill et al. 1995), the last of which is arguably the most appropriate comparison. It is not clear what causes these variations in $y$, but clearly an estimate of the triose phosphate cycling in the cambium is needed to utilize tree-ring models for climate reconstruction (Hill et al. 1995). The fact that all three species (alder, birch, and cottonwood) from this study produced equivalent results and that the oak stem tissue from Hill et al. (1995) showed a similar fraction of triose-P cycling implies that a common $f_O$ value is likely for cellulose formation in the cambium of trees.

The value for $f_H$ was slightly lower (0.36) than $f_O$, but still consistent with the conclusions of Luo and Sternberg (1992) who estimated from empirical relationships that $f_H \approx f_O$. The biochemical basis for exchange of hydrogen during cellulose synthesis is less well documented than for oxygen, however a similar analysis to equation 4 should be appropriate. Although the C-H hydrogen is not exchangeable with medium water, there are a number of opportunities for exchange during metabolism (Yakir 1992). The greatest potential for H exchange with medium water occurs during triose phosphate isomerization and interconversion between the products fructose-6-P and glucose-6-P where 50% of the carbon bound hydrogen atoms of each hexose could be exchanged (Yakir 1992). For sugars that do not enter into the triose-P cycle, we estimate that 2 out of 14 hydrogen atoms in sucrose (that eventually become C-H bonded in cellulose) exchange with medium water if the isomerization between glucose-6-P and fructose-6-P is rapid. If we assume 50% exchange for hexose phosphates that go through triose-P cycling (Yakir 1992) and inserting these exchange parameters for hydrogen into Eq. 4, we estimate 43% triose-P cycling which is similar to the 38% estimated using oxygen.

It may not be necessary to invoke futile cycling to explain $f_H$ and $f_O$ values if carbohydrates in general contain a similar portion of exchangeable hydrogen and oxygen atoms (L.S.L. Sternberg, personal communication). At present there is no test of the futile cycle exchange hypothesis and we are unable to distinguish between possible explanations for $f_H$ and $f_O$. More work is needed to estimate the amount of triose-P cycling in various tissues and metabolic pathways as well as the amount of exchange that occurs during sucrose synthesis and whether the same atoms exchange during cellulose synthesis.

Our experimental results enhance an understanding of the mechanistic relationships between the isotopic composition of source water and tree-ring cellulose. The results presented here are directly applicable to dendrological studies, being derived from trees that were grown for an entire growing season under controlled conditions, rather than cellulose produced over much shorter periods by plants that are unlikely to act as tree-ring analogs (potato, avocado, carrot, and water ferns). Although the hypothesis of complete isotopic exchange with xylem water at the time of cellulose synthesis in the cambium is attractive in its simplicity, it is not supported by the results of this study. Nor is it supported by other laboratory and field studies. Clearly, the isotopic composition of tree-ring cellulose reflects source water in a linear fashion. However, the slope may not under all environmental conditions be equal to 1.0, and other environmental parameters such as humidity can alter that slope. This complicates the straightforward interpretation of the δD and $\delta^{18}O$ in tree-ring records as reflecting precipitation inputs only. Although the signal within tree rings becomes more complicated, it also becomes richer in information. For example, the variation around the 1:1 relationship between source water and tree-ring cellulose in previous studies (25–50‰ in δD, Epstein and Yapp 1977; Yapp and Epstein 1982) may represent additional information such as humidity rather than simply noise. With the development of a mechanistic model of tree-ring isotope ratios (Roden et al. 1999), we may be able to clarify inferred relationships between tree-ring isotopic composition and environmental parameters derived from dendrogeochemical studies.

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