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Hydrogen and oxygen isotope ratios of tree ring cellulose for field-grown riparian trees

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Abstract The isotopic composition of tree ring cellulose was obtained over a 2-year period from small-diameter riparian-zone trees at field sites that differed in source water isotopic composition and humidity. The sites were located in Utah (cool and low humidity), Oregon (cool and high humidity), and Arizona (warm and low humidity) with source water isotope ratio values of –125/–15‰ ($\delta D/\delta^{18}O$), –48/–6‰, and –67/–7‰, respectively. Monthly environmental measurements included temperature and humidity along with measurements of the isotope ratios in atmospheric water vapor, stream, stem, and leaf water. Small riparian trees used only stream water (both δD and $\delta^{18}O$ of stem and stream water did not differ), but δ values of both atmospheric water vapor and leaf water varied substantially between months. Differences in ambient temperature and humidity conditions between sites contributed to substantial differences in leaf water evaporative enrichment. These leaf water differences resulted in differences in the δD and $\delta^{18}O$ values of tree ring cellulose, indicating that humidity information was recorded in the annual rings of trees. These environmental and isotopic measurements were used to test a mechanistic model of the factors contributing to δD and $\delta^{18}O$ values in tree ring cellulose. The model was tested in two parts: (a) a leaf water model using environmental information to predict leaf water evaporative enrichment and (b) a model describing biochemical fractionation events and isotopic exchange with medium water. The models adequately accounted for field observations of both leaf water and tree ring cellulose, indicating that the model parameterization from controlled experiments was robust even under uncontrolled and variable field conditions.

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Introduction

Tree rings have been used extensively to quantify past climatic variation through measurements of ring widths (see review in Fritts 1976) and more recently through stable isotope analyses of cellulose (see review in Switsur and Waterhouse 1998). The δD and $\delta^{18}O$ values in tree ring cellulose are derived from water and reflect, to a first approximation, precipitation inputs (Yapp and Epstein 1982; Lawrence and White 1984; White et al. 1994). Regressions of the variations in δD and $\delta^{18}O$ values in tree ring cellulose have been used for temperature reconstruction (Schiegl 1974; Gray and Thompson 1976; Epstein and Yapp 1977; Feng and Epstein 1994), since the isotopic composition of precipitation is a direct function of condensation temperature (Dansgaard 1964). However, a perfect 1:1 relationship between the isotopic composition of tree ring cellulose and source water is seldom observed, implying that tree rings may record additional information related to biological processes that respond to local environmental variation. Many studies have used correlation analyses to determine which environmental parameters (water source, humidity, temperature) might be recorded in the cellulose of annual growth rings (Schiegl 1974; Gray and Thompson 1976; Epstein and Yapp 1977; Burk and Stuiver 1981). However, substantial uncertainty still remains as to what biological and environmental information is contained in the isotopic composition of tree ring cellulose (DeNiro and Cooper 1990; Edwards 1990). For example, some studies report that tree rings may contain information about local humidity (Edwards and Fritz 1986; Lipp et al. 1993; Saurer et al. 1997), while others find no evidence for a humidity signal (DeNiro and Cooper 1989; White et al. 1994; Terwilliger and DeNiro 1995). It is important to determine how these contrasting results can be reconciled if we are to make ecological interpretations from tree ring isotope data.

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. Roden et al. (2000) presented a model to describe the various fractionation events at each step along the way. This model has been shown to adequately account for the various results in the literature (Roden et al. 2000) as well as those obtained from a long-term experimental system where tree rings were produced under controlled environmental conditions (Roden and Ehleringer 1999a).

The δD and $\delta^{18}O$ values of tree ring cellulose ultimately originate from the water taken up by the roots. Although there is no fractionation upon uptake for either δD or $\delta^{18}O$ (White et al. 1985; Dawson 1993; although mangroves growing in sea water are an exception: Lin and Sternberg 1993), there are numerous points along the pathway to a tree ring where the δ values of the source water may be altered. The model, described in detail by Roden et al. (2000), has two major components. Firstly, a modified version (Flanagan et al. 1991a) of the Craig and Gordon (1965) evaporative enrichment model was used to estimate the δD or $\delta^{18}O$ of leaf water in the chloroplast using environmental information and a given isotopic input of source water and atmospheric vapor. Secondly, a biochemical model (developed from the general models of Sternberg et al. 1986; Yakir and DeNiro 1990; Luo and Sternberg 1992) that predicts cellulose isotopic composition based on autotrophic (carbohydrate synthesis associated with photosynthesis) and heterotrophic (carbohydrate metabolism associated with cellulose synthesis in the stem) fractionation factors as well as the extent of isotopic exchange between substrates and medium water in the steps leading to cellulose synthesis:

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

$$\delta^{18}O_{cx}=f_O(\delta^{18}O_{wx}+\varepsilon_O)+(1-f_O)(\delta^{18}O_{wl}+\varepsilon_O) \quad (2)$$

where the subscripts cx, wx, and wl refer to the xylem cellulose, xylem water, and leaf water, respectively. Yakir and DeNiro (1990) calculated the autotrophic fractionation factor for hydrogen (ε_{HA}) to be –171‰. The autotrophic fractionation factor for oxygen (ε_O) is +27‰ from the carbonyl-water interaction during biosynthesis (Sternberg and DeNiro 1983). ε_{HH} and ε_O are the heterotrophic fractionation factors for the enzyme-mediated exchange or addition of either hydrogen or oxygen. For hydrogen, ε_{HH}=+158‰ (Yakir and DeNiro 1990) and since the fractionation factor is the same for autotrophic and heterotrophic metabolism for oxygen (Hill et al. 1995), there is no need to distinguish between the two (ε_O=+27‰). *f* is the proportion of the carbon-bound hydrogen or oxygen that undergoes exchange with the medium water at the site of cellulose synthesis (see also a slightly different formulation using *f* as a damping factor, as in Saurer et al. 1997). A spreadsheet version of the model is available at ftp://ecophys.biology.utah.edu/treering/.

These models were tested and parameterized on saplings of three tree species (alder, birch, and cottonwood) using a hydroponics system that controlled source water isotopic composition, and greenhouses that controlled the temperature and humidity environment and therefore leaf water evaporative enrichment (Roden and Ehleringer 1999a). However, it is also important to determine if the results obtained in controlled environments are applicable to 'real world' situations in the field. Although a hydroponics system can provide a substantially larger range in source water isotopic composition than natural systems, field verification of the model parameters is critical for the application of the model for climate reconstruction or ecological comparisons. Therefore, the objective of this study was to determine if the models of Roden et al. (2000) can predict the isotopic composition of leaf water and tree ring cellulose for trees grown in the field under varied source waters and relative humidities.

Materials and methods

Plant material and field sites

Field sites were located along stream courses in four locations. The locations were (a) the Bill Williams River Wildlife Reserve near Lake Havasu, Arizona (34°16' N 114°02' W) containing cottonwood (*Populus fremontii* Wats), (b) Cascade Head Field Station near Otis, Oregon (45°02' N 123°55' W) containing red alder (*Alnus rubra* Bong), (c) the Weber River near Ogden, Utah (41°08' N 111°54' W) containing cottonwood (*P. fremontii*), and (d) the Red Butte Canyon Nature Reserve near Salt Lake City, Utah (40°47' N 111°48' W) containing both alder (*A. incana* L. Moench) and water birch (*Betula occidentalis* Hook). The sites were chosen for their proximity to Salt Lake City, similar tree species, differences in growing season humidity and differences in stream water isotopic composition with the Utah sites being substantially more depleted than either the Oregon or Arizona sites. All trees (five to seven individuals per site) were streamside and less than 10 cm in diameter to ensure that they were still using the stream water rather than deeper sources (Dawson and Ehleringer 1991). The same trees were followed over two growing seasons (1996 and 1997) except when trees were damaged (by beavers) or died, and then a replacement tree was selected.

Isotope sampling and environmental measurements

At monthly intervals, approximately 5 ml of stream water was sampled at each site. During the growing season, in addition to stream water, monthly samples of twigs and leaves were collected for water extraction. Leaf material, with the midvein removed, and approximately 5–10 cm of suberized twig material were placed into separate glass vials, sealed with parafilm and brought back to the laboratory on dry ice, and then placed into a freezer (–5°C) until the water could be extracted for isotopic analysis. At the time of leaf and stem water collection, the ambient relative humidity and air temperature were measured and the atmospheric water vapor was sampled using a pump to draw air through a glass trap submerged in a mixture of ethanol and dry ice (–78°C). Relative humidity was measured with both a sling psychrometer and a Vaisala humidity sensor, since Vaisala sensors are less accurate at high humidity (Oregon) and sling psychrometers are less accurate at very low humidity (Arizona) (Rundel and Jarrell 1989). At the end of each growing season, cores of the main stems were taken from each cardinal direction. The annual growth ring produced during that year was cut out, dried and ground to pass a 40-mesh screen using a Wiley mill (Thomas, Philadelphia, Pa.). The ring was not subdivided due to the relatively large amount of material needed

for cellulose nitration and the fact that these trees were obtaining water from streams that had little seasonal variation in δD or $\delta^{18}O$, reducing some of the differences between earlywood and latewood.

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 (–190°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 streams, 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 (Socki et al. 1992). 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.) with a precision of ±1‰ for δD and ±0.2‰ for $\delta^{18}O$.

The $\delta^{18}O$ recorded in tree rings was 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 was then placed in a silver capsule and converted to CO by pyrolysis (Saurer et al. 1998) in a hot (1,100°C) high-purity alumina combustion column (Carla-Erba interface) and separated from other gases in a 1-m mole sieve 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‰.

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 trinitrated 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 Zn catalyst for hydrogen reduction as described above.

Throughout this paper we use the conventional 'delta' notation which expresses the isotopic composition of a material relative to that of a standard on a per mil (‰) deviation basis:

$$\delta=(R_{sample}/R_{standard}-1)1{,}000 \quad (3)$$

where δ is referred to as the isotope ratio (δD for hydrogen and $\delta^{18}O$ for oxygen). The standard for both hydrogen and oxygen is standard mean ocean water (SMOW).

Results

Figures 1 and 2 show the δD and $\delta^{18}O$ values of atmospheric water vapor along with stream, leaf, and xylem sap water. The water taken up by the trees was virtually identical isotopically to the stream water, implying that the trees were not tapping deeper water sources. The cottonwood trees near the Bill Williams River in Arizona did tap some deeper water sources, since the surface stream dried up fairly early in the season. However, there was evidence that the stream was simply flowing below the river bed which is common in desert riparian zones.

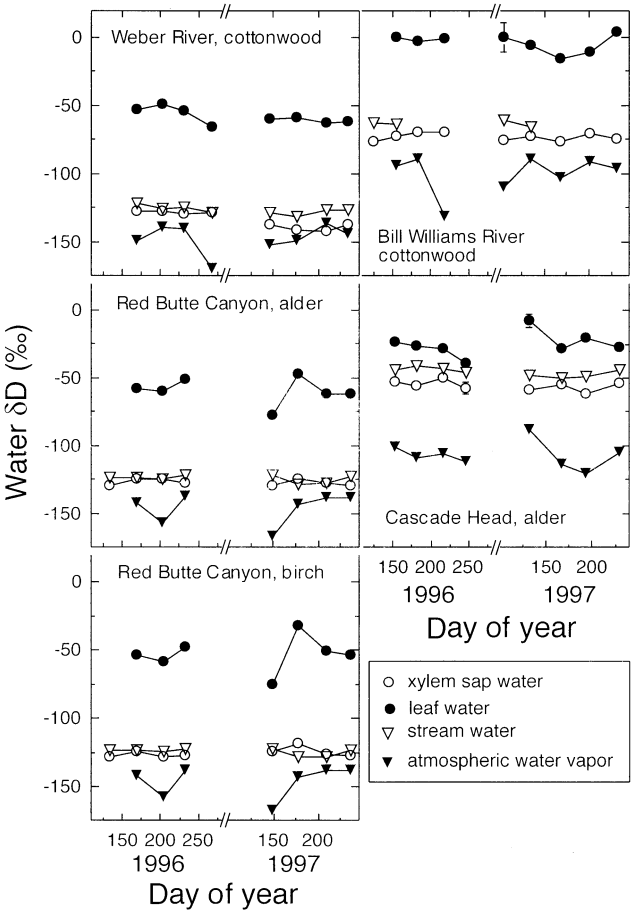

Fig. 1 The hydrogen isotope composition of stream, stem, and leaf water along with atmospheric water vapor during the growing season over a 2-year period. Values are means±SE (where replication was possible)

The similar isotopic values of xylem sap throughout the year for the Arizona cottonwoods implies that these trees were simply tapping the below-ground stream flow. Both the Arizona (Bill Williams River) and Oregon (Cascade Head) sites had substantially more enriched source water (–67/–7‰ and –48/–6‰, δD/$\delta^{18}O$, respectively) than the Utah sites (Weber River and Red Butte Canyon Creek water, –131/–15‰ and –125/–15‰, δD/$\delta^{18}O$, respectively). The stream water for these sites did not vary much throughout the winter months (data not shown), indicating that the source water used for cellulose synthesis in these trees was not subject to large seasonal variations in meteoric water isotopic composition which could complicate the interpretation of tree ring results (requiring the annual ring to be subdivided).

As expected, the leaf water δD and $\delta^{18}O$ values were more enriched than the source water. However, the leaf water values were much more variable than those of source water (Figs. 1, 2). Leaf water varied within a single season by as much as 50‰ and 15‰ in δD and $\delta^{18}O$, respectively. The higher humidities and lower temperatures at the Oregon site (Cascade Head; Table 1) reduced the amount of leaf water evaporative enrichment com-

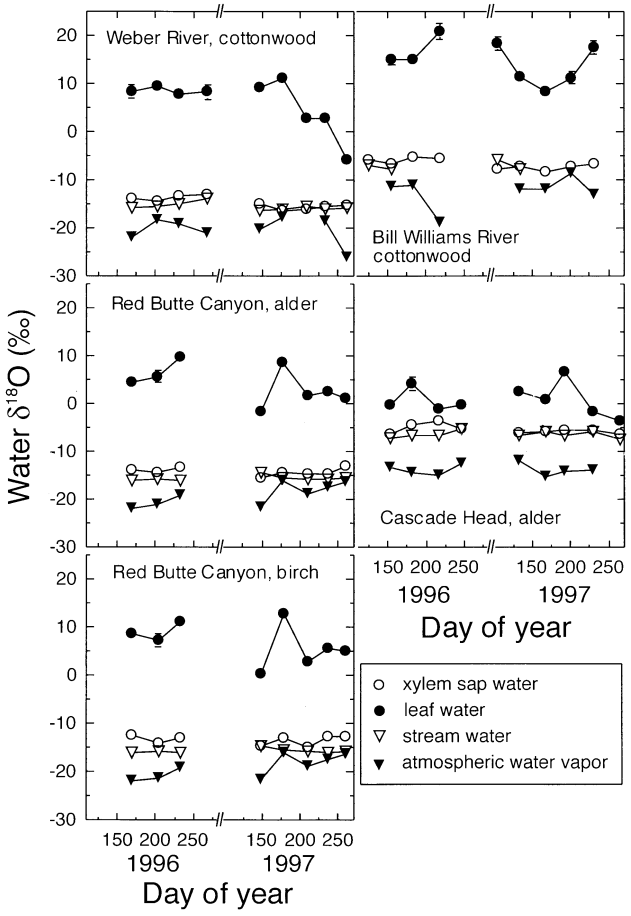

Fig. 2 The oxygen isotopic composition of stream, stem, and leaf water along with atmospheric water vapor during the growing season over a 2-year period. Values are means±SE (where replication was possible)

Table 1 The mean growing season temperatures and relative humidities at the four sites in this study. Values were obtained from monthly measurements during the summers of 1996 and 1997

Site	Temperature (°C)	Relative humidity (%)
Cascade Head (Oregon)	17.4	84.4
Bill Williams River (Arizona)	34.0	36.0
Red Butte Canyon (Utah)	23.6	35.6
Weber River (Utah)	24.5	36.3

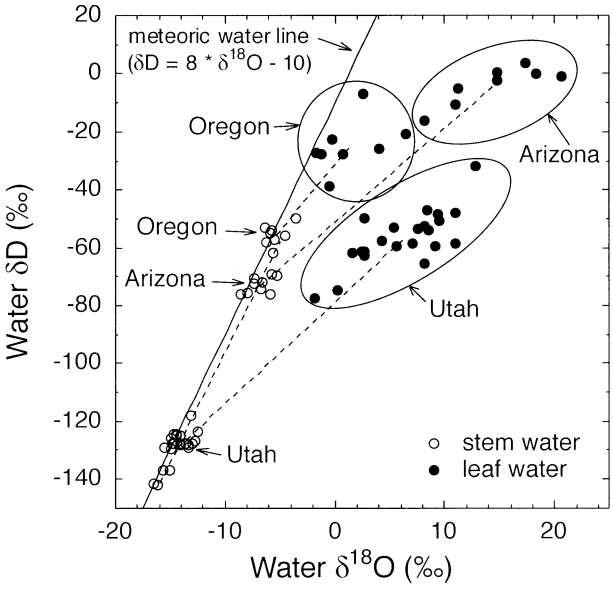

Fig. 3 The relationship between growing season stem and leaf water $\delta^{18}O$ and δD at three sites

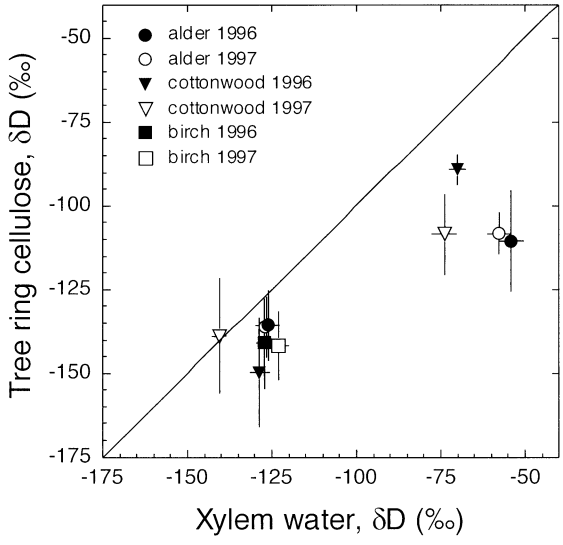

Fig. 4 The relationship between source water and tree ring cellulose δD for three species at three sites over a 2-year period. Data are distinguished by species and year. Sites are distinguished by species and source water δD values with the most depleted waters from the Utah sites and the more enriched water from the Arizona (cottonwood) and Oregon (alder) sites. Values are means±SD

pared to the other sites (Figs. 1, 2). A plot of the δD versus $\delta^{18}O$ for both leaf and xylem sap water (Fig. 3) demonstrates that the source water being utilized by these trees fell on the same slope as the meteoric water line ($\delta D = 8\delta^{18}O - 10$). However, the leaf water for these trees fell off the meteoric water line by an amount dependent on the site environmental conditions. The warm temperatures and low humidity at the Arizona site (Table 1) resulted in highly enriched leaves that fell on a line between source water and leaf water with a slightly smaller slope (m=3.2) than the low humidity but cooler conditions of the Utah sites (m=3.5) and a much smaller slope than the cool and humid Oregon site (m=4.6; Fig. 3).

The δ values of atmospheric water vapor were from 5 to 75‰ and 2 to 13‰ more depleted than stream water in δD and $\delta^{18}O$, respectively (Figs. 1, 2). Within a single season, the isotopic composition of atmospheric water vapor varied by as much as 43‰ and 8‰ in δD and $\delta^{18}O$, respectively (Figs. 1, 2). However, on average, large deviations in atmospheric water vapor may have been associated with the intensity and origin of storm events. Whatever the cause, the heavy isotopes in atmo-

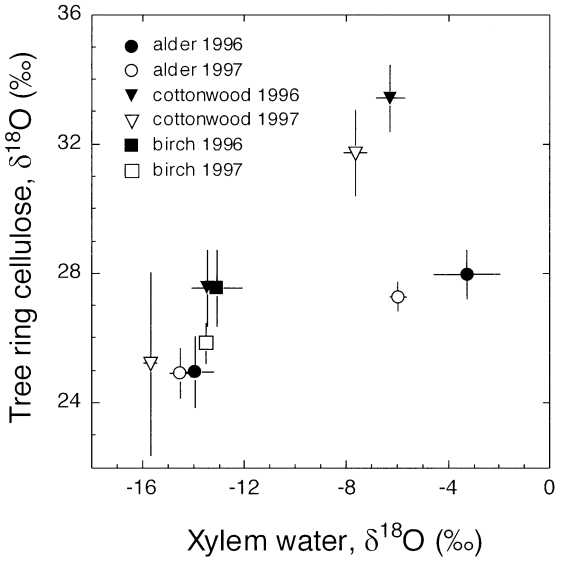

Fig. 5 The relationship between source water and tree ring cellulose $\delta^{18}O$ for three species at three sites over a 2-year period. Data are distinguished by species and year. Sites are distinguished by species and source water $\delta^{18}O$ values with the most depleted waters from the Utah sites and the more enriched water from the Arizona (cottonwood) and Oregon (alder) sites. Values are means±SD

spheric water vapor are clearly not constant and may not always covary with plant source water especially if trees are not dependent on precipitation events for water.

The δD values of tree ring cellulose were dependent on source water δD, but not in a simple 1:1 relationship (Fig. 4). The most depleted values were from the Utah sites and the more enriched alder and cottonwood values were from the Oregon and Arizona sites, respectively. Even though the Arizona site had more depleted water sources than the Oregon site, the tree ring cellulose was similar or even more enriched in δD than at the Oregon site. Thus, the differences in humidity and temperature between the two sites, which altered leaf water isotopic composition (Fig. 1), affected the isotopic composition of the cellulose laid down. The differences between the Oregon and Arizona sites were even more pronounced when the $\delta^{18}O$ values of source water and tree ring cellulose were compared (Fig. 5). This is likely due to the greater effect of evaporative enrichment on leaf water $\delta^{18}O$ than δD, as seen in deviations from the meteoric water line (Fig. 3).

The mixture of three species with similar water sources at the Utah sites (the most depleted xylem waters; Figs. 4, 5) as well as the data from the Arizona site show substantial variation in tree ring cellulose δ values between years (open versus closed symbols) and this variation can be as large as that between species. Thus, the incorporation of stable isotopes into the cellulose of tree rings may indeed record valuable environmental information which is sensitive to differences in climate between years.

Discussion

Although the lack of a 1:1 relationship between the hydrogen isotopic composition of tree ring cellulose and source water (Fig. 4) would seem to differ from many other studies (Epstein and Yapp 1977; Yapp and Epstein 1982; White et al. 1994; Terwilliger and DeNiro 1995), the variation around the 1:1 line is within that found in some of those studies. For example, in our results, the data obtained from the Oregon site were about 50‰ more depleted in deuterium than predicted by a 1:1 relationship. The studies of Epstein and Yapp (1977) and Yapp and Epstein (1982) described a 1:1 relationship over a wide range of environmental water isotopic compositions (>180‰) with a maximum deviation from the 1:1 line of 25–50‰, respectively. The results from the Oregon and Arizona sites (Fig. 4) indicated that humidity differences can affect tree ring isotopic composition. Therefore, much of the variation around the 1:1 relationship observed in other studies could be related to additional environmental factors unaccounted for by models that predict the δD of tree ring cellulose as a simple function of source water (e.g., Terwilliger and DeNiro 1995). Humidity differences would affect the oxygen isotope ratios of tree ring cellulose (Fig. 5) to an even greater extent, since evaporation causes a greater enrichment in leaf water $\delta^{18}O$ than δD (Fig. 3).

These data collected from natural field situations were used to test the Roden et al. (2000) models that were previously parameterized based on controlled experimental systems (Roden and Ehleringer 1999a). The monthly observations of leaf water were used to test a modified version (Flanagan et al. 1991a) of the Craig and Gordon (1965) evaporative enrichment model. Roden and Ehleringer (1999b) found that a modified Craig-Gordon model was robust over a wide range of leaf waters, although other studies have found that the Craig-Gordon model predicted somewhat greater than observed isotopic enrichment (Allison et al. 1985; Leaney et al. 1985; Flanagan and Ehleringer 1991; Flanagan et al. 1991b; Wang and Yakir 1995). The leaf water model uses vapor pressures in combination with the δ values of source water and atmospheric water vapor as primary inputs (which were all measured at each site at the time of leaf water collections) as well as kinetic and equilibrium fractionation factors from Flanagan et al. (1991a). Leaf water models are sensitive to humidity inputs (Dongmann and Nürnberg 1974; Flanagan et al. 1991a; Roden and Ehleringer 1999b) and since canopies can substantially modify the water vapor microenvironment of a leaf, empirical relationships were developed using humidity measurements made in the open and within the canopy to correct humidity observations collected in the open. The corrections were generally very small (5–7% for δD) but helped bring closer agreement between predicted and observed δD and $\delta^{18}O$ values for leaf water (Figs. 6, 7).

Using Eqs. 1 and 2 and known autotrophic and heterotrophic fractionation factors, the proportion of the hy-

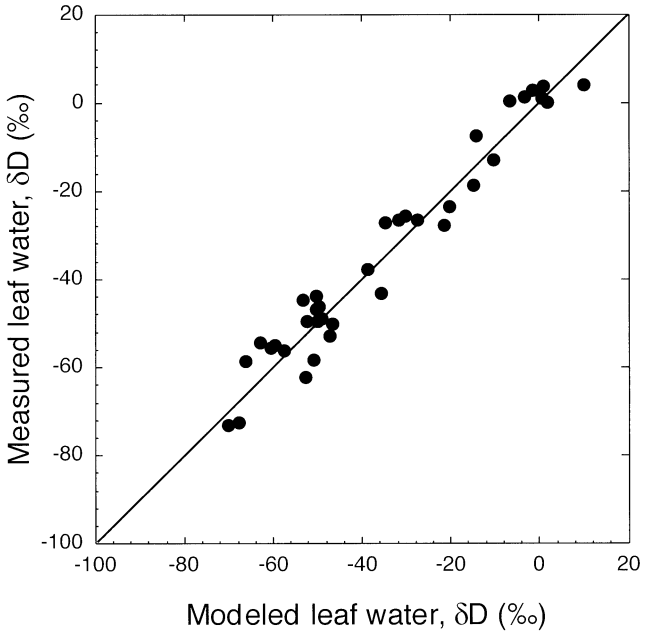

Fig. 6 The relationship between the δD of modeled and measured leaf water for field-grown riparian-zone trees. Variations in leaf water were due to different stream water δD values and temperature and humidity differences between sites. The *line* represents a 1:1 relationship

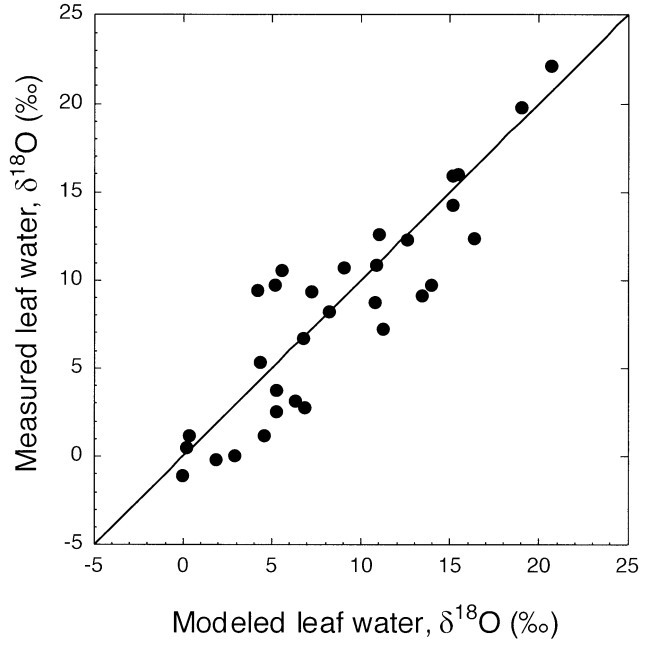

Fig. 7 The relationship between the $\delta^{18}O$ of modeled and measured leaf water for field-grown riparian-zone trees. Variations in leaf water were due to different stream water $\delta^{18}O$ values and temperature and humidity differences between sites. The *line* represents a 1:1 relationship

drogen or oxygen that undergoes exchange with medium water (f_H and f_O) can be calculated from the closest agreement between field cellulose observations and model predictions (Roden et al. 2000). We used the hydrogen isotope biological fractionation factor for autotrophic carbohydrate metabolism (ε_{HA}=–171‰) from Yakir and DeNiro (1990) as well as the biochemical fractionation factor associated with heterotrophic synthetic reactions (ε_H=+158‰) from Yakir and DeNiro (1990), which is similar to the mean multispecies value of +155‰ from Luo and Sternberg (1992). The near-equivalent magnitudes and opposite signs of ε_A and ε_H indicate that the hydrogen isotope ratio values of tree ring cellulose might be expected to approach that of source water (1:1 relationship) as has been commonly observed by many previous studies (Epstein and Yapp 1977; Burk and Stuiver 1981; Yapp and Epstein 1982; Lawrence and White 1984; White et al. 1994). For oxygen, the fractionation factor is the same for both heterotrophic and autotrophic metabolism (ε_O=+27‰; Sternberg 1989; Yakir and DeNiro 1990). The mechanism for the 27‰ enrichment is the carbonyl-water interaction during biosynthesis (Sternberg and DeNiro 1983). We have also shown (J.S. Roden and J.R. Ehleringer, unpublished data) that these fractionation factors are not temperature dependent.

The cellulose model was constrained to use only the f_O and f_H values that had been derived from the tightly controlled greenhouse experiments (Roden and Ehleringer 1999a). For oxygen isotope ratios in cellulose, there was a very strong agreement between the best-fit f_O value from field observations and the greenhouse experiments. Both experiments predicted that f_O values should be 0.42. For the field observations, it is important to recognize that the leaf water values associated with cellulose synthesis may not have been exactly the same as those values collected at midday on a particular day of the month. Therefore, our modeling analyses considered the range of possible predictions based on the range of leaf waters observed rather than only the average leaf water observed. The range of predicted cellulose $\delta^{18}O$ ratios is depicted in Fig. 8 as the gray band. For hydrogen isotopes in field cellulose samples, the best-fit f_H value was 0.31 (Fig. 9), which is slightly lower than had been observed in the well-controlled greenhouse experiment (0.36). When we constrain the model to use only the greenhouse observed f_H value of 0.36, the relationship between the model predictions and field observations is shown by the right-hand edge of the gray band in Fig. 9. The left-hand edge of this gray band corresponds to the lower range of possible values, based on known field variations in leaf water values as previously described. It is important to recognize that the model is inherently more sensitive to variations in f_H than f_O values, because for oxygen, the heterotrophic and autotrophic fractionation factors are identical, but they differ for hydrogen (Roden et al., in press). The predicted differences in f_H values may be real or possibly associated with the lack of constant environmental control in the field observations relative to the precise controls available under greenhouse conditions.

The results of DeNiro and Cooper (1989) for oxygen and Terwilliger and DeNiro (1995) for hydrogen imply that complete isotopic exchange with xylem water oc-

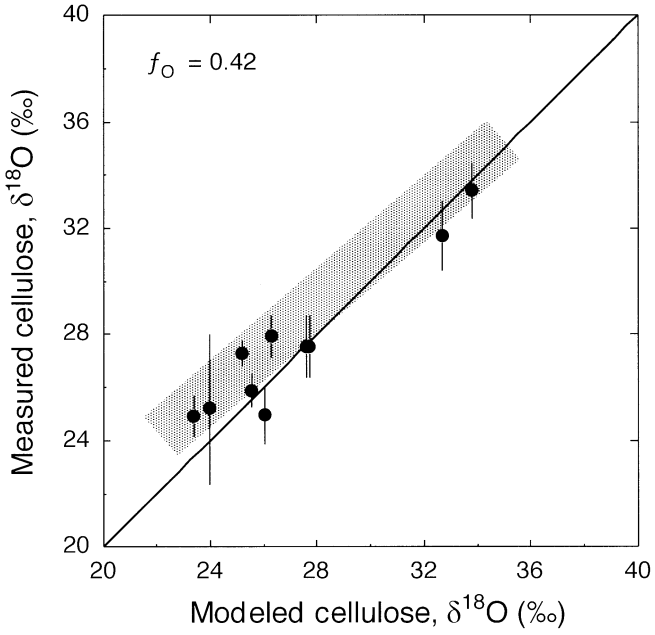

Fig. 8 The relationship between the $\delta^{18}O$ of modeled and measured tree ring cellulose for field-grown riparian-zone trees. Variations in tree ring cellulose were due to different stream water $\delta^{18}O$ values and temperature and humidity differences between sites. Values are means±SD. The *line* represents a 1:1 relationship. The *gray band* represents the predictions of the model using the measured range of leaf water values rather than the average leaf water value for a sampling date

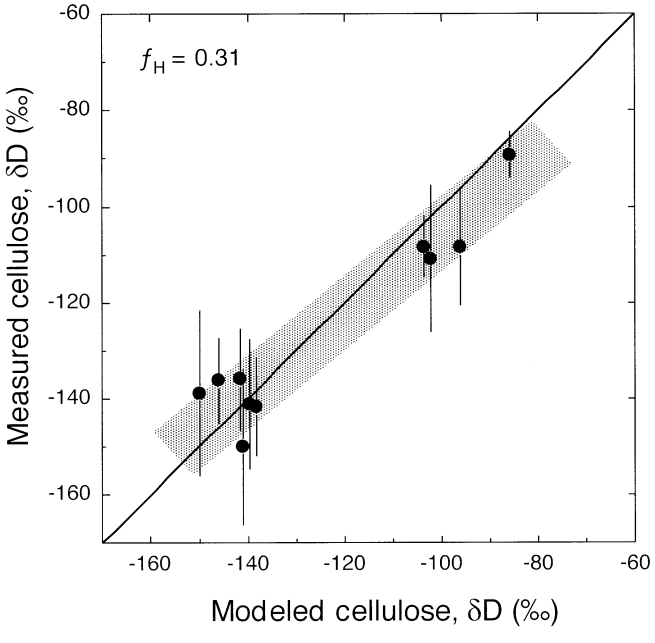

Fig. 9 The relationship between the δD of modeled and measured tree ring cellulose for field grown riparian zone trees. Variations in tree ring cellulose were due to different stream water δD values and temperature and humidity differences between sites. Values are means±SD. The *line* represents a 1:1 relationship. The *gray band* represents the predictions for cellulose isotope ratios if f_H was constrained to a value of 0.36 and if we include the observed range of leaf water values

curs during cellulose synthesis (that is, both f_H and f_O=1) and thus a strong correlation should be observed between source water and tree ring cellulose. We observed that f_H and f_O were less than 1 (f_H=0.31, f_O=0.42), indicating incomplete isotopic exchange between carbohydrate substrate and medium water during cellulose synthesis. These results agree with the observations of Luo and Sternberg (1992) that f_H and f_O are roughly similar in magnitude, and that when cellulose is derived from starch $f_H \approx f_O \approx 0.34$. Other studies have also reported that both f_H and f_O are substantially less than 1 (0.4–0.5; Sternberg et al. 1986; Yakir and DeNiro 1990; Yakir 1992) and are dependent on the carbohydrate substrate (lipids vs starch). Although Saurer et al. (1997) use a similar term (*f*, a damping factor) and obtained a similar result (*f*=0.4–0.5) for the $\delta^{18}O$ of cellulose in tree rings of three coniferous species, their model differs from that presented here. Their damping factor (*f*) includes both leaf water heterogeneity and isotopic exchange of sucrose with medium water, whereas our model distinguishes the two factors and is thus not strictly comparable. The differences between the Oregon and Arizona site also indicate that humidity information is recorded in tree ring cellulose, which differs from the predictions of DeNiro and Cooper (1989) and Terwilliger and DeNiro (1995), but agrees with other studies (Burk and Stuiver 1981; Edwards and Fritz 1986; Lipp et al. 1993).

Of course, this modeling analysis could have assumed that f_H and f_O are well established in the literature (≈0.4; Yakir and DeNiro 1990) and tested the literature estimates of ε_{HH}, ε_{HA}, and ε_O. However, as seen above, the estimates of f_H and f_O and the concept of isotopic re-equilibration during cellulose synthesis is still the most critical concept open to debate. The value of ε_O has been well established (Sternberg and DeNiro 1983; Sternberg 1989), and very similar values for ε_{HH} have been found in two studies (Yakir and DeNiro 1990; Luo and Sternberg 1992). Estimates of ε_{HA} are more difficult to determine, and using field observations would not be the most appropriate system to test both ε_{HH} and ε_{HA} since Eq. 1 would have two unknowns.

The fact that these parameters provided the greatest predictive power in both our field and experimental studies (Roden and Ehleringer 1999a) indicates that increased confidence in the literature estimates of biochemical fractionation is warranted.

Model predictions of the isotopic composition of tree ring cellulose from field-grown trees are complicated by limited knowledge as to the prevailing environmental conditions at the time of cellulose synthesis. Monthly measurements of source water and atmospheric water vapor δD and $\delta^{18}O$ along with estimates of leaf microclimate are likely insufficient to capture all the possible environmental variation for modeling purposes. Our data show that the isotopic composition of leaf water and atmospheric water vapor can vary substantially over short time periods (Figs. 1, 2). White and Gedzelman (1984) have shown that the δD of atmospheric vapor can vary by as much as 60‰ within a season and by even as much

as 30‰ over a 24-h period. As such, the isotopic composition of atmospheric vapor may not always be in equilibrium with local environmental water and can vary with the meteorological setting and the degree of mixing with upper air masses (White and Gedzelman 1984). Seasonal variation in source water δD and $\delta^{18}O$ could further complicate model predictions, which was avoided in this study by using small streamside trees where source water did not substantially vary throughout the sampling period (Figs. 1, 2). Most of these environmental uncertainties would be integrated into leaf water isotopic signatures, and thus our results include a range of possible predictions (gray band; Figs. 8, 9) based on the range of leaf waters observed. Techniques for subsampling individual tree rings (Loader et al. 1995) to quantify seasonal variations in cellulose δD and $\delta^{18}O$ along with intensive sampling of leaf microclimate and the δD and $\delta^{18}O$ of source water, leaf water, and atmospheric water vapor could greatly enhance our understanding of the way environmental variation is incorporated into a tree ring and how much information is lost through averaging measurements and bulking samples.

These results obtained from field-grown trees enhance our understanding of the relationships between the isotopic composition of source water and tree ring cellulose. Although the hypothesis of complete isotopic exchange with stem water at the time of cellulose synthesis is attractive in its simplicity, it is not supported by the results of this study. Clearly, the isotopic composition of tree ring cellulose reflects source water. However, the slope may not be equal to 1.0 under all environmental conditions and other environmental parameters such as humidity can alter that relationship. Although this complicates the straightforward interpretation of the δD and $\delta^{18}O$ values in tree ring records as reflecting precipitation inputs only, it also makes those records richer in information. The results presented here corroborate that the mechanistic model of tree ring isotope ratios (Roden et al. 2000), developed and tested under controlled environments, is robust enough to account for observed δD and $\delta^{18}O$ values of cellulose in trees grown under contrasting field conditions. The results of this study help to clarify inferred relationships between tree ring isotopic composition and environmental parameters derived from dendrogeochemical studies and correlation analysis. Field confirmation of these models will enhance their use as a tool for studying climate change, historical patterns of seasonal precipitation, long-term reconstruction of the sensitivity of plants to changes in moisture source and humidity, and water relation factors that contribute to immigration and emigration of species from a region.

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