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Leaf cellulose δD and $\delta^{18}O$ trends with elevation differ in direction among co-occurring, semiarid plant species

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Abstract—The potential to reconstruct paleoclimate from analyses of stable isotopes in fossil leaf cellulose could be enhanced by adequate calibration. This potential is likely to be particularly great in mid-latitude deserts, where a rich store of fossil leaves is available from rodent middens. Trends in δD and $\delta^{18}O$ of leaf cellulose were examined for three species growing across climatic gradients caused by elevation and slope aspect in southeastern Utah, USA. The species differed in morphology (*Pinus edulis* vs. *Yucca glauca*), photosynthetic pathway (C_3 *Y. glauca* vs. CAM *Yucca baccata*) or both (*P. edulis* vs. *Y. baccata*). The δD_{LCN} (leaf cellulose nitrate) and $\delta^{18}O_{LC}$ (leaf cellulose) values of *P. edulis* decreased with elevation. Stem water δD values either increased (in spring) or did not change with elevation (in summer). Needle water δD values usually decreased with elevation and differed greatly with leaf age. These results suggest that δ cellulose values of *P. edulis* record the effects of climate on the isotopic composition of leaf water but not climate effects on meteoric water. In contrast to *P. edulis*, δD_{LCN} values of *Y. glauca* increased with elevation. The $\delta^{18}O_{LC}$ values of *Y. glauca* also increased with elevation but less significantly and only on south-facing slopes. The δ cellulose values in both *P. edulis* and *Y. glauca* were most significantly related to changes in temperature, although temperature and precipitation were negatively correlated in the study area. Where all three species co-occurred, their δD_{LCN} values differed but their $\delta^{18}O_{LC}$ values were the same. The disparity in δD_{LCN} between *Y. baccata* and the other species corresponds to differences in biochemical fractionations associated with photosynthetic pathway. Biochemical fractionations may also contribute to differences between the two C_3 species. Knowledge of factors affecting responses of individual plant species to environment may be required to infer climate from δD_{LCN} and $\delta^{18}O_{LC}$. *Copyright © 2002 Elsevier Science Ltd*

1. INTRODUCTION

The potential for hydrogen and oxygen isotopic analyses of durable fractions of plants to yield inferences about past climates has long been recognized (Epstein and Yapp, 1976; Epstein et al., 1976, 1977; Yapp and Epstein, 1977). This potential derives from two factors: First, meteoric water reflects the effects of temperature on the extent to which hydrogen and oxygen fractionate as water changes state (Dansgaard, 1964; Gat, 1980; Rozanski et al., 1993). Second, water is the original source of hydrogen and, via exchange with CO_2, much of the oxygen for the synthesis of plant tissue (Sternberg et al., 1989).

Most efforts to reconstruct past climates from δD and $\delta^{18}O$ values of plants have been spent on the durable cellulose of tree rings (e.g., Burk and Stuiver, 1981; Edwards and Fritz, 1986; Feng and Epstein, 1994; White et al., 1994). Climatic reconstructions spanning longer periods of time are possible, however, from isotopic analyses of plant remains in fossil deposits, most notably rodent middens present in midlatitude deserts (Betancourt et al., 1990). Few efforts have been made, however, to reconstruct climate from δD and $\delta^{18}O$ of plant macrofossils in rodent middens (Siegel, 1983; Long et al., 1990; Jennings and Elliott-Fisk, 1993). Preliminary studies have yielded hopeful but inconclusive results, in part because species and plant parts were not held constant throughout the chronologies (for exceptions, see Pendall et al., 1999; Pendall, 2000).

Despite the physical effects of temperature on δD and $\delta^{18}O$ values of water, there remain uncertainties about climatic signals in celluloses between species and between plant parts (e.g., White et al., 1994; Buhay et al., 1996). Specifically, the δD and $\delta^{18}O$ values of cellulose can be affected by additional external factors and also internal factors. External factors include humidity and heterogeneity in isotopic composition of soil waters (Tang et al., 2000). Humidity can affect δD and $\delta^{18}O$ values of leaf water (modification of Craig and Gordon, 1965 by Dongmann et al., 1974), and this enrichment can be recorded in cellulose (Edwards and Fritz, 1986; Terwilliger and DeNiro, 1995; Roden et al., 2000). Physiologic and biochemical factors can cause internal fractionations (Sternberg, 1989b; Yakir, 1992). Furthermore, interactions can occur between these factors, such as differences in humidity effects on δ values among plant parts or variations in magnitude of internal fractionations among species. The potential for reconstructing climate from δD and $\delta^{18}O$ analyses of plant celluloses would be maximized by better understanding and quantifying these factors.

Leaf δ values have greater potential to yield high-resolution climatic information than any other plant part in rodent middens. This is because δ values of plant parts vary between species (Cooper and DeNiro, 1989), and leaves are the plant remains found in rodent middens that can be most readily sorted by species.

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Three questions need to be addressed to define better the certainty and resolution with which climatic inferences can be made from δD and $\delta^{18}O$ analyses of leaves: (1) To what extent can factors other than temperature affect δ values of leaves in the field? For example, humidity may have a larger influence on leaf than stem (xylem) δD and $\delta^{18}O$ values because of smaller fractionations from heterotrophic isotope effects and little input from non-enriched stem water (Yakir, 1992; Terwilliger and DeNiro, 1995). (2) What are the minimum differences in climate that can be extracted from δD and $\delta^{18}O$ values of leaf cellulose, given that other factors may also cause variation in these values within species? (3) Are there differences in the resolution with which climate shifts can be detected among species?

We address these three questions in this study by examining trends in δD and $\delta^{18}O$ values of leaves within species along a systematic climatic gradient and comparing the trends between species of different functional groups. Specifically, trends in δD and $\delta^{18}O$ of cellulose are examined from leaves collected over a large climatic gradient created by changes in elevation and over smaller variations in climate caused by differences in slope aspect. The species studied are common in ancient packrat middens of the southwestern United States (Wells, 1983; Betancourt et al., 1990). They include piñon pine (*Pinus edulis* Engelm.); a non-woody C_3 perennial, small soapweed (*Yucca glauca* Nutt.); and a member of the same genus that exhibits Crassulacean acid metabolism (CAM) (*Yucca baccata* Torr.).

2. STUDY SITE AND MATERIALS AND METHODS

2.1. Study Site

P. edulis, *Y. glauca*, and *Y. baccata* were collected on north- and south-facing slopes every 150 m within a 1220 to 2745 m elevation transect in southeast Utah, USA. Specifically, *P. edulis* was collected along its range from 1525 to 2745 m; *Y. glauca*, along its range from 1220 to 2135 m; and *Y. baccata*, along its range from 1830 to 2440 m. The transect began near the Colorado River at Blue Notch Canyon (37°44′52″ N, 110°23′24″ W, 1220 m), included Natural Bridges National Monument, and rose to the top of Bear's Ears Mesa in the Manti La Sal National Forest (37°37′31″ N, 109°52′29″ W, 2745 m). Vegetation at the lowest elevations is desert scrub. The transition to piñon pine-juniper woodland begins at 1500 m, and ponderosa pine-gambel oak communities appear above 2300 m. The exposed rimrock is Permian Cedar Mesa Sandstone and Organ-Rock Tongue of the Cutler Formation, Triassic Chinle Formation, and the Jurassic Wingate Formation (Stokes, 1986; Hintze, 1988). Collections were made only from interfluve areas.

2.2. Sampling Design

Leaves from which cellulose was extracted were sampled during early summer 1995 as part of a separate research effort (Van de Water, 1999). Needles of *P. edulis* were from the 1994 cohort. Current leaves of the *Yucca* species were sampled but might represent previous years' growth as well. Where possible, leaves were sampled from five individuals per aspect at a given elevation over the full range of each species along the transect. To achieve a homogeneous representation of the isotopic variation within each individual (Leavitt and Long, 1983), samples were equal proportions of leaves collected from all four cardinal directions of outer, sun-lit locations of each plant.

To examine variation in δD and $\delta^{18}O$ of plant source waters, needles and stem xylem samples of *P. edulis* were collected in 1997. Stem xylem samples were collected with a 6.25-mm increment borer from five trees per aspect at two elevations (2135 and 2740 m) in late May 1997. In early August, the full range of *P. edulis* was more fully represented by collecting stem xylem from these trees and from an additional five trees per aspect at 1677 m. Bark and phloem cells were discarded from each stem core, and the remaining sample was sealed in a glass tube with a rubber septum. Needles were collected from the same trees as stems close to dawn and again in the early afternoon. Both newly emerging needles and needles from the 1996 cohort were collected as separate samples in May. Only needles from the 1997 cohort were collected in August. All samples were frozen with dry ice immediately upon collection until they could be brought to a freezer.

2.3. Climate

The specific climatic variables examined in this study were temperature and precipitation, the latter providing an indication of humidity. Climate data from 18 weather stations in the sampling region and ranging from the lowest elevation studied (1220 m) to 2150 m were obtained from Western Region Climate Data Center records. Some weather stations ranged in location from <1 km to >10 km from the nearest point on the sampling transect. These data were used to establish adiabatic trends in temperature and precipitation that we used to estimate climates of the elevations sampled.

2.4. Cellulose Extraction and Nitration

Dry needles and leaves were ground to 60 mesh in a Wiley mill. Waxes and resins were removed in a hot ethanol/toluene wash, using the batch method of Leavitt and Danzer (1993). Alpha cellulose was extracted using hot sodium chlorite solution, glacial acetic acid, and a final soak in sodium hydroxide as described in Sternberg (1989a). For δD analyses, exchangeable hydrogens in hydroxyl groups were removed by nitration using acetic anhydride and fuming nitric acid as per Sternberg (1989a).

2.5. Isotopic Analyses

Water was extracted quantitatively from needles and stem xylem of *P. edulis* by cryogenic vacuum distillation as per Epstein and Mayeda (1953). Specifically, all water was extracted from the sample tubes by attaching them to the vacuum lines via needles inserted into their rubber septa. The $\delta^{18}O$ values of plant waters were determined on a GasBench with a CombiPAL autosampler (CTC), coupled to a Finnigan Delta Plus stable isotope ratio mass spectrometer (ThermoFinnigan), from injections of CO_2 that had been fully equilibrated with 0.5-mL water samples in sealed vials by shaking for 48 h at 25°C. For analyses of their δD values, hydrogen gas was obtained from water samples by passing them over uranium at

Table 1. Air temperatures and precipitation at different elevations near the study gradient in southeastern Utah (from http://www.wrcc.dri.edu). Data include annual and growing season average air temperatures and precipitation totals for 1994 (when samples for cellulose extraction were taken) and 1997 (when *Pinus edulis* was sampled for water extraction), long-term mean annual and mean growing season values, and time periods over which long-term means were calculated. Growing season values encompass April–October and have a "G" in the label.

Weather station	Elevation (m)	Temperature (°C)						Precipitation (mm)						Period
		1994	1994G	1997	1997G	Mean	MeanG	1994	1994G	1997	1997G	Mean	MeanG	
Moab	1220	15.1	21.8	13.9	20.8	13.1	20.2	172	105	333	249	230	139	1889–1999
Green River	1240	13.3	20.7	12.1	18.4	11.4	19.0	198	147	229	180	158	106	1893–1999
Dewey	1260	13.5	21.0	12.2	19.0	12.2	19.4	211	102	268	201	247	138	1967–1999
Mexican Hat	1290	14.7	22.0	13.6	20.4	13.6	20.6	133	92	166	130	163	97	1948–1999
Bluff	1314	12.8	19.7	12.2	18.7	12.5	19.4	145	81	241	184	198	113	1928–1999
Hanksville	1360	13.0	20.6	12.0	19.1	11.8	19.3	139	98	221	164	135	95	1948–1999
Aneth Plant	1410	19.1	21.3	9.5	19.2	13.1	20.0	182	124	114	61	226	119	1959–1999
Canyonlands, Needle	1540	12.1	19.4	12.8	19.4	12.0	18.7	191	131	262	180	215	144	1965–1999
Monument Valley	1590	*	*	*	*	13.6	20.2	*	*	*	*	186	121	1961–1989
Hovenweep	1600	12.4	18.4	11.1	16.9	10.8	17.6	200	143	282	208	278	159	1957–1999
Capitol Reef	1680	12.3	19.4	11.5	17.9	12.1	18.9	173	112	293	247	194	134	1967–1999
Castle Dale	1710	11.0	16.9	8.5	15.3	8.3	15.2	148	130	298	252	197	131	1928–1999
Canyonlands, Neck	1800	13.2	19.5	11.3	18.1	11.4	18.4	136	87	270	202	236	153	1965–1999
Ferron	1810	10.2	17.3	9.3	16.1	9.1	15.9	174	138	337	286	217	141	1948–1999
Blanding	1840	12.1	18.6	11.4	17.6	10.1	16.4	374	239	509	331	337	187	1948–1999
Natural Bridges	1980	10.9	17.8	10.3	16.3	10.3	16.7	233	177	362	237	315	196	1965–1999
Cedar Point	2060	9.0	15.5	8.1	14.2	8.2	14.5	395	227	548	331	385	221	1957–1999
Monticello	2150	8.4	14.7	7.4	13.7	7.8	14.0	443	246	543	319	385	231	1948–1999

750°C (Northfelt et al., 1981) and collecting aliquots of the gas in borosilicate glass breakseals.

The $\delta^{18}O$ values of cellulose were obtained from all but 10 of the samples after combustion with mercuric chloride at 600°C for 3 h in evacuated Vycor tubes to yield a mixture of CO_2, CO, and HCl. The CO was converted to CO_2 and C via a 5000-V discharge under vacuum; Cl was removed by exposure to hot zinc; and total CO_2 was then cryogenically isolated into borosilicate breakseals (Sauer and Sternberg, 1994). Later, the opportunity to automate the analyses of $\delta^{18}O$ values on an online system arose. We used online methods to reanalyze 10 outliers and 10 samples that had resulted in insufficient yield using the offline method. Using the online method, three 0.2- to 0.4-mg samples per tree were pyrolized on nickelized carbon using an elemental analyzer (EA1108, Carlo Erba Instruments), and the resulting CO was carried to a stable isotope ratio mass spectrometer (Optima, Micromass) on a 99.9996% pure He gas stream (as per Wang et al., 1998).

The δD values were obtained from cellulose nitrate by combustion with cupric oxide at 900°C for 3 h. The water of combustion was then isolated under vacuum with a dry ice/isopropanol slush (see Sternberg, 1989a) and reduced to hydrogen gas as described for water samples. All δD values and most of the $\delta^{18}O$ values of cellulose were obtained on the dual inlet of a Delta-S mass spectrometer (Finnigan). All δ values are expressed relative to SMOW. The precisions of the isotopic analyses were ± 0.2 and 1‰ for oxygen and hydrogen, respectively.

Tissues were ground at the Laboratory of Tree Ring Research, University of Arizona. All other preparations and offline gas extractions were performed at the University of Kansas. Dual inlet mass spectrometry was performed at the Stable Isotopes Laboratory, University of Nebraska. $\delta^{18}O$ analyses of *P. edulis* waters were performed at the SIMSL Laboratory, Kansas State University. On-line $\delta^{18}O$ analyses of cellulose were performed at the Department of Environmental Science and Energy Research, Weizmann Institute, Rehovot, Israel.

2.6. Statistical Analyses

Comparisons of two groups of samples were made using t-tests. Parameters that might be autocorrelated, such as temperature and precipitation, were examined by Pearson's correlation analysis. Simple linear regressions were used to examine dependence of one variable on another. Relationships of δ values to physical variables were examined by analyses of covariance and tests for parallel slopes when some variables, such as aspect, were nominal. When variables were all ordinal, stepwise model selection followed by multiple regression were performed. Analyses of measurements repeated on the same individual such as paired t-tests were performed only if the measurement was repeated on the same module. For example, a δD value of a stem's water in August was paired with the δD value of the corresponding stem in May, whereas needle waters were not paired. When independent variables were significantly correlated, such as for temperature and precipitation, residuals from the linear regression of one were plotted against values of the other to examine whether both had influences on the dependent variable. ANCOVAS and related tests were performed using Statistica v.5.5 (StatSoft). All other statistical analyses were performed using Minitab v.12.2, (Minitab).

3. RESULTS

3.1. Climate

The change in long-term mean annual temperatures with elevation was -0.5°C 100 m^{-1} ($p < 0.0005$, $r^2 = 0.61$) and for growing seasons was -0.6°C 100 m^{-1} ($p < 0.0005$, $r^2 = 0.74$) (Table 1). Mean annual precipitation increased 15 mm 100 m^{-1} ($p < 0.0005$, intercept at origin) and 9 mm 100 m^{-1} during the

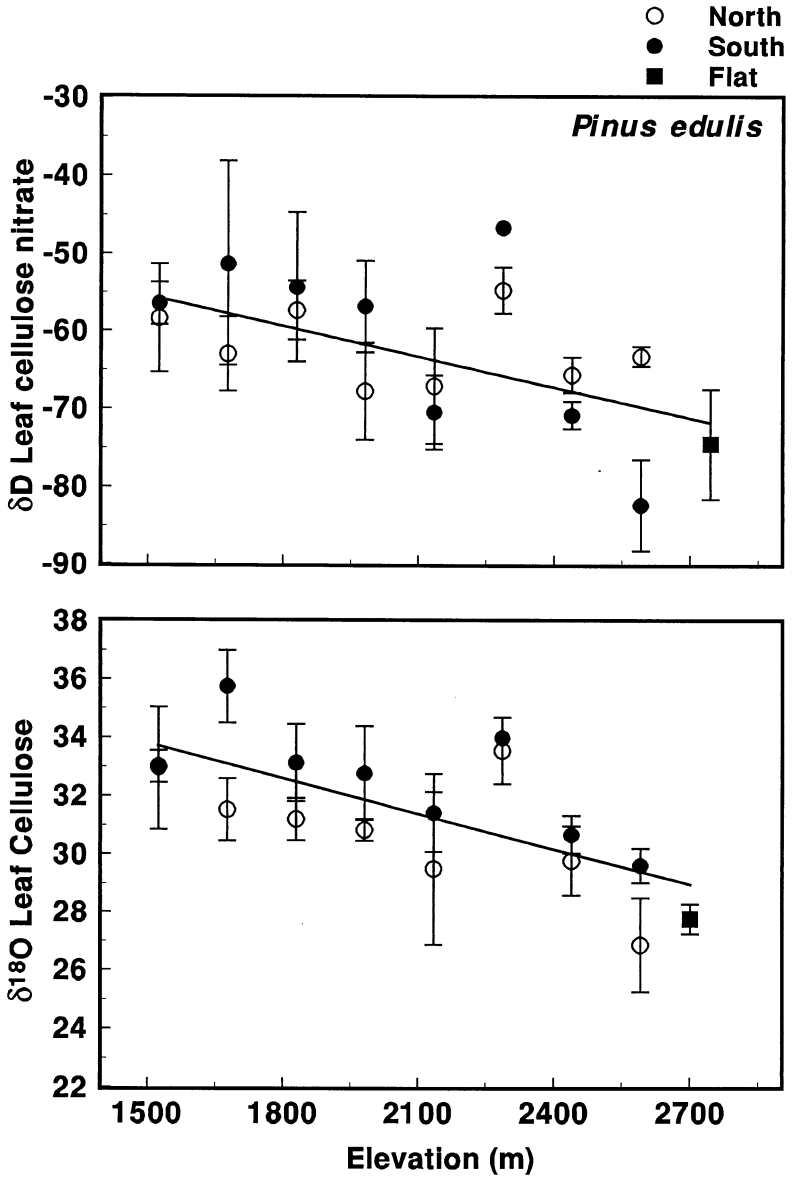

Fig. 1. Changes in δD and $\delta^{18}O$ in the celluloses of *P. edulis* needles with elevation. Data are means for a given aspect (± SE) ($n = 5$ except at 2290 m where south $n = 1$ and north $n = 8$). Values do not differ by aspect, and regression lines are for all values (for δD, $p < 0.002$, $r^2 = 0.14$; for $\delta^{18}O$, $p < 0.0005$, $r^2 = 0.22$).

growing season (data in Table 1). The gradient in mean temperature in 1994 was -0.6°C 100 m^{-1} ($p < 0.0005$) and -0.5°C 100 m^{-1} in 1997. The precipitation gradient was 14 mm 100 m^{-1} in 1994 and 20 mm 100 m^{-1} in 1997. Growing season (April–October) was defined as temperatures above freezing. Nonetheless, all species studied were evergreens and capable of carbon gains at close to freezing temperatures (Teskey et al., 1995; Maragni et al., 2000). We caution that, although these data are adequate for comparing the sensitivities of δ values to climate, more error has been introduced into our estimates of the absolute sensitivities of δ values to climate than would have been introduced had site-specific data on climate been feasible to obtain.

3.2. Variation of Cellulose δD and $\delta^{18}O$ with Topography and Climate: *P. edulis*

Both δD of leaf cellulose nitrate (LCN) and $\delta^{18}O$ of leaf cellulose (LC) in *P. edulis* decreased significantly as elevation increased (Fig. 1) (for δD, $p < 0.002$; for $\delta^{18}O$, $p < 0.0005$). The samples that were run both online and from offline gas extractions had similar $\delta^{18}O_{LC}$ values, so the 10 samples that were run exclusively online were included in this analysis. Other studies have found the two methods to give similar results as well (e.g., Sauer et al., 1998). δD_{LCN} values decreased on average by -1.3‰ 100 m^{-1} increase in elevation and $\delta^{18}O_{LC}$ values decreased by -0.4‰ 100 m^{-1}. Variation in

the relationship between δ values and elevation was high, however, and higher in δD_{LCN} ($r^2 = 0.14$) than in $\delta^{18}O_{LC}$ ($r^2 = 0.22$) values. Neither δD_{LCN} nor $\delta^{18}O_{LC}$ values differed between north and south facing slopes, however. δD_{LCN} values were not significantly related to $\delta^{18}O_{LC}$ values either when examined as a whole or at each individual elevation.

The direction of changes in δD_{LCN} and $\delta^{18}O_{LC}$ were what would be expected of water over a climatic gradient from hot and dry to cooler and wetter. The minimum change in temperature that would be detectable from δD_{LCN}, and $\delta^{18}O_{LC}$ trends at this level of replication was 7.3°C (at 2.3‰ $°C^{-1}$ for hydrogen and 0.7‰ $°C^{-1}$ for oxygen), and the minimum change in precipitation was 170 mm (based on the minimum elevation range over which significant changes in δ occurred and the climate trends over that range obtained from linear regressions of the 1994 data in Table 1).

There are weak statistical indications that the relationships of δD_{LCN} and $\delta^{18}O_{LC}$ to elevation are more associated with temperature than with precipitation (and thus humidity). Caution must be added to this statement, however, because temperature and precipitation were highly correlated on an annual average ($r = -0.65$, $p < 0.004$) and especially during a growing season ($r = -0.8$, $p < 0.0005$, data in Table 1). Both δD_{LCN} and $\delta^{18}O_{LC}$ were positively related to mean annual as well as growing-season temperatures for 1994 when the needles were produced (for δD_{LCN}, $p < 0.029$, $r^2 = 0.104$; for $\delta^{18}O_{LC}$, $p < 0.025$, $r^2 = 0.127$, δ values regressed against temperatures of similar elevations listed in Table 1). The relationships of δ values with mean annual temperature are the same as to mean growing-season temperature (parallel line test, $p > 0.8$). There was no significant relationship between δD_{LCN} values and either annual or growing-season precipitation for 1994. $\delta^{18}O_{LC}$ values decreased with increase in precipitation, although the relationship was less robust than between $\delta^{18}O_{LC}$ and temperature ($p < 0.038$, $r^2 = 0.11$). Precipitation did not significantly influence the relationship of δD_{LCN} or $\delta^{18}O_{LC}$ to temperature (based on δD_{LCN} or $\delta^{18}O_{LC}$ vs. temperature residuals regressed against precipitation).

3.3. δD and $\delta^{18}O$ in *P. edulis* Stem Water

The δD values of stem (xylem) water (SW) of *P. edulis* did not reflect climate trends with elevation (Fig. 2). In the spring samples, δD_{SW} values increased slightly (8‰ on average) but significantly from 2135 to 2740 m (1 tailed t-test, $p < 0.02$). The δD_{SW} samples collected in summer were significantly higher than those collected in May (paired t-test, $p < 0.0005$) but did not vary significantly with elevation, even though a larger gradient (1670–2740 m) was sampled. Neither May nor August δD_{SW} samples varied systematically with aspect.

We were unable to extract sufficient water from many stems to determine accurately their $\delta^{18}O$ values. No clear trend in $\delta^{18}O$ emerged with elevation in the samples we analyzed, however (Fig. 2).

3.4. δD and $\delta^{18}O$ of Needle Water in *P. edulis*

Needle water (LW) δD and $\delta^{18}O$ trends with elevation did not correspond to those of stem water in *P. edulis* (Fig. 3). In contrast to δD_{SW} values, the δD_{LW} and $\delta^{18}O_{LW}$ values of newly emerging needles decreased significantly with elevation in May (−3.3‰ 100 m^{-1} on average for δD_{LW}, one tailed t-test, $p < 0.02$; −0.9‰ 100 m^{-1} on average for $\delta^{18}O_{LW}$, $p < 0.00005$). In August, both δD_{LW} and $\delta^{18}O_{LW}$ values decreased with elevation (−2.0‰ 100 m^{-1} on average for δD_{LW}, $p < 0.0005$, $r^2 = 0.67$; −0.9‰ 100 m^{-1} on average for $\delta^{18}O_{LW}$, $p < 0.0005$, $r^2 = 0.64$). In contrast to δD_{SW} trends, δD_{LW} and $\delta^{18}O_{LW}$ values waters sampled in May did not vary with elevation.

In addition to elevation, we examined whether δD and $\delta^{18}O_{LW}$ values were related to slope aspect, time of day of sampling, and needle age. Needle waters were slightly but significantly more enriched in deuterium (8‰ on average) and ^{18}O (1.5‰ on average) in the early afternoon than before midmorning in August, and the magnitude of this difference was the same at all three elevations sampled (ANCOVA, parallel line test, $p < 0.0005$). The greatest differences in δD_{LW} or $\delta^{18}O_{LW}$ values occurred between needles of different ages (ANCOVA, $p < 0.0005$) (Fig. 3). Newly emerging needles of May had at least 30‰ lower δD_{LW} and 10‰ lower $\delta^{18}O_{LW}$ values than the oldest needles (also May). No effect emerged for slope aspect.

As a measure of the extent to which δD_{LW} changes with elevation reflect deuterium enrichment patterns in leaves, we quantified deuterium enrichment in bulk leaf water relative to source (stem) water (ΔD) as follows:

$$\Delta D_{LW\text{-}SW} = 1000\,(\alpha - 1) \qquad (1)$$

where $\alpha_{LWSW} = R_{LW}/R_{SW}$ and $R = [D]/[H]$. $\Delta D_{LW\text{-}SW}$ was computed from data using the equation

$$\Delta D_{LW\text{-}SW} = 1000\,(\delta D_{LW} - \delta D_{SW})/(\delta D_{SW} + 1000) \qquad (2)$$

As would be expected from climate trends, $\Delta D_{LW\text{-}SW}$ always decreased with increasing elevation (August samples, $p < 0.0005$, $r^2 = 0.41$; newly emerging leaves, one-tailed t-test, $p < 0.0005$; older leaves in May, one-tailed t-test, $p < 0.0014$) (Fig. 4). No significant differences emerged in $\Delta D_{LW\text{-}SW}$ between needle water of north- and south-facing slopes. We did not have a sufficient number of $\delta^{18}O_{SW}$ values to perform a similar analysis of ^{18}O enrichment patterns in leaves.

Although δ values of both leaf water and cellulose decreased with elevation, one important difference occurred between δ_{LW} and δ cellulose trends. In contrast to δD_{CN} and $\delta^{18}O_{LC}$, δD_{LW} was significantly related to $\delta^{18}O_{LW}$ ($p < 0.0005$, $r^2 = 0.59$) (Fig. 5).

3.5. Variation in δD_{LCN} and $\delta^{18}O_{LC}$ with Topography and Climate: *Yucca spp.*

The δD_{LCN} and $\delta^{18}O_{LC}$ values of *Y. glauca* were related to elevation (Fig. 6); however, the relationships did not coincide with those of *P. edulis*. Specifically, δD_{LCN} increased by an average of 4.0‰ 100 m^{-1} ($p < 0.0005$), and $\delta^{18}O_{LC}$ values on south-facing slopes increased by 0.7‰ 100 m^{-1} ($p < 0.033$). The $\delta^{18}O_{LC}$ values on north-facing slopes did not vary with elevation. The δD_{LCN} and $\delta^{18}O_{LC}$ values did not vary systematically with elevation in *Y. baccata* (Fig. 7).

As in *P. edulis*, the δD_{LCN} values of *Y. glauca* were unrelated to slope aspect. In contrast to *P. edulis*, however, the

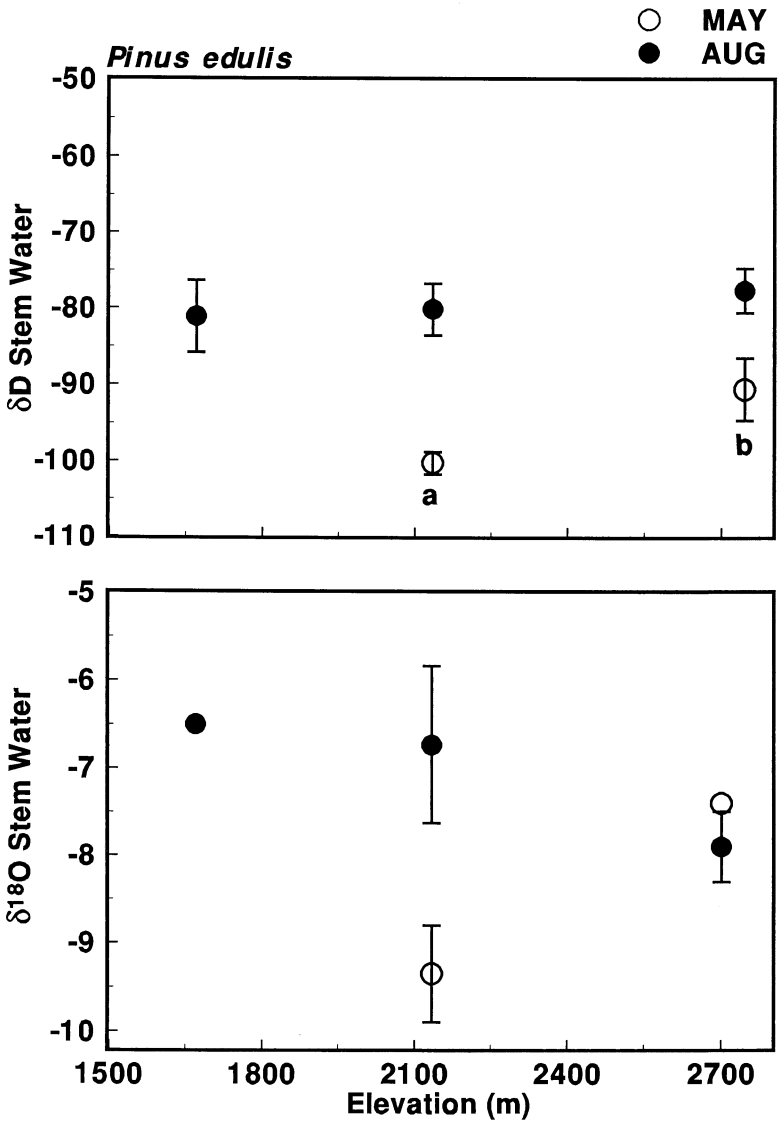

Fig. 2. δD and $\delta^{18}O$ values of the stem water of *P. edulis* at different elevations and times of year. Data points are means for a given elevation (± SE, $n = 10$ for δD, $n = 2$–6 for $\delta^{18}O$, no error bar if $n = 2$). Open symbols are for samples collected in May and closed circles are for samples collected in August 1997. Different letters show where values differed significantly with elevation (May δD values only; one tailed t-test, $p < 0.02$).

$\delta^{18}O_{LC}$ values of *Y. glauca* were significantly higher on south- than on north-facing slopes (ANCOVA, parallel line test, $p < 0.05$). *Y. baccata* occurred at both aspects of a single elevation (2290 m) and showed higher δD_{LCN} and $\delta^{18}O_{LC}$ values on the south-facing than on the north-facing slope (t-test, $p < 0.02$). δD_{LCN} values could detect a minimum change in temperature of 3.7°C and 86 mm change in precipitation (based on the minimum elevation range over which significant changes in δ occurred and the climate differences over that range using Table 1, 1994 data).

As with *P. edulis*, the δD_{LCN} and $\delta^{18}O_{LC}$ values of *Y. glauca* were more closely associated with changes in temperature than with precipitation, although the directions of the relationships were opposite to one another in the two species. Specifically, both $\delta^{18}O_{LC}$ on south-facing slopes and δD_{LCN} values decreased significantly as annual and growing-season temperatures for both the long term and 1994 increased (for δD_{LCN} vs. annual and growing-season temperatures, $p < 0.0005$, $r^2 = 0.23$; for $\delta^{18}O_{LC}$ south-facing slopes, $p < 0.02$, $r^2 \geq 0.56$; δ values regressed against temperature of similar elevation in Table 1). δD_{LCN} values increased significantly with increase in annual precipitation and growing-season precipitation for 1994, although the relationships were less robust than between δD_{LCN} and temperature (for annual precipitation, $p < 0.04$, $r^2 = 0.10$; for growing-season precipitation, $p < 0.005$). $\delta^{18}O_{LC}$ values were not significantly related to amount of precipitation at either aspect. Amount of precipitation did not significantly influence the relationship of δD_{LCN} or $\delta^{18}O_{LC}$ on south-facing slopes to temperature (residuals of δ values vs. temperature regressed against precipitation). As with *P. edulis*, δD_{LCN}

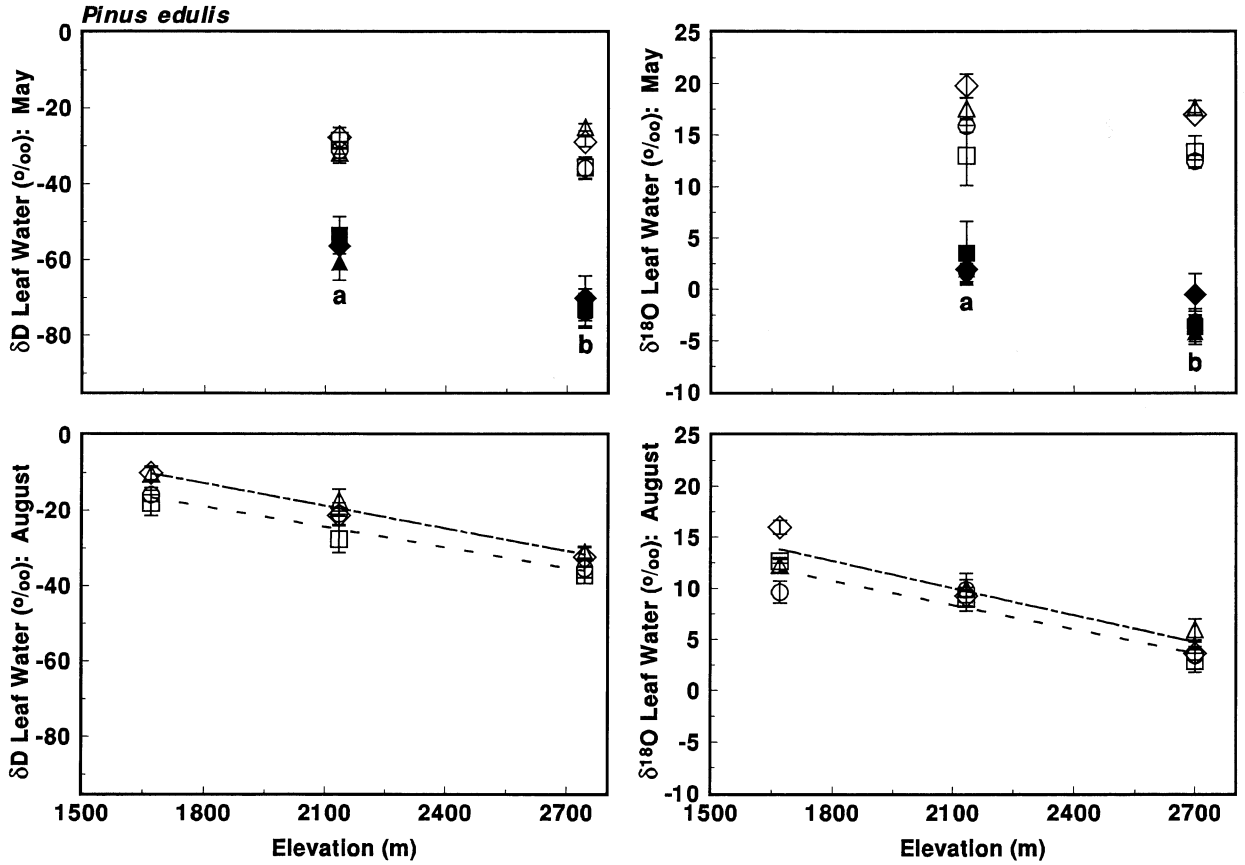

Fig. 3. δD and $\delta^{18}O$ values of water of *P. edulis* needles at different elevations, times of year, leaf ages (newly emerging = closed symbols, fully expanded = open symbols), times of day, and aspects (circles = morning samples on north aspects, triangles = afternoon samples on north aspects, squares = morning samples on south aspects, diamonds = afternoon samples on south aspects. Data are means (± SE, $n = 5$). As denoted by different letters, δ values of newly emerging leaves differed significantly between the two elevations sampled in May (t-tests, $p < 0.02$ for δD and $p < 0.00005$ for $\delta^{18}O$). In the August plots, the dotted line is for morning samples and the dot-dashed line is for afternoon samples (regressions, $p < 0.0005$; $r^2 = 0.64$ for morning δD, $r^2 = 0.82$ for afternoon δD; $r^2 = 0.59$ for morning $\delta^{18}O$, $r^2 = 0.77$ for afternoon $\delta^{18}O$).

values of *Y. glauca* were not significantly related to their corresponding $\delta^{18}O_{LC}$ values, even if considered separately by aspect and elevation (ANCOVA).

4. DISCUSSION

4.1. Comparison of minimum differences in climate detectable in δD_{LCN} and $\delta^{18}O_{LC}$

The δDLCN and $\delta^{18}O_{LC}$ values of *P. edulis* and *Y. glauca* cellulose varied significantly, albeit in differing directions with elevation and hence temperature (Figs. 1, 6). Temperature, in turn, was negatively correlated with amount of precipitation and, by logical extension, humidity. As suggested by the following evidences, amount of precipitation had a less discernable effect on δ values of cellulose than temperature, however. In no species were both δD_{LCN} and $\delta^{18}O_{LC}$ related to estimated precipitation amounts. Where significant relationships did emerge between δ values and precipitation amount, precipitation accounted for less of the variation among δ values than did temperature.

Even though the relationship of δD_{LCN} to temperature was counterintuitive in direction in *Y. glauca*, δD_{LCN} values varied significantly over smaller changes in temperature (minimum of 3.7°C) than in *P. edulis* (7.3°C). We caution that because our climate trends are not from the specific sampling sites, the magnitude of the temperature differences may, in reality, be greater or smaller. *Y. baccata* δD_{LCN} and $\delta^{18}O_{LC}$ values showed no relationship to elevation or climate but spanned only a 3.0°C temperature gradient (Fig. 7). The δD_{LCN} values of *Y. baccata* were simply much higher than δD_{LCN} values of the other species.

The aforementioned information provides an affirmative answer to the question of whether there are differences in the resolution with which climate shifts are recorded in the δ values in the cellulose of different species. It also establishes some minimum differences in climate from δD_{LCN} and $\delta^{18}O_{LC}$ with the caveat that many replicates are needed to achieve a credible level of resolution in any climatic reconstruction. Thus, for example, credible inferences about past climate from δ values of rodent middens would require separating tissue from a single species, then conducting several analyses for each date to be examined. This is analogous to the method of obtaining moving

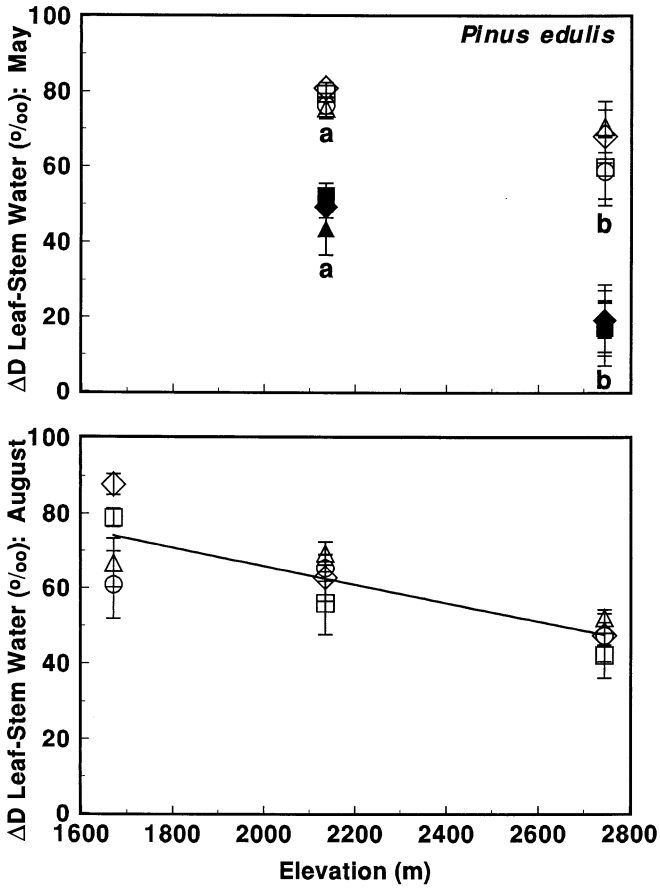

Fig. 4. Changes in discrimination of leaf from stem water (ΔD) values of *P. edulis* with elevation. Data points are means (± SE, $n = 5$). Symbols are for the same categories described for Fig. 3. Letters represent that ΔD differed with elevation for newly emerging and mature needles in May (t-tests, newly emerging needles, $p < 0.0005$; mature needles, $p < 0.014$). The line shows the trend in elevation for August ΔD values (regression, $p < 0.0005$, $r^2 = 0.41$).

averages of δ values over multiyear time sequences to better extract climate trends from other sources of noise in tree ring chronologies (Feng and Epstein, 1994, 1995).

The remaining question is, to what extents can factors other than temperature affect δ values of leaves in the field? The rest of our discussion addresses this question. First, we examine the influence of climate on plant water δ values and the extent to which this influence may be recorded in δ values of *P. edulis* needles. Then we evaluate possible reasons for the differences in relationships of climate to δ values among all three species.

4.2. Climate and δ Values in *P. edulis*

δD_{LCN} and $\delta^{18}O_{LC}$ values generally changed as expected with climate in *P. edulis*, decreasing from hotter and drier to colder and wetter elevations (Fig. 1). Numerous studies have found that the δD and $\delta^{18}O$ values of precipitation also decrease from warmer, drier, and lower to cooler, wetter, and higher elevations (e.g., Smith et al., 1979; Siegenthaler and Oeschger, 1980; Burk and Stuiver, 1981). Our results do not suggest that temperature effects on δ values of meteoric water produced the trends we observed in δ values of needle celluloses, however. Climate signals from meteoric water would had to have been preserved in the δ values of stem water for them to have been recorded in cellulose. Neither δD nor $\delta^{18}O$ values of stem water decreased with elevation (Fig. 2).

The following data suggest that seasonal changes in δD_{MW} and $\delta^{18}O_{MW}$ may have influenced the δ values of stem waters at a given elevation, however. The closest station with isotopic records of meteoric water is Flagstaff, Arizona (IAEA, 2001). In the 15 yr that these records span (1961–1976), mean δD and $\delta^{18}O$ values of precipitation were significantly lower in May than in August (t-tests, $p < 0.01$). Similarly, Smith et al. (1979) found δD values of meteoric water to be lower in winter and spring than in summer within a region encompassing the not too distant Sierra Nevada Mountains in California and Nevada. Pendall (1997) found soil and xylem water δD values to be lower in spring than in summer at locations in Arizona, Nevada, and New Mexico within our sites' median elevation.

Tang and Feng (2001) concluded that although $\delta^{18}O$ values of both precipitation and water in shallow soil were lower in spring than in summer in climatically dissimilar New Hampshire, the summer soil values reflected both the effects of evaporative enrichment as well as inputs of summer rain. Because the tree species they studied had shallow roots, en-

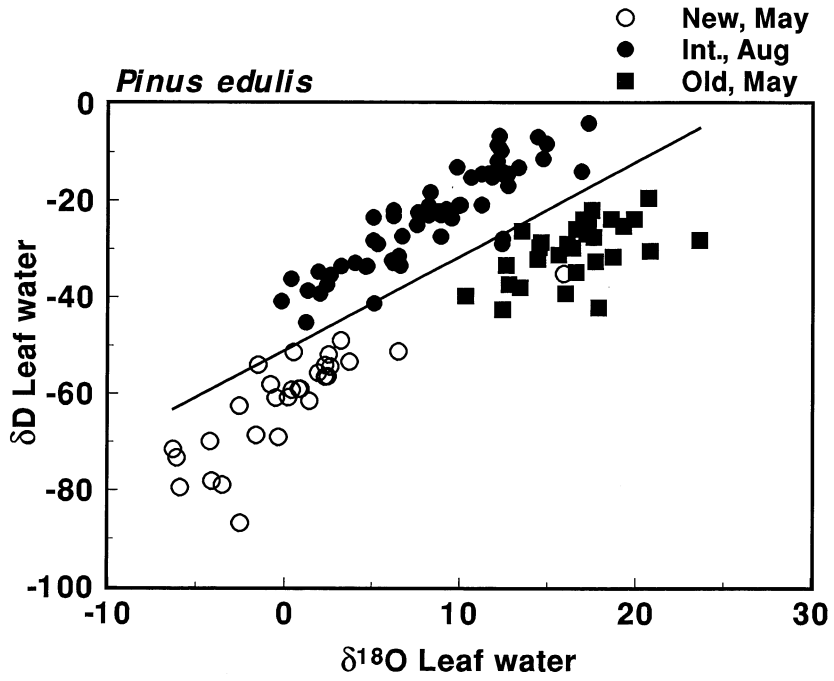

Fig. 5. Relationship of δD to $\delta^{18}O$ values in needle waters of *P. edulis* ($p < 0.0005$; $r^2 = 0.59$). If the relationship of δD to $\delta^{18}O$ and needle age are considered, then $r^2 = 0.90$ (i.e., slopes of relationships differ between age/time of year groups; ANCOVA, $p < 0.0005$; parallel line test).

richment effects could be accounted for in efforts to extract a climate signal from stem water. The δD and $\delta^{18}O$ values of soil water change with soil depth so that enrichment effects may be more difficult to quantify in deeply rooted species that are capable of acquiring water from a variety of depths. From the available evidences, we can only conclude that any climate effects on δ_{MW} other than what may occur seasonally were masked by external factors such as variation in evaporative enrichment in soil waters before their uptake by *P. edulis*.

More compelling is the argument that trends in δ values of needle celluloses with elevation reflect leaf responses to climate. Leaf response to climate, as given by $\Delta D_{LW\text{-}SW}$, decreased as would be expected with elevation (Fig. 4). The response was large enough in needles of a given age to mask the tendency for the source of water (stem) to the leaf to either not vary or increase with elevation. These results suggest that the decrease in δD_{LCN} with elevation was more firmly associated with needle water response to climate than to exterior effects of temperature on the source of water to leaves in *P. edulis*.

δD_{LCN} and $\delta^{18}O_{LC}$ values varied greatly among trees growing close to one another at a given elevation. The results we have discussed thus far suggest that one source of this variation may be differing evaporative enrichments in water taken up by the plant. Two other possibilities may also contribute to this variation. First, the δD and $\delta^{18}O$ values of water may vary spatially within a leaf and throughout the course of leaf development. Spatially, δD and $\delta^{18}O$ values may vary between symplastic and apoplastic water compartments (Yakir et al., 1989; Yakir 1992), bulk leaf and chloroplast (specific photosynthetic region) water (Wang et al., 1998), and with distance from the petiole (Helliker and Ehleringer, 2000). Our results showed δD and $\delta^{18}O$ values to be lower in bulk waters of newly emerging needles than in adjacent, mature needles of *P. edulis*. The possibility that variations in δ values of the water specifically used in cellulose synthesis contributed to differences in δD and $\delta^{18}O$ values of *P. edulis* leaf celluloses within given sites cannot be discounted.

Another source of variation in the δ values of *P. edulis* needle cellulose may be because some cellulose is synthesized from immediate products of photosynthesis and some from organic reserves. For simplicity, call cellulose synthesized from immediate products of photosynthesis, autotrophic (A) cellulose and cellulose from organic reserves, heterotrophic (H). If all of a leaf's cellulose was autotrophic, its δD or $\delta^{18}O$ value ($\delta_{\text{Leaf cellulose}}$) would be affected by the water involved in its synthesis and a biologic fractionation (ϵ_A) as given by the equation:

$$\delta_{\text{Leaf cellulose}} = \delta_{LW} + \varepsilon_A. \quad (3)$$

In the theoretical case that all of a leaf's cellulose was heterotrophic, its δD or $\delta^{18}O$ value would be affected by a biologic fractionation to water involved in its synthesis (ϵ_H) and an organic reserve source (OR) as given by approximation (modified from Luo and Sternberg, 1992):

$$\delta_{\text{Leaf cellulose}} = n(\delta_{LW} + \varepsilon_H) + (1 - n)\delta_{OR}, \quad (4)$$

where *n* is a proportion.

There are both heterotrophic and autotrophic inputs to leaf growth (Terwilliger et al., 2001b). This means that Eqn. 1 and 2 must be combined such that a certain proportion (*p*) of a leaf's δD_{LCN} or $\delta^{18}O_{LC}$ will be $\delta_{LW} + \epsilon_A$, another proportion (*q*) will be approximately $\delta_{LW} + \epsilon_H$, and the remainder (1-*p*-*q*)

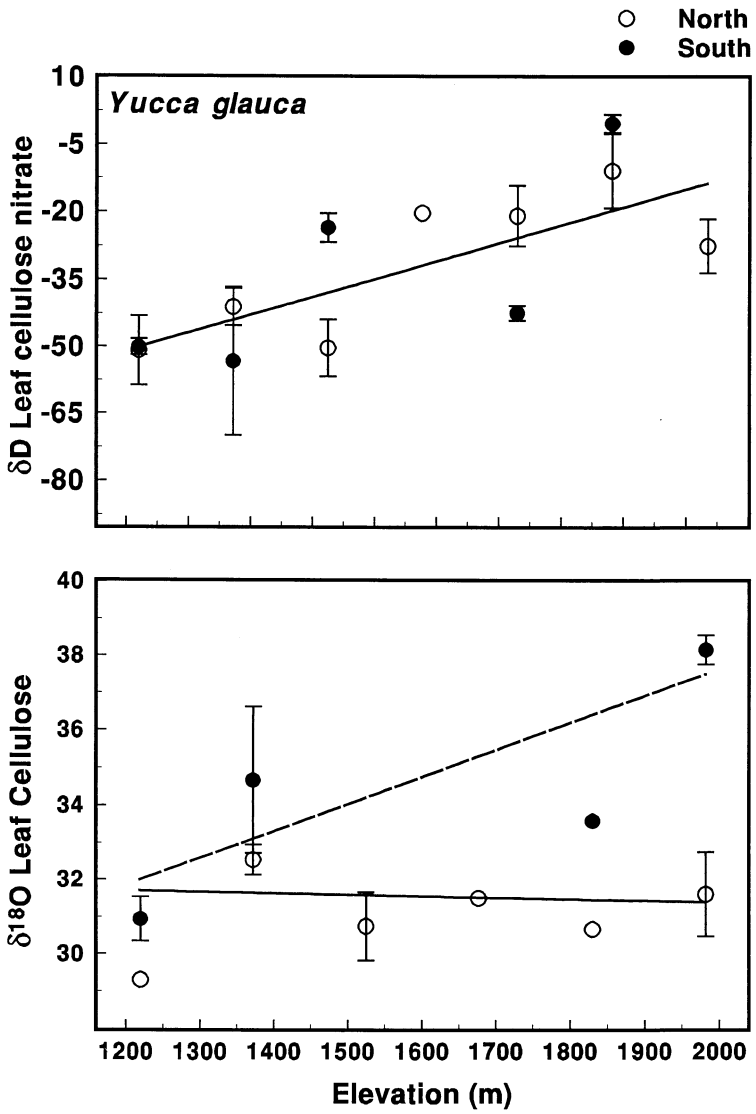

Fig. 6. Changes in δD and $\delta^{18}O$ in the celluloses of *Y. glauca* leaves with elevation. Data points are means (± SE, $n = 5$, where no bars, $n \leq 2$). Lines indicate significant trends with elevation. The solid line is for all δD values ($p < 0.0005$, $r^2 = 0.35$) and the dashed line is for $\delta^{18}O$ values on south aspects ($p < 0.033$, $r^2 = 0.50$).

will be δ_{OR}. The combined heterotrophic and autotrophic inputs to leaf cellulose synthesis can be expressed as the simple linear model (from Yakir and DeNiro, 1990; Terwilliger and DeNiro, 1995):

$$\delta_{\text{Leaf cellulose}} = [(p + q)\delta_{LW}] + [p\varepsilon_A + q\varepsilon_H + (1 - p - q)(\delta_{OR})]. \quad (5)$$

Accepted values for ϵ_A and ϵ_H values for carbon-bound hydrogen in cellulose are −171 and +158‰, respectively (Roden and Ehleringer, 1999, 2000; Roden et al., 2000). ϵ_A and ϵ_H are identical for oxygen in cellulose and probably equal to +27‰ (Sternberg 1989b). If δD_{LW} is linearly related to $\delta^{18}O_{LW}$ and the proportions of autotrophic and heterotrophic inputs to cellulose synthesis are the same in all leaves of a species, then δD_{LCN} and $\delta^{18}O_{LC}$ will also be linearly related.

δD and $\delta^{18}O$ values of needles of *P. edulis* were very significantly related in our study (Fig. 5), in the work of Pendall (1997), and in the leaves of many other species (Cooper and DeNiro, 1989). We found no relationship between δD_{LCN} and $\delta^{18}O_{LC}$ of *P. edulis*, however. Differences in heterotrophic inputs to leaf growth among trees would cause this lack of a relationship, particularly by increasing the variation among δD_{LCN} values. Differences in heterotrophic inputs to leaf growth might also explain why there was a somewhat tighter relationship between $\delta^{18}O_{LC}$ and elevation than between δD_{LCN} and elevation.

Differences in heterotrophic inputs to leaf growth have been found among individuals of a given species and within species (Terwilliger et al., 2001a, 2001b). Little is known about the possible causes and adaptive significance of these differences, although light levels and defoliation are two probable influ-

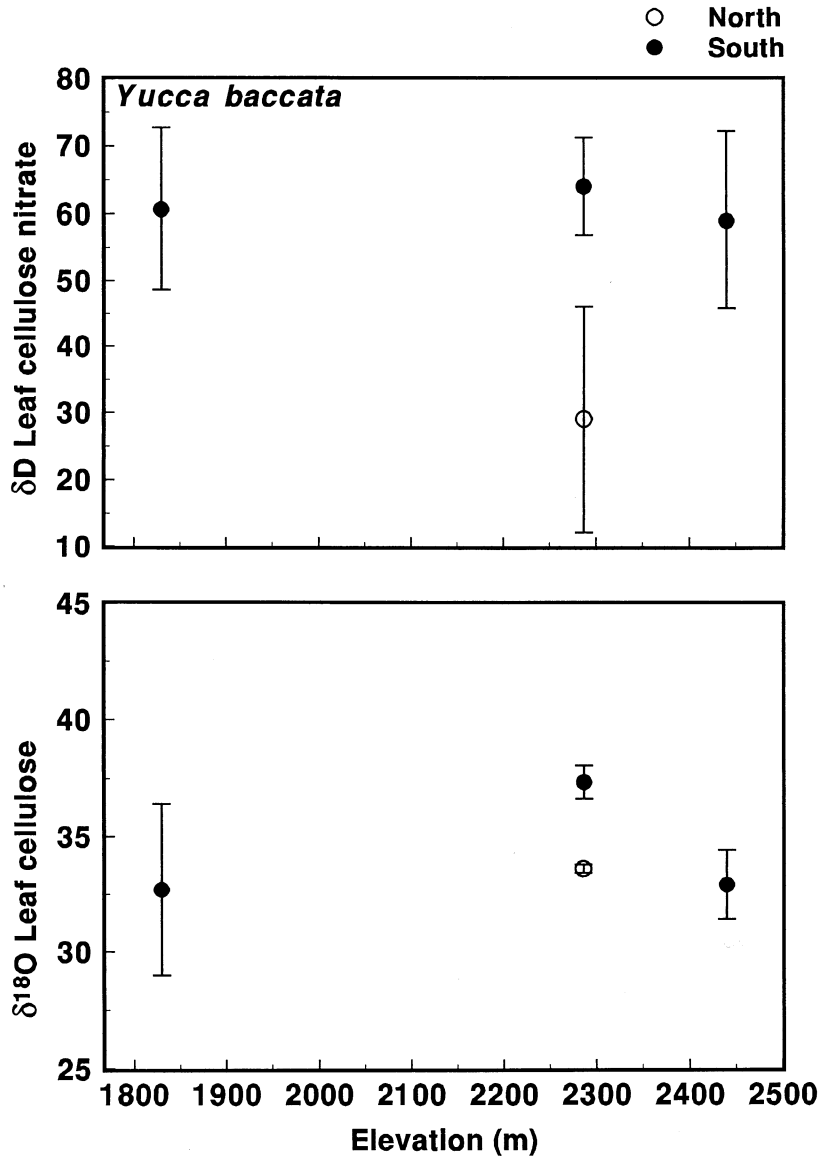

Fig. 7. δD and $\delta^{18}O$ in the celluloses of *Y. baccata* leaves at different elevations and aspects. Data points are means (± SE, $n = 5$, where no bars, $n \leq 2$).

ences (Le Roux-Swarthout et al., 2001). If differences in heterotrophic inputs to leaf growth contributed to some of the variation in δ cellulose values of *P. edulis*, evidence suggests that the differences are small relative to external influences on δ cellulose. The only information present in δ_{LW} that was lost concerned differences in evaporative forces that might have been obtainable had significant slopes of relationships between δD_{LCN} and $\delta^{18}O_{LC}$ emerged.

4.3. Why Relationships between δ Cellulose and Climate May Differ between Species?

The possible causes of intrasite variation in δD_{LCN} and $\delta^{18}O_{LC}$ for *P. edulis* may also provide reasons for the differences in directions of relationships of δ values with climate between the species we studied. The high intrasite variation in, as well as intersite differences in, δ cellulose trends might simply be recording variation in the waters used for cellulose synthesis among plants. Alternatively, differences in physiologic or biochemical factors such as heterotrophic inputs to growth may be important causes of disparities in trends among species. The present research was an effort to take advantage of a unique set of existing plant tissues sampled over a definable climate gradient on similar substrata. This labor-intensive study and existing literature can address these possibilities to a limited degree but point to a need for follow-up work where δD and $\delta^{18}O$ are sampled in celluloses and waters of all three species across climate gradients. Gains in automated δD and $\delta^{18}O$ analyses should soon make such work less prohibitively labor intensive.

Although it is functionally possible, no one has yet demon-

strated that isotopic changes in plant waters over environmental gradients can be opposite in direction between species. Cooper and DeNiro (1989) found disparities in δD_{LW} of as much as 120‰, in $\delta^{18}O_{LW}$ of 48‰, and different slopes in relationships between δD_{LW} and $\delta^{18}O_{LW}$ between species growing close to one another. Intrasite variation in δD and $\delta^{18}O$ of environmental waters was unlikely to have caused these disparities in at least one of their study sites, which was on the well-watered loam of a botanical garden. Similarly, Wang et al. (1998) found up to 21‰ differences in $\delta^{18}O_{LW}$ values among co-occurring plants in a botanical garden.

The *Yucca* species have a large, shallow system of tubers for storage of water and organic carbon (Wallen and Ludwig, 1978). This source of water to the leaf may differ in isotopic composition from a tree stem. Furthermore, as Figure 2 suggests, some enrichment in waters taken up by plants may, for whatever reason, occur with increase in elevation at some times of the year. Less isotopic change of leaf waters in the *Yucca* species than in *P. edulis* could result in δ cellulose values that are more reflective of waters taken up by the *Yucca* plants.

Nonetheless, if differences in δD and $\delta^{18}O$ of the waters used in cellulose synthesis were the sole reasons for the differences in direction and resolution of trends of δ cellulose with climate between species, these differences should also arise where the species co-occur. A comparison of δ cellulose values among species at overlapping elevations suggests that variation in δ water values is unlikely to be the primary cause of the different trends in δ cellulose values among species. Differences in δD_{LCN} did indeed occur between species where they overlapped in elevation. The δD_{LCN} values of *Y. baccata* were at least 50‰ higher than in *Y. glauca* at the one elevation (1830 m) where the two species overlapped (see Figs. 6, 7) and in the elevations in which it co-occurred with *P. edulis* (Figs. 1, 7) (t-tests, $p < 0.01$). Furthermore, δD_{LCN} values of *Y. glauca* differed significantly from those of *P. edulis* over the range of elevation in which the two species overlapped (Figs. 1, 6) (t-test, $p < 0.0005$). Nevertheless, no significant differences in $\delta^{18}O_{LC}$ occurred among species where they co-occurred.

The large difference in δD_{LCN} between the CAM *Y. baccata* and the two C_3 species (Figs. 1, 6, 7) can almost certainly be explained by differences in biochemical fractionations associated with photosynthetic pathways. Deuterium enrichment in CAM plants was first reported by Ziegler et al. (1976). Several lines of evidence pinpointed differences in biochemical fractionations between CAM and C_3 plants rather than isotopic composition of the source water isotopic as the cause of this enrichment (reviewed by Sternberg, 1989b). Of greatest pertinence to the present study was the observation that $\delta^{18}O_{LC}$ values of CAM plants were not significantly different from those of adjacent C_3 plants, while corresponding δD_{LCN} values of CAM were markedly enriched in deuterium (Sternberg et al., 1989). Differences in biochemical fractionations may explain why δD_{LCN} but not $\delta^{18}O_{LC}$ differed between the CAM and C_3 *Yucca* species.

In addition, δD_{LCN} but not $\delta^{18}O_{LC}$ values may also have varied between the two C_3 species due to differences in biochemical fractionations. It is functionally possible that *Y. glauca* and *P. edulis* differ in their capacity to contribute stored organic carbon to leaf growth. It is also possible that at the coolest, moistest elevation of its range on our transect, *Y. glauca* relied more on heterotrophic reallocation of organic carbon for leaf growth than at its hottest, driest, lowest reaches. Changing biochemical fractionations may have significantly influenced the increase in δD_{LCN} with increase in elevation, causing the trend to be more robust than for corresponding $\delta^{18}O_{LC}$ values.

5. CONCLUSION

This study shows that, given sufficient replicate samples, δD_{LCN} and $\delta^{18}O_{LC}$ trends with climate can be statistically robust yet different from one another in leaves of species of the same photosynthetic pathway. Therefore, knowledge of the direction of trends in δD_{LCN} and $\delta^{18}O_{LC}$ with climate of a particular species is a necessary prerequisite to reconstructing climate from its ancient leaf remains. In addition, our findings suggest that differences in the direction of δ cellulose trends with elevation and climate may be caused by differences in relative influences of factors causing the trends. The most likely influences are variation in δ values of source waters for cellulose synthesis and variation in biochemical fractionations. Most intriguing is the possibility that climate may affect biochemical fractionations producing a somewhat cleaner, if counterintuitive trend in δD_{LCN} with climate than in $\delta^{18}O_{LC}$ in some species.

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