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Needle cell elongation and maturation timing derived from pine needle cellulose $\delta^{18}O$

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ABSTRACT

Estimates of the timing of *Pinus arizonica* Engelm. needle development in 1998 and 1999 were derived from the leaf-cellulose $\delta^{18}O$ of weekly growth increments. Significant correlations were noted between time series of local humidity and leaf-cellulose $\delta^{18}O$ for needles growing near Tucson, Arizona. Correlations with temperature were also significant, but much lower, suggesting these variations in cellulose $\delta^{18}O$ were determined mostly by changes in humidity. The timing of all significant correlations lags the timing of the appearance of the new needle growth, and is interpreted as indicating 16–23 d were required for cell enlargement in 1998 and 13–17 d in 1999. Similarly, properties of the environmental time series, when significantly correlated, are interpreted as indicating the duration of cellulose deposition (7–27 d in 1998, 13–21 d in 1999). Variations in stable-isotope back diffusion (the Péclet effect) and the synthesis of cellulose using stored photosynthate are discussed as explanations for departures from a Craig and Gordon-type model of leaf water $\delta^{18}O$. The Péclet effect, use of stored photosynthate, and variations in the growing-season source-water $\delta^{18}O$, probably confound the development of a high-resolution paleohumidity proxy from subfossil needle cellulose $\delta^{18}O$ in this region.

Key-words: $\delta^{18}O$; cell enlargement; cell wall maturation; humidity; needle elongation; oxygen isotope ratio; Péclet effect; secondary cell wall.

INTRODUCTION

The fractionation of the stable isotopes of oxygen ($\delta^{18}O$) in precipitation and plant tissues has been studied for many years, with most research addressing questions of climate reconstruction or plant physiology. The $\delta^{18}O$ research on carbohydrates in trees has mostly involved analysis of xylem cellulose from the stem. Water taken up by the roots provides the source water for photosynthesis, and the $\delta^{18}O$ of that water is unchanged, in most cases, until the water reaches the leaves. The source-water $\delta^{18}O$ of trees usually changes gradually, as precipitation infiltrates and mixes with soil water from previous precipitation events. However, the $\delta^{18}O$ in the xylem cellulose is a mixture of source water $\delta^{18}O$ and the $\delta^{18}O$ of leaf water enriched by evaporation, with additional exchange occurring during cellulose synthesis. Local changes in the temperature and humidity around the leaf and exchange of leaf water with atmospheric water vapour can change the $\delta^{18}O$ of plant tissues by modifying the conditions at the point of evaporation. A portion of the modified leaf water may then be used in photosynthesis, and some of the photosynthate may be subsequently used in the synthesis of plant tissues.

Like stem cellulose, the $\delta^{18}O$ in leaf cellulose is influenced by these factors, but the proximity of leaf tissues to the atmosphere suggests that the $\delta^{18}O$ of leaf cellulose is likely to incorporate a smaller proportion of the non-evaporated source water than does the stem cellulose. However, changes in the leaf-water $\delta^{18}O$ can occur rapidly, because leaf-water $\delta^{18}O$ at the point of evaporation depends mostly on stomatal conductance, atmospheric humidity and temperature, and the $\delta^{18}O$ of atmospheric moisture.

This paper investigates whether the $\delta^{18}O$ in cellulose from weekly measured growth increments of *Pinus arizonica* Engelm. needles covaries with time series of atmospheric humidity and temperature. Covariance of leaf cellulose $\delta^{18}O$ and the climate parameters mentioned may be used to quantify the timing of leaf cell enlargement and maturation, by assessing the temporal characteristics of the climate time series. Identification of weekly covariance might also allow the development of a high-resolution climate proxy.

Cells in the leaves of members of the genus *Pinus* are formed sequentially and linearly, with the older cells at the needle tip and the younger cells at the needle base; deposition along a single easily recognizable vector. Needles from many long-leafed *Pinus* species attain lengths greater than 10 cm in a single growing season, allowing subdivision at weekly or shorter time steps. In addition, the material forming *Pinus* needles is usually deposited continuously, thereby potentially providing a continuous record of conditions at the leaf–atmosphere interface. Characteristics of the rate and duration of the cellulose deposition are diffi-

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cult to directly assess *post facto*. Yet, the presence of any significant correlations among climate time series and time series of some physical property of the needles would indicate low variance in the rate of the cellulose deposition, and a duration of deposition similar to or less than the time step analysed.

If significant correlations are identified between the needle cellulose $\delta^{18}O$ and an environmental time series, then comparison of the timing of the highest significant correlation and the timing of measured needle elongation may provide phenological information. Any offset between the timing of cell elongation measurements and the timing of significant correlations among the needle cellulose $\delta^{18}O$ time series and environmental parameters would suggest the duration of the cell elongation phase. Similarly, the temporal input to each data point of the environmental time series with the highest significant correlation (e.g. 7-day mean) may suggest the duration of the cell maturation phase.

The factors controlling leaf-water $\delta^{18}O$ and leaf-cellulose $\delta^{18}O$ have gradually been identified and quantified over the years, resulting in increasingly sophisticated physiological models (Dongmann *et al*. 1974; Flanagan, Comstock & Ehleringer 1991; Farquhar & Lloyd 1993; Aucour, Hillaire-Marcel & Bonnefille 1996; Roden, Lin & Ehleringer 2000; Gan *et al*. 2003). These developments allow mechanistic interpretations of any significant correlations among time series of leaf-water $\delta^{18}O$ (or leaf cellulose $\delta^{18}O$) and environmental parameters. A modified version of the model of Flanagan *et al*. (1991) was used in this study as an aid in interpreting the leaf-cellulose $\delta^{18}O$. In addition, equations from recent research (Wang, Yakir & Avishai 1998; Barbour *et al*. 2004) were used to assess the importance of back diffusion from the point of evaporation, the Péclet effect, to leaf-water enrichment in this system.

Plant cell maturation

Celluloses (α-cellulose and hemicelluloses) are the chemical components of plant tissues usually chosen for stable isotope analysis, because of the dominant proportion of cellulose in most plant tissues, and because of the environmental stability of the cellulose molecule. Celluloses are present in the primary cell walls of all leaf cells, but the active expansion of the primary cell walls necessarily limits the thickness of the cellulose microfibril layers. As a consequence, most leaf cellulose is deposited after cell expansion is completed, in cell types with secondary cell walls, and in a few types of cells whose primary cell walls are thickened after cell expansion ceases. These include the sclerenchyma and collenchyma, and some cell types in certain complex tissues (e.g. xylem, phloem, and transfusion tissue). Needle tissues consisting primarily or entirely of these cell types include the vascular bundle, the transfusion tissue, and the hypodermis. Other needle tissues, including the mesophyll and the epidermis, have only primary cell walls and therefore contribute much less cellulose to the analysed sample.

Inter-relationship of new needles and existing components

The extension of new needles in pines begins early in the growing season, immediately following bud break, the needle primordiae having been initiated the previous season (Sacher 1954). The photosynthate used to initiate new needles is mainly transported from existing needles retained from previous years (Ursino, Nelson & Krotkov 1968; Ziemer 1971), or from storage in other nearby tissues (Dickmann & Kozlowski 1968), not from the main stem or roots. Photosynthate produced in existing needles prior to bud break is translocated to the roots. However, the translocation to the roots is apparently terminated at bud break, and does not resume until the new needle photosynthesis exceeds the growth needs (Gordon & Larson 1968). The material used to form the new needles may be either photosynthate produced during the current growing season, remobilized starch formed in an earlier time period, or some combination of both sources.

Stable isotopes in leaf water of conifers

Evaporation of leaf water causes changes in the ratio of ^{18}O to ^{16}O in the remaining leaf water. The changes are caused by differences between the vapour pressures (Bottinga & Craig 1969) and diffusion rates (Cappa *et al*. 2003) of $H_2{}^{18}O$ and $H_2{}^{16}O$. If the stable isotope ratios of the source water and the atmospheric water vapour are fairly constant, then the dominant influence on the leaf-water $\delta^{18}O$ will be relative humidity (Wang *et al*. 1998). Many models of leaf-water $\delta^{18}O$ have been developed by combining the equation derived by Craig & Gordon (1965), for a free water surface, with terms for the physical properties of leaf geometry (e.g. Dongmann *et al*. 1974). However, these models tend to overestimate the heavy isotope enrichment of evaporated leaf water, an offset that increases with increasing transpiration (Barnes & Walker 1989; Flanagan *et al*. 1994).

One solution, initially suggested by Farquhar & Lloyd (1993), involves the addition of a back-diffusion component counter to the advective flux of the transpiration stream, a Péclet effect, to allow the mixing of water from evaporation sites with non-evaporated leaf water. A Péclet effect occurs during free evaporation from a body of water, but rapid mixing of the surface water with an effectively infinite reservoir limits the influence of back-diffusion on the $\delta^{18}O$ of the surface water.

Stable oxygen isotope studies of conifer needle tissues

Currently, only two studies have considered $\delta^{18}O$ in needle cellulose. Of these, one analysed bulk cellulose, and linked the results to changes in climate (Terwilliger *et al*. 2002). The other study subdivided conifer needles of *Picea abies* into 3–4 parts per needle (Jäggi *et al*. 2003), and considered the relationships among whole-needle $\delta^{18}O$, whole-wood $\delta^{18}O$, and local seasonal climate.

MATERIALS AND METHODS

Field

The tree sampling site is located at 2300 m elevation; on a WSW-facing slope in the Santa Catalina Mountains north of Tucson, Arizona. The closest vehicular access is Sollers Road, a gated United States Forest Service road about 2000 m ESE of the Palisades Ranger Station. This site was chosen, in part, because of ongoing stable isotope research, which included 5 years of $\delta^{18}O$ and δD seasonal data from soil, stem, and leaf water samples. In addition, $\delta^{18}O$ and δD were determined for bulk precipitation samples collected at the Palisades Ranger Station on at least a weekly/bi-weekly basis during the years of this study. A portable Campbell weather station (Campbell Scientific, Logan, Utah, USA) was installed within 200 m of the tree site, recording temperature, relative humidity, precipitation amount, measured hourly, and soil moisture, measured every 6 h.

The precipitation regime in Southern Arizona is strongly bimodal, with a hyper-arid period from April to June, followed consistently by an increase in humidity and 3 months of intermittent thunderstorms termed the North American Monsoon (e.g. Higgins, Chen & Douglas 1999). The 1971–2000 mean annual rainfall at the Palisades Ranger Station was slightly less than 800 mm per year. Of this amount, 34% fell in the period January to March, 9% in April to June, 37% in July to September, and 20% in October to December

The local forest is mixed conifer with a semi-closed canopy. *Pinus arizonica* and *Pseudotsuga mensiezii* dominate, with *Pinus ponderosa* replacing *Pinus arizonica* at the higher elevations. *Pinus strobiformis* and a few *Juniperus deppeanna* are also present. The age structure is mixed, ranging from seedlings to mature trees over 250 years old. A heavily fractured granite substrate is covered with a thin layer of soil, usually no more than 30–40 cm deep.

Before the beginning of the 1998 growing season, branches were selected from orthogonal directions on five mature *Pinus arizonica* trees, and marked with flagging tape. Three subcanopy trees were selected for ease of access, with lowest foliage heights ranging from 1.5 to 3 m. The crown heights ranged from 3 to 5 m. Despite their small stature, the age of all trees selected was greater than 30 years.

Weekly visits to the site began in mid-May, 1998, immediately after receiving reports of budbreak, but needle extension was not measurable until the first week of June. Measurements began at the end of the first week of June, using a metric ruler with millimetre resolution, with the longest and shortest needle lengths on each branch tip being recorded. Measurements continued through the growing season at intervals ranging from 6 to 9 d, although most measurements followed 7 d of growth (Fig. 1).

Selection of additional branches for extension of the study into the 1999 growing season was necessitated by the harvesting of the branch tips in 1998. All other experimental considerations were identical to those for the 1998 field season. Coincidentally the 1999 needle extension also began during the first week of June

In both seasons, the needles were harvested after needle measurements were unchanged for 2 weeks, the last week of September in both years. The harvested needles were placed in labelled reclosable plastic bags, double-bagged, and stored in a freezer until processing. Weekly time series of the shortest and longest needles on each tree are presented for both 1998 and 1999, for the trees analysed in each year (Fig. 1). These values are the averages of the four orthogonal directions.

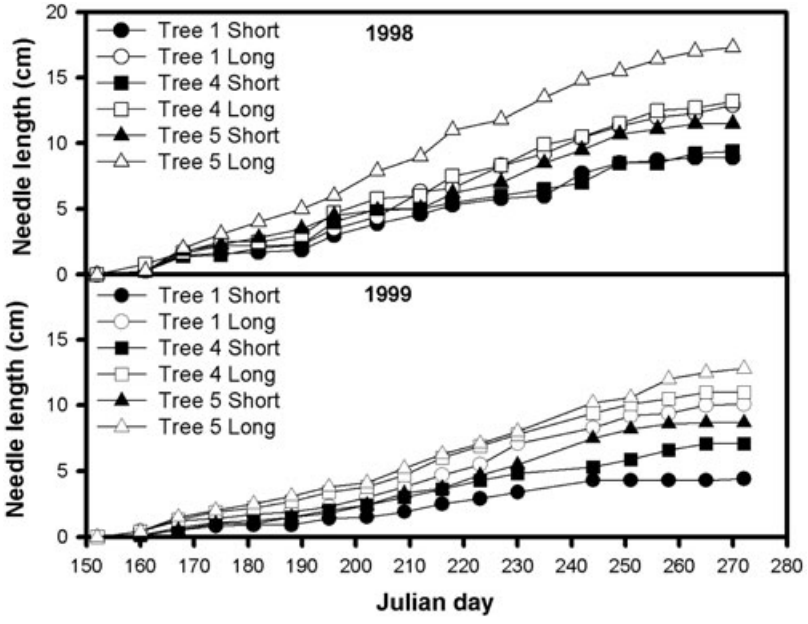

Figure 1. Weekly *Pinus arizonica* needle extension measurements. Short and long refer to the needle extension of the shortest and longest needles on each tree. The plotted values are composites of the measurements made orthogonally on each tree.

Laboratory

The weekly growth increments for the non-measured needles were determined by interpolating between the shortest and longest measurements from each field assessment for each tree, thereby assuming that the *relative* needle growth rates within each tree were constant across each growing season. The total needle length and associated interpolated growth increments were then used to determine where to subdivide the needles. The needles were subdivided by placing them next to a metric ruler, with the needle tip (the oldest material) at zero, cutting the needles at the interpolated lengths using a scalpel with a no. 15 blade. Sample homogeneity was ensured by macerating the growth increments to 40 mesh using an intermediate Wiley Mill (Thomas Scientific, Swedesboro, New Jersey, USA).

Available resources limited the number of $\delta^{18}O$ time series that could be produced, so we chose to analyse cellulose from the same two sides of the same tree for both years (the north and west sides of tree no. 4), and a composite of all four sides from a *different* tree for each year (trees no. 1 and no. 5). Combining the appropriate growth increments and macerating them together produced the composites used in the analyses. The intent was to determine the mean needle biomass $\delta^{18}O$ by branch tip or by tree, depending on the method of compositing, so input to the composites was not corrected for individual needle mass differences. Therefore, results of $\delta^{18}O$ analyses for each sample are the mean $\delta^{18}O$ for that growth increment for either an entire needle cohort, or the composite mean for four needle cohorts.

Holocellulose was isolated from the composited subdivisions using a variation of the method described by Leavitt & Danzer (1993). The $\delta^{18}O$ was determined for the holocellulose fraction at the Research School of Biological Sciences of the Australian National University, using the online pyrolysis to carbon monoxide method (e.g. Farquhar, Henry & Styles 1997). The standard error reported by the laboratory at the Australian National University was less than 0.3‰ for the internal beet sugar standard.

Alpha-cellulose is now the standard choice for stable isotope analysis, but limitations in sample sizes forced us to consider using holocellulose, a mixture of alpha-cellulose and hemicelluloses. To determine whether holocellulose could be used in this study, 12 samples of *wood* were split, analysed for both fractions, and then regressed against each other. The result was a slope that was statistically indistinguishable from 1 : 1 (Fig. 2): t-stat = 0.14; $P(T = t)$ two tails = 0.89, suggesting that there is a high probability that the population means are the same. Note that subsequent analyses performed on the cellulose after 2 years of storage suggest exchange of some of the oxygen in holocellulose can occur over time. This is probably caused by exchange of the carbonyl group in the hemicelluloses (John Roden, personal comm.). Re-analysis after extraction of the stored samples to alpha-cellulose returned the original holocellulose $\delta^{18}O$ values, supporting the accuracy of the results reported here, but holocellulose should only be used in cellulose oxygen isotope research when the samples are dried immediately and stored in sealed containers, with the analyses performed soon afterward.

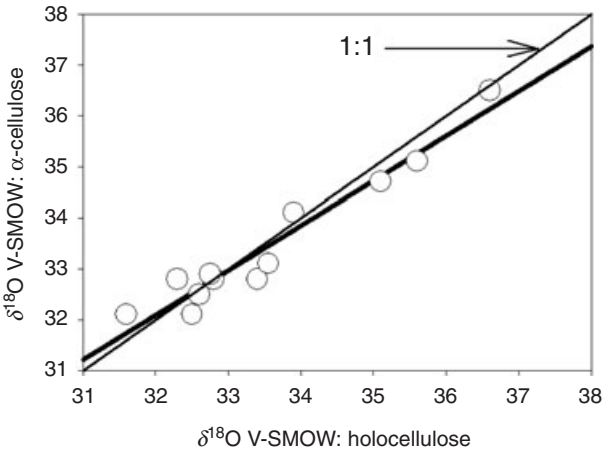

Figure 2. Simple linear regression of $\delta^{18}O$ values for holocellulose and α-cellulose from *Pinus arizonica* wood samples [α-cellulose = 0.874 ± 0.08 · (Holo $\delta^{18}O$) +4.4 ± 2.5]. The two sample populations are statistically identical [t-stat = 0.14; $P(T, = t)$ two tail = 0.89].

Analysis

Meteorological data

Power problems and related programming problems with the Campbell portable weather station at the tree site resulted in discontinuous onsite meteorological data in 1998 and 1999. Furthermore, preliminary data analysis in late 1999 revealed a trend in the relative humidity data from late 1998 to late 1999. The humidity sensor was replaced before the beginning of the 2000 growing season, and all variables were measured continuously for the year 2000.

The daily mean 1998 and 1999 humidity and temperature data were estimated using relationships derived from simple linear regression analysis of daily meteorological data for the year 2000 from the on-site Campbell weather station and radiosonde data from the Tucson International Airport. Radiosonde data soundings are taken twice daily at the airport, 30 km to the south-east of the tree site. The radiosonde data was converted to the desired variables using the following equations

Saturation vapour pressure (*Bolton* 1980)

$$e_s = 0.6112 \exp\left(\frac{17.67T}{T + 243.5}\right)$$

Vapour pressure (*Bolton* 1980) $e = 0.6112 \exp\left(\frac{17.67T_d}{T_d + 243.5}\right)$

Relative humidity $h\frac{e}{e_s}$

Mixing ratio (≈ specific humidity) $q = 0.622(P - e)$

The mixing ratio equation can usually be used to provide an estimate of specific humidity, because the density of water vapour is much less than the density of dry air

The atmospheric pressure at the tree site is roughly 77.5 kPa, so we initially assessed correlations using radiosonde estimates from both the 70.0 kPa and 85.0 kPa pressure heights. Significant correlations were noted between the tree-site measurements and the 85.0 kPa measurements, for both temperature and humidity. Correlations with the 70.0 kPa time series were not significant.

Data from the 0Z and 12Z soundings were regressed separately, and as an average of values from the two soundings. The terms 0Z and 12Z refer to Greenwich Mean Time and correspond to 1800 and 0600 h, respectively, in Tucson, Arizona. The average of the 85.0 kPa 0Z and 12Z soundings provided the highest correlations, so data from these soundings were used to estimate the missing tree-site meteorological variables.

A simple linear regression was used to estimate the temperature [$T_{site} = 0.785(T_{radiosonde}) - 2.06$; $N = 119$; $r = 0.88$; $P < 0.05$] and the specific humidity [$SH_{site} = 0.969(SH_{radiosonde}) + 1.67; N = 113; r = 0.86; P < 0.05$]. A third-order polynomial was required to estimate the relative humidity [$RH_{site} = 2.7734(RH_{radiosonde}^3) - 4.95\ (RH_{radiosonde}^2) + 3.3382\ (RH_{radiosonde}) - 0.0606$; $N = 114$; $r = 0.87$; $P < 0.05$], because the on-site relative humidity reached saturation before the free-air measurements, resulting in a curvilinear relationship. The next step was regression of the needle-cellulose $\delta^{18}O$ time series against the estimated relative humidity, specific humidity and temperature time series. Initially, the length of time over which the environment could influence the $\delta^{18}O$ for any particular needle segment was not known, so many time series of running means were produced for each variable (e.g. each subsequent data point in a given time series was the mean of the same number of days, lagged forward by 1 week). Running correlations were then calculated among the $\delta^{18}O$ time series and environmental time series, using simple linear regression.

The term 'window' is used in this paper to describe the number of days included in the running mean used to produce each environmental time series. The term 'lag' is used in this paper to describe the difference between the timing of the highest correlation with environmental variable and the timing of the needle extension measurements.

Leaf-water $\delta^{18}O$ and the Péclet effect

Equations from Barbour *et al*. (2004) and Wang *et al*. (1998), including a modified version of the Flanagan leaf water model (1991), were used to estimate the Péclet numbers pertinent to each sampling date, to calculate the effective mixing path lengths, and to assess the influence of a Péclet effect on this system. The version of the Flanagan leaf water model used in this study was acquired through an internet link provided in the text of the Barbour *et al*. (2004) paper.

Measured parameters used as model input were $\delta^{18}O$ in precipitation, in stem water, and in leaf water, as determined for samples collected at the study site during the early summer and the late summer of the years 1998 to 2000, and wind speed. Estimated parameters were temperature, relative humidity, water vapour $\delta^{18}O$ and stomatal conductance. Parameters treated as constants were barometric pressure (77.5 kPa; interpolated from the mean pressure/elevation relationship of the radiosonde data) and boundary-layer conductance (2.5 mol m^{-2} s^{-1}; Zhang *et al*. 1997).

All leaf samples were collected between 0900 and 1100 h, so data from the 1000 h reading from the Sollers Road weather station for June to September of AD 2000 and from the 12Z radiosonde (0600 h) were used to estimate the temperature ($T_{site} = 0.943(T_{12Z}) - 2.348$; $N = 115$; $r = 0.82$; $P < 0.05$) and the relative humidity ($RH_{site} = 1.121(RH_{12Z}) + 0.086$; $N = 118$; $r = 0.85$; $P < 0.05$). Note that unlike the estimations of the daily mean relative humidity previously mentioned, a simple linear regression (SLR) was appropriate between the 12Z radiosonde data and the 1000 h Sollers road data, because the atmosphere in the morning was never saturated.

Atmospheric water vapour $\delta^{18}O$ for Tucson, Arizona, was estimated from a SLR of precipitation $\delta^{18}O$ values and atmospheric water vapour values, as determined for Tucson, Arizona during the warm seasons of 1996 and 1998. This data, from Dr Austin Long of the University of Arizona, was collected only during the years 1996 and 1998, and is currently unpublished. The SLR from Tucson precipitation and atmospheric water vapour $\delta^{18}O$ values was combined with the SLR of warm-season Tucson precipitation $\delta^{18}O$ values and warm-season precipitation $\delta^{18}O$ values from Palisades Ranger Station (PRS), for common events (Wright 2001), to estimate the atmospheric water vapour $\delta^{18}O$ values for PRS. The common event sample included events from most years between 1995 and 2003. Significant correlations in both regressions supported using this technique for the estimation of the atmospheric water vapour $\delta^{18}O$ at the tree site ($\delta^{18}O_{Tucson\ Vapour} = 0.76 \times \delta^{18}O_{Tucson\ Precipitation} - 10.6$, $r = 0.86$, $N = 15$, $P < 0.005$; $\delta^{18}O_{PRS\ Precipitation} = 0.87 \times \delta^{18}O_{Tucson\ Precipitation} - 3.3$, $r = 0.94$, $N = 15$, $P < 0.005$).

Stomatal conductance was estimated by using on-site measurements of soil water potential and relative humidity to first estimate tree-water deficit, following the model of Zweifel. Zimmerman & Newberry (2005; see Appendix). A linear relationship between stomatal conductance and tree-water deficit was assumed. Another assumption was that the growing-season extremes of tree-water deficit, as calculated using the model, represented maximum and minimum stomatal conductance. These assumptions are certainly inaccurate in terms of *absolute* stomatal conductance, but the estimates provide a stomatal conductance value for any particular day that is correct relative to the estimate for other days (higher or lower), and it is the relative stomatal conductance that is required to assess the influence of a Péclet effect. These estimates of stomatal conductance were used as input to the Flanagan *et al*. (1991) model to estimate the transpiration rate. The mod-

Table 1. Inter- and intra-tree needle cellulose $\delta^{18}O$ correlation coefficients

Correlation coefficients (r)	Within-tree (All values)	Between trees (All values)	Within-tree (weeks 3–16)	Between trees (weeks 3–16)
1998	0.99	0.97	0.94	0.77
Sample size	16	16	14	14
P	<0.0001	<0.0001	<0.0001	<0.005
1999	0.97	0.94	0.85	0.82
Sample size	16	16	14	14
P	<0.0001	<0.0001	<0.0001	<0.0005

The table shows statistics related to simple linear regressions between time series of needle-cellulose $\delta^{18}O$ extracted from 1998 and 1999 needle cohorts from different sides of the same trees, and different trees. Correlation coefficients based on weeks 3–16 are provided to indicate the importance of the extreme $\delta^{18}O$ values in the first 2 weeks of growth to the significance of the correlation (Fig. 3).

elled transpiration rate was then plotted against the fractional difference between measured leaf-water enrichment and the enrichment as predicted by the Craig and Gordon model (Barbour *et al*. 2004).

RESULTS

Leaf cellulose $\delta^{18}O$ time series

Three time series of $\delta^{18}O$ were produced from weekly growth increments of needle cellulose for 1998 and for 1999, including two time series from different aspects of the same tree in both years (tree no. 4), and one time series from composited orthogonal samples each year, tree no. 5 in 1998 and tree no. 1 in 1999. Each time series consisted of 16 data points, one data point for each week of the growing season.

The time series of needle cellulose $\delta^{18}O$ were highly intercorrelated during the same year; both for different aspects of the same tree, and between trees (Table 1). However, the first two values in the time series are very different from the remainder of the values (Fig. 3), and correlations can be strongly influenced by extreme values, so the time series were also regressed after removing the values for the first two weeks to indicate the strength of the covariance in the remainder of the time series. The resulting correlation coefficients were lower, but still highly significant (Table 1). The similarity of the time series within each year suggested that subsequent analyses could be done using the mean of the three time series for each year.

Simple linear regression of temperature, relative humidity, and specific humidity time series against the mean needle cellulose $\delta^{18}O$ time series revealed significant correlations, but only for subsets of the 16-week mean needle cellulose $\delta^{18}O$ time series. Significant correlations with temperature, relative humidity and specific humidity were found for weeks 1–8 of 1998, and weeks 4–14 of 1999. For 1998, the correlations dropped below the level for significance if any data points from additional weeks were added to weeks 1 to 8. For 1999, many more lags and window sizes

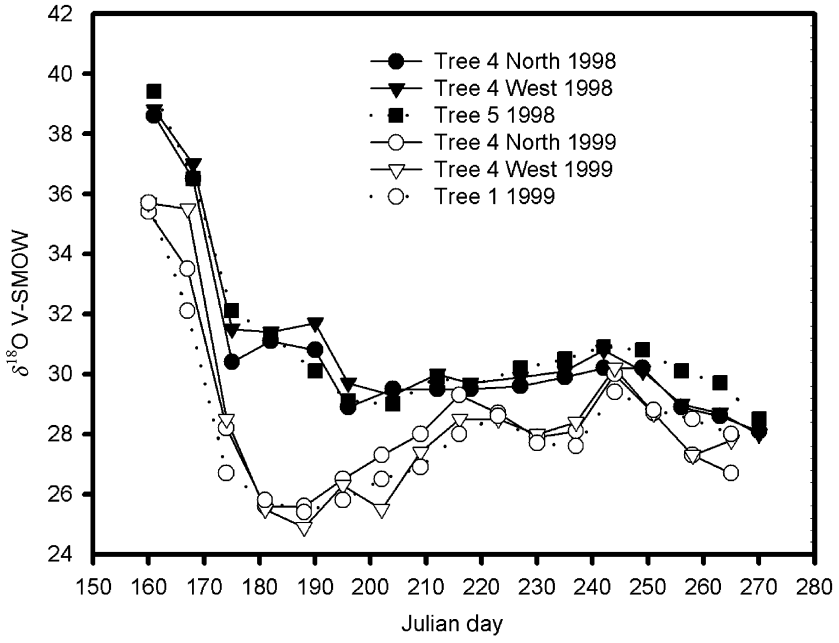

Figure 3. The 1998 and 1999 needle cellulose $\delta^{18}O$ from weekly growth increments. Time series from trees 1 and 5 are composites of branches sampled orthogonally. Different trees were chosen for the composites for each year so that more than two trees were represented in the analysis. Note the similar patterns in the same years between trees and between different sides of each tree (see Table 1).

Table 2. The highest significant correlation coefficients among needle cellulose $\delta^{18}O$, temperature, relative humidity and specific humidity are presented

	Weeks 1–8		
1999	Highest correlation coefficient. $N = 8$	Lag (within 95% conf. limits of highest correlation)	Window (within 95% conf. limits of highest correlation)
T	0.948	20 (11–22)	19(15–31 +)
RH	–0.991/–0.998	17/23 (17, 22–23)	13/25 (13, 23–27)
SH	–0.997/–0.995	17/23 (16–17, 22–23)	13/25 (7–15, 23–27)
	Weeks 4–14		
1999	Highest correlation coefficient. $N = 11$	Lag (within 95% conf. limits of highest correlation)	Window (within 95% conf. limits of highest correlation)
T	0.791	16 (9–29)	15 (All)
RH	–0.950	16 (13–30)	19 (All)
SH	–0.986	16 (13–17)	19 (13–21)

The variables are temperature (*T*), relative humidity (*RH*) and specific humidity (*SH*). Lag refers to the shift in days from the timing of needle measurement that yielded the reported correlation. Window refers to the number of days averaged to produce each data point of the environmental time series that yielded the reported correlation coefficient. The significant correlation coefficients for the humidity-related parameters in 1998 reach maxima at two different points in time. Numbers separated by slashes indicate the two maxima for the correlation coefficient, the lag and the window. The numbers in parentheses indicate the lags and windows that fall within the 95% confidence limits for the highest correlation coefficient, indicating that there is an equal probability that those lags and windows are the actual values for the needle elongation phase (lag) and the cellulose deposition phase (window). Note that in 1999 only the specific humidity calculations are able to resolve a range of windows

had significant but much lower correlations if any data points from weeks 1 to 3 were added to the time series. Additional lags and windows reduced the interpretability, so results for weeks 4 to 14 are considered in the discussion.

The highest significant correlations are listed in Table 2 for all variables analysed for both years. Correlations for other lags and windows were also significant, so the 95% confidence intervals for the highest correlation for each variable for each year were used to indicate which lags and windows were statistically equivalent. All correlations that fell between the 95% confidence intervals for the highest correlation were considered equally probable as measures of the true lag and window for each year and are listed in Table 2.

Assessing the Péclet effect

As expected, Péclet numbers estimated for the tree site were highly correlated with a time series of leaf-water $\delta^{18}O$ values from June and August of 1998, 1999 and 2000 ($r = 0.97$, $P < 0.005$, $N = 6$). Barbour *et al*. (2004) suggest that the importance of a Péclet effect may be assessed by plotting the reciprocal of the fractional difference between measured and modelled leaf water against the transpiration rate. The Flanagan *et al*. (1991) model estimates of these parameters yielded a positive slope with a significant correlation ($r = 0.90$, $P < 0.01$, $N = 6$), indicating that a Péclet effect must be considered when interpreting the leaf water $\delta^{18}O$, and therefore the leaf cellulose $\delta^{18}O$, at this site. Without the inclusion of a stomatal conductance estimate, as calculated from estimates of tree water deficit (Zweifel *et al*. 2005), the relationship had a negative slope and was not significant ($r = 0.63$). With a maximum stomatal conductance of 0.25 mol m^{-2} s^{-1}, and the assumption that the stomata were completely closed (stomatal conductance = 0.0 mol m^{-2} s^{-1}) only under extreme conditions, the calculated effective path lengths ranged between 30 and 51 mm. However, these are only rough estimates, because absolute values for stomatal conductance were not available. See the Appendix for modelling details.

DISCUSSION

This study site is located at 2300 m elevation on a 'Sky Island' in the Sonoran Desert of the US south-west, a region where the North American Monsoon dominates the warm season climate (e.g. Higgins, Yao & Wang 1997). The source of moisture fuelling the warm season precipitation in the region is the subtropical Eastern Pacific/Gulf of California (e.g. Douglas *et al*. 1993; Adams & Comrie 1997; Wright *et al*. 2001). Moisture enters the region in surges lasting for many days or weeks, resulting in broad-scale changes in atmospheric humidity. Precipitation during the warm season falls mostly from small convective cells, resulting in a spatially heterogeneous pattern of precipitation amounts.

Low variability in the moisture source region and in daily temperatures (the 1σ range for daily maximum temperature during summer of the year 2000, as measured at the tree site, was 25.5 to 32.5 °C) are in contrast to the large changes in the atmospheric humidity (the 1σ range for daily maximum relative humidity during summer of the year

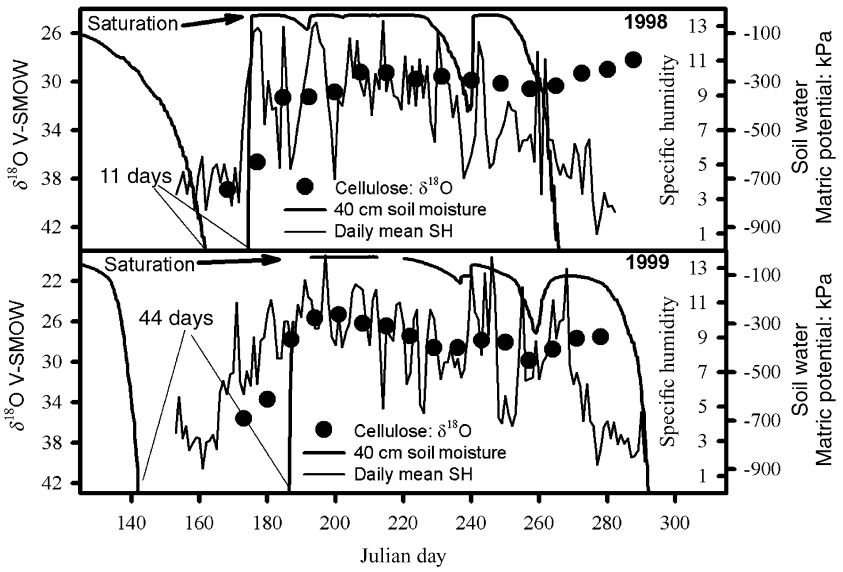

Figure 4. The 1998 and 1999 soil water matric potential at 40 cm, needle cellulose $\delta^{18}O$, and daily specific humidity. The needle cellulose $\delta^{18}O$ is placed at the lag that yielded the highest correlation with specific humidity (Table 2). Note that the extreme soil moisture deficit in the early part of 1998 lasts only 11 d, but is 44 d in 1999. In addition, the soil at 40 cm is near saturation for many more days in 1998 than in 1999. Note that the needle cellulose $\delta^{18}O$ axis is inverted to emphasize the similarity to the specific humidity data. The breaks in the 1999 soil moisture data were caused by power failures. The specific humidity is estimated from radiosonde data.

2000, as measured at the tree site was 65 to 100%), suggesting that changes in humidity should dominate leaf water fractionation in this environment. Tree-ring cellulose $\delta^{18}O$ time series co-vary with seasonal humidity in this setting (Wright 2001), so weekly subdivisions of needle cellulose were expected to do likewise.

The first growing season studied in this research, 1998, occurred during the strong El Niño of 1997/1998. The El Niño–Southern Oscillation teleconnection to the region (e.g. Simpson & Colodner 1999) is winter dominant and usually results in increased cold season precipitation. During the winter of 1997/1998, the Santa Catalina Mountains developed a very heavy snowpack. Consequently, there was adequate soil moisture until well into the usually hyper-arid pre-summer months of April to June (Fig. 4). The soil water matric potential at 40 cm was less than –1000 kPa for only 11 d in the spring of 1998, from 22 June (Julian Day 174; JD 174) to 2 July (JD 183). The 11-day extreme of soil moisture in 1998 is in contrast with 44 d below –1000 kPa in the spring of 1999 (Fig. 4) and 58 d in the spring of 2000 (not shown). One consequence of the cool wet spring of 1998 was a late beginning to the growing season, with budburst occurring in late May rather than late April, as is more typical (Fritts 1976). Initially, needle extension was slow, immeasurable until the weekly visit on 9 June (JD 160), but the extension rate increased rapidly after the summer rains began (Fig. 1). Soil moisture reached field capacity by 4 July of 1998 (JD 185), and remained there until 11 September (JD 254) the soil water was at field capacity. By 17 September 1998 (JD 259), the humidity had dropped sharply and the rains had ended, causing a swift depletion of soil moisture, and by 26 September 1998 (JD 269), the soil water matric potential had dropped to –1000 kPa and needle extension had ceased. Despite the late bud burst and a dry September, needle lengths recorded at the end of the 1998 growing season were 50% longer than those recorded the following year.

The growing season of 1999 was preceded by a dry warm winter with almost no snowpack. As a consequence, the soil water matric potential at 40 cm was less than –1000 kPa by 14 May (JD 134) remaining there until 27 June (JD 178). Budburst in 1999 occurred in late May, the same as in 1998, despite the difference in the conditions preceding the growing season, and needle extension was not measurable until the weekly visit on 8 June (JD 159). The rate of needle extension increased with the beginning of the summer rains, but more slowly than in 1998. The soil moisture reached field capacity by about 1 July (JD 182) and remained high through the growing season, finally dropping to –1000 kPa on 11 October (JD 285). Relative humidity also remained high until 30 September (JD 272).

This study set out to investigate whether leaf cellulose $\delta^{18}O$ values, as determined from weekly growth increments of conifer leaves sampled in a semi-arid environment, could be demonstrated to co-vary with atmospheric temperature or humidity. Significant correlations between time series of $\delta^{18}O$ from tree-ring cellulose and temperature, humidity, or precipitation $\delta^{18}O$ have previously been noted at an annual resolution by many researchers working in a range of environments (Luckman & Gray 1990; Lipp *et al*. 1993; Buhay & Edwards 1995; Saurer, Aellen & Siegwolf 1997; Saurer, Borella & Leuenberger 1997; Anderson *et al*. 1998, 2002; Saurer, Cherubini & Siegwolf 2000; Robertson *et al*. 2001). Although the many factors that can influence the $\delta^{18}O$ of the moisture prior to uptake by the roots of trees may be somewhat constant when viewed as annual means, changes can be highly variable over shorter time spans. Examples include shifts in the moisture source $\delta^{18}O$, differences in the rainout history of each air parcel, admixing with evaporated moisture, changes in the lifting condensation level temperature, and secondary evaporation of the rain during throughfall. For significant relationships between the $\delta^{18}O$ of leaf cellulose and the local environment, either the soil water $\delta^{18}O$ must be fairly constant, thereby allowing the

local conditions to determine the fractionation of the leaf water, or a single factor must control both the soil water and leaf water $\delta^{18}O$. The latter is probably true for moisture-stressed trees in the warm season in the US south-west, where there is evidence of strong secondary evaporation of rain during cloud-to-ground throughfall and during transpiration (Wright 2001).

In addition, interpretations of leaf cellulose $\delta^{18}O$ at a weekly resolution are potentially complicated by changes in physiological processes. Growing season shifts in the relative timing of photosynthate production and the use of the photosynthate in cellulose synthesis would reduce and possibly erase any statistical evidence of covariance between changes in the atmosphere surrounding the needles and changes in the $\delta^{18}O$ of the photosynthate. Examples of variable physiological processes that could change this relative timing are photosynthate storage and reuse, leaf respiration, and stomatal conductance.

Finally, changes in the plant water status can modify the leaf water $\delta^{18}O$ by changing the transpiration rate. The rate of water movement out of a tree influences the amount of back diffusion of $H_2{}^{18}O$ from the point of evaporation, the Péclet effect. This change, when controlled by soil moisture, would cause changes in the leaf water $\delta^{18}O$ that may be independent of changes in the atmosphere.

Despite these potentially confounding influences, significant correlations were found for 8 of 16 weeks of the growing season in 1998, and 14 of 16 weeks in 1999. Existence of these correlations implies that changes in the soil water $\delta^{18}O$ and the Péclet effect were not controlling influences during those weeks, or those changes co-varied with the leaf water $\delta^{18}O$, and suggests that the temporal relationship between photosynthate production and use in cellulose synthesis was fairly constant.

Duration of cell enlargement

Needle cellulose $\delta^{18}O$ values in the growth increments for weeks 1 and 2 of the growing season were much higher than at any other time in the growing season. The weather was hot and dry during weeks 1 and 2 of both years (JD 150 to JD 164), and soil moisture was very low. By the fifth week of needle measurements in 1998 (JD 178 to JD 185), and the sixth week in 1999 (JD 186 to JD 193), the humidity had doubled, the soil moisture was at field capacity, and the resulting needle cellulose $\delta^{18}O$ values were about 8‰ lower. However, the needle cellulose $\delta^{18}O$ values were also about 8‰ lower for needle segments measured during the previous 2 weeks, weeks 3 and 4, when humidity was low and the soil moisture was depleted. This seeming discrepancy is interpreted as an indication of the time lag between the appearance of the needle cells and the time when cellulose was synthesized in those cells

Although no significant correlations were found between the environmental time series and needle cellulose $\delta^{18}O$ time series when samples from all weeks were included (potential explanations are offered below), significant correlations were found for both years for all time series regressions on certain subsets of weeks. Correlations between 1998 time series of specific humidity and weeks 1 to 8 of the needle cellulose $\delta^{18}O$ were at their maximum when the $\delta^{18}O$ time series was shifted forward by 23 d (Fig. 5), while the maximum correlation for 1999, for weeks 4 to 14, required a shift of 16 d. However, lower correlations at many other lags were also significant, so a 95% confidence interval calculated for the highest correlation was used as the statistical cut-off point, above which the lags for the additional correlations were considered equally probable to represent the actual mean number of days required for the cells to enlarge (Table 2). If all lags within the confidence limits are included, then the range of days indicated for the cell enlargement phase is similar for both years; 16–23 d for 1998 and 13–17 d for 1999. The results for specific humidity are stated here, because the analyses using specific humidity yielded a tighter grouping of significant correlations with the $\delta^{18}O$ than did relative humidity; probably caused by the temperature component of relative humidity, which had much lower correlations with the cellulose $\delta^{18}O$ time series.

Duration of cellulose deposition

At the beginning of this study, the number of days of photosynthate production that might be contributing to the $\delta^{18}O$ for any particular needle segment was unknown. To investigate this question, we produced environmental time series of running daily means of 3–31 d, called 'windows' in this paper. We then calculated running correlations among the $\delta^{18}O$ time series and environmental time series. The window size at the point of highest correlation was interpreted as the minimum number of days over which the environment was influencing the cellulose $\delta^{18}O$ in each weekly growth increment. The results suggest that the cellulose for weeks 1 to 8 of 1998 was produced using mostly photosynthate from a 25-day period. However, one would expect cellulose synthesis to 'ramp up' in the beginning of the cell wall maturation phase, and 'ramp down' at the end of the phase, and the small amount of cellulose deposition at the beginning and end would probably not affect the correlations, so the number of days suggested statistically for cell wall maturation must be considered a minimum estimate, indicating the number of days when *most* of the cellulose was synthesized. At present there is no way to quantify the percentage of the total that is synthesized over the 25 d indicated by the highest correlation. Furthermore, as with the lagged relationships, there were additional significant correlations at many other windows. The same 95% confidence interval that was used to estimate the duration of the cell enlargement was used here as the statistical cut-off point. Values above the lower 95% confidence limit were considered equally likely to represent the actual mean number of days required for the cell walls to mature (Table 2). If all lags within the confidence limits are included, then the range of days indicated for the cellulose deposition phase is 7–27 d for 1998 and 13–21 d for 1999. The results for specific humidity are stated

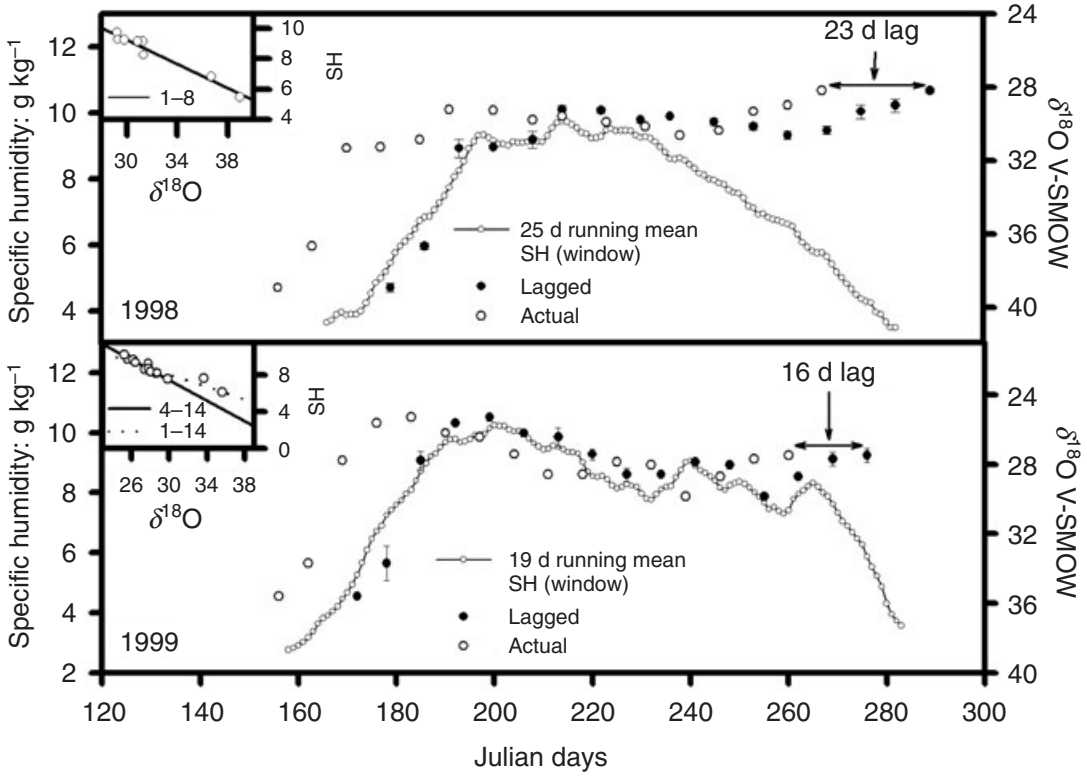

Figure 5. The 1998 and 1999 needle cellulose $\delta^{18}O$ mean values and estimated mean tree site specific humidity. The needle cellulose $\delta^{18}O$ values are plotted at the actual temporal placement, and at the lag yielding the highest correlation with the estimated mean tree site-specific humidity. Error bars for the standard error of the mean are presented for each of the $\delta^{18}O$ mean values. The same data are also shown as simple linear regressions (insets), with the following correlation coefficients: (1) 1998, weeks 1 to 8, $r = 0.98$, $P < 0.0005$. (2a) 1999, weeks 1 to 14, $r = 0.92$, $P < 0.0005$ and (2b) 1999, weeks 4 to 14, $r = 0.99$, $P < 000.5$, $N = 11$. The needle cellulose $\delta^{18}O$ axis is inverted to emphasize the similarity to the specific humidity data.

here, for the same reasons mentioned in the previous section.

Explaining departures in covariance

The leaf cellulose $\delta^{18}O$ values from the needle segments produced during weeks 9 to 16 of the 1998 growing season and from weeks 15 and 16 of the 1999 growing season do not co-vary with atmospheric humidity or temperature. The most likely explanations for the lack of covariance involve either a change in the influence of back diffusion from the point of evaporation (the Péclet effect), or use of stored photosynthate. An increase (decrease) in the transpiration rate will decrease (increase) the proportion of leaf water that has been enriched by evaporation in the leaf by decreasing (increasing) the Péclet effect, the result being a decrease (increase) in the $\delta^{18}O$ of the water used in photosynthesis, until the stomata close entirely and evaporative enrichment ceases. However, photosynthesis in conifer needles also ceases soon after stomatal closure.

Similarly, storage of photosynthate as starch in older needles may have occurred during the favourable growth conditions that occurred earlier in the current growing season (Fig. 4). Starch produced under favourable growing conditions (high humidity, high soil moisture) will have $\delta^{18}O$ values closer to those of the soil water, because there will be less evaporative enrichment of the leaf water. Less favourable conditions in the growing season may reduce or eliminate photosynthesis, requiring the mobilization of stored starch to provide the material required for cell wall maturation, maintenance respiration and growth respiration. Some exchange of the oxygen isotopes in the mobilized starch with leaf water oxygen isotopes would then occur, but the stress that necessitates the mobilization of starch would also likely result in partial closure of the stomata, reducing evaporative fractionation and resulting in lower leaf-water $\delta^{18}O$ values during the exchange. The end result would be lower $\delta^{18}O$ values in the cellulose than would be produced if net photosynthesis were occurring with open stomata.

In this study, the soil moisture at 40 cm below the surface at the study site was at or near field capacity during weeks 9 to 12 of the 1998 growing season (Fig. 4), the weeks when the 1998 leaf-cellulose $\delta^{18}O$ values first depart from the humidity trend (Fig. 5). Moisture stress should not have been high enough to cause stomatal closure during those weeks, so the leaves should still have been photosynthesizing. Similarly, during weeks 15 and 16 of the 1999 growing

season (Fig. 4), the weeks when the 1999 leaf-cellulose $\delta^{18}O$ values depart from the humidity trend (Fig. 5), the soil moisture at 40 cm below the surface at the study site was high, although not saturated, and the same reasoning holds. In both years the atmospheric humidity began to decrease at the same time as the time series departures (Fig. 5). A decrease in humidity, without high plant water deficit, would result in a higher transpiration rate, thereby reducing the back diffusion component of the leaf water $\delta^{18}O$, and causing the leaf water $\delta^{18}O$ to remain the same or decrease. Such a decrease in the leaf cellulose $\delta^{18}O$ is seen in the 1998 growing season for needle segments matured during weeks 13 to 16. However, soil moisture at 40 cm below the surface during these weeks was extremely low. Lower cellulose $\delta^{18}O$ and very dry soil at 40 cm suggests that either the trees were accessing moisture sources below 40 cm, and continued photosynthesizing with an additional decrease in the back diffusion component, or net photosynthesis was stopped and the needle cellulose was produced from stored photosynthate. In summary, the departures in the 1999 growing season, and during weeks 9 to 12 of the 1998 growing season, were likely caused by an increase in the Péclet effect, but the cause of subsequent departures in 1998 is unclear given the available data.

High resolution humidity proxy

The needle cellulose $\delta^{18}O$ values in the first half of each growing season were offset by 3‰, an amount equal to the difference in soil water $\delta^{18}O$ as determined for samples collected in late June of each year. Variations in the soil water $\delta^{18}O$ cannot be determined from the needle cellulose $\delta^{18}O$ values, and covariance of needle cellulose $\delta^{18}O$ and atmospheric humidity was not apparent during all portions of the two growing seasons analysed, suggesting that a high resolution proxy for atmospheric humidity from needle cellulose $\delta^{18}O$ will not be possible in this setting. However, a large early growing-season shift in needle cellulose $\delta^{18}O$, as caused by the transition from a hyper-arid pre-monsoon environment to the humid monsoon, should dominate other potential influences on the needle cellulose $\delta^{18}O$, so analyses of sub-fossil needles may provide some evidence for the existence of such a transition in the past.

CONCLUSIONS

Simple linear regressions of needle-cellulose $\delta^{18}O$ time series against environmental time series suggest that humidity is the dominant factor determining the intraseasonal variance of the stable oxygen isotope ratios in *Pinus arizonica* needle cellulose from the semi-arid US Desert South-west. The exception to this finding is $\delta^{18}O$ ratios produced in the late summer of 1998 and possibly the last 2 weeks of growth in 1999. The lack of covariance between the late-growing-season cellulose $\delta^{18}O$ and atmospheric humidity during certain weeks was probably caused by changes in the back diffusion of enriched leaf water, the Péclet effect, although the remobilization of starch stored from earlier time periods may also be involved.

Correlations between specific humidity and needle-cellulose $\delta^{18}O$ apparently provide phenological information about the timing and duration of needle cell enlargement and needle cell-wall development (the period of cellulose deposition). The time lag between the onset of needle elongation and the highest correlation with specific humidity provides an estimate of the time period required for cell enlargement. The size of the specific humidity window at the highest correlation provides an estimate of the time period over which most of the secondary cell wall is synthesized. Phenological information should be recoverable from any long-needle conifer growing in a semi-arid setting, using the same techniques, and may also be applicable in more mesic or controlled settings. A study using finer needle subdivisions should provide better estimates of the duration of the enlargement and maturation phases, and may allow intra-annual changes to be quantified.

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APPENDIX

Assessing the Péclet effect

Starting with an equation in Farquhar & Lloyd (1993),

$$\Delta L = \frac{\Delta e\left(1-e^{-\wp}\right)}{\wp} \tag{1}$$

the following equality may be determined.

$$\frac{\Delta L}{\Delta e} = \frac{\delta_{LW}-\delta_s}{\delta_e-\delta_s} = \frac{1-e^{-\wp}}{\wp} \tag{2}$$

where ΔL is the laminar mesophyll water ^{18}O enrichment above source water, Δe is the evaporation site ^{18}O enrichment above source water, δ_{LW} is the laminar mesophyll water ^{18}O ratio relative to V-SMOW (Vienna Standard Mean Ocean Water), δ_s is the source water ^{18}O ratio relative to V-SMOW, δ_e is the evaporation site ^{18}O ratio relative to V-SMOW, and $\wp$ is the Péclet number, a dimensionless value that describes the ratio of diffusion to convection. A similar equation is found in Wang *et al*. (1998). Values for δ_{LW} and δ_s were determined from samples collected at the tree site, and δ_e was determined using the following equation from Barbour *et al*. (2004)

$$\delta_e = \delta_s + \varepsilon_k + \varepsilon_{eq} + (\delta_v - \delta_s - \varepsilon_k)\frac{e_a}{e_i} \tag{3}$$

where ε_k is the combined kinetic fractionation term for water vapour diffusing through the stomata and through the boundary layer, ε_{eq} is the liquid-vapour equilibrium fractionation factor, δ_v is the oxygen isotope composition of the atmospheric water vapour, e_a is the partial pressure of water vapour in the atmosphere, and e_i is the partial pressure of water vapour in the leaf intercellular spaces.

The term ε_k was calculated using equations from Appendix C of Buhay, Edwards & Aravena (1996), accounting for dissected leaf morphology, and incorporating on-site measurements of mean late-morning wind speed. The kinetic fractionation factor used was the revised value for oxygen isotopomers of water from Cappa *et al*. (2003). [The experimental values from Cappa *et al*. (2003) are very close to those returned by the kinetic theory of gases (e.g. Craig & Gordon 1965), and differ from the values presented by Merlivat (1978).] The term ε_{eq} was calculated from the equations of Majoube (1971). The term δ_v was estimated using equations from the regression of Tucson, Arizona, atmospheric vapour $\delta^{18}O$ against Tucson, Arizona, precipitation $\delta^{18}O$, and against the precipitation $\delta^{18}O$ of common events from Palisades Ranger Station, Santa Catalina Mountains (see Methods). For the term α_{kb}, diffusion through the boundary layer, the value $\alpha_\kappa^{2/3}$ was used (Flanagan *et al*. 1991). The terms e_i and e_a were calculated using values for temperature and relative humidity estimated for the tree site from radiosonde data, following the equations of Ball (1987).

After determining values for the left side of equation 2, we solved for $\wp$. We also used the ratio $\Delta L/\Delta e$ regressed against the modelled transpiration rate (see below) to assess the importance of a Péclet effect in this system (Barbour *et al*. 2004). With values for $\wp$ and transpiration rate, we were able to estimate the effective path length by rearranging the following equation from Farquhar & Lloyd (1993):

$$\wp = \frac{EL}{CD} \tag{4}$$

where E is the evapotranspiration rate (mol m^{-2} s^{-1}), L is the effective mixing path length in metres, C is the molar density of water (5.55×10^4 mol m^{-3}), and D is the diffusivity of $H_2{}^{18}O$ in water (2.66×10^{-9} m^2 s^{-1}). (This equation differs from the classic equation for the Péclet number, because evapotranspiration rate replaces velocity in the numerator, thereby requiring molar density in the denominator so the units will cancel and yield a Péclet number that is dimensionless.)

Estimating *relative* stomatal conductance

Stomatal conductance was estimated from a model of tree water deficit (Zweifel *et al.* 2005). Inputs were soil water potential (ψ_{soil}) and vapour pressure deficit (VPD). The former was available for the site from 1998 to 2000. The latter was estimated for these years using the vapour pressure and saturation vapour pressure calculated for the Flanagan *et al*. leaf water model (see Analysis; Flanagan *et al*. 1991).

$$\Delta W_E = \left(VPD\frac{\Psi_{soil}}{k_1}\right)k_2 \tag{5}$$

The k_1 parameter is a weighting of the impact of VPD on ΔW_E relative to the impact of Ψ_{soil}. The k_2 parameter relates changing stem radius to changing water potential. Zweifel *et al*. (2005) determined k_1 and k_2 for *Pinus sylvestris* as follows

$$k_{1Pinus} = 3.86 + 0.08|\Psi_{soil}| + 0.0018\left(|\Psi_{soil}|^2\right) \tag{6}$$

$$\frac{k_{2Pinus}}{k_{1Pinus}} = 20.1\left(|\Psi_{soil}|^{-0.57}\right) \tag{7}$$

The soil water matric potential at the *Pinus arizonica* site in this study ranged from saturation, at about –25 kPa, to greater than –1000 kPa. Vapour pressure deficit ranged

from 0 kPa to about 2.8 kPa. The minimum and maximum tree water deficit from the tree water deficit model, for the years 1998 to 2000, were assumed to represent maximum and minimum stomatal conductance. This assumption is not valid for absolute values, but does provide a relative measure of drought stress, and can be used to estimate the relative stomatal conductance, and therefore to estimate transpiration, the critical missing variable for assessing the influence of a Péclet effect.

Equations developed in the Swiss Alps cannot provide accurate measures of absolute drought stress in the semi-arid conditions of the US South-west, but they do provide a measure of relative drought stress. We calculated ΔW_E values for each day for the tree site, and then calculated the percentage of the highest value calculated that the value represented for the 3 year period analysed. The results then ranged from zero water stress to maximum measured stress, and because stomatal conductance has been shown to correlate well with soil water and VPD for *Pinus ponderosa* (Panek & Goldstein, 2001), a species very closely related to *Pinus arizonica* (Peloquin 1984), the results could be used to roughly indicate stomatal conductance. However, stomatal conductance is inversely related to moisture stress, so the percentage was subtracted from 1, multiplied by the assumed maximum stomatal conductance value of 0.25 mol m^{-2} s^{-1} (Mission, Panek & Goldstein 2004), and then substituted into the modified Flanagan leaf water model (Flanagan *et al*. 1991). The maximum stomatal conductance value used in the model was measured in California, in a more mesic environment than semi-arid southern Arizona, so the use of this value as an estimate of maximum stomatal conductance may underestimate the absolute transpiration, but the relationships between the samples at the tree site should have the correct sign relative to the other sampling times, and the relative differences between the transpiration rates should be roughly correct.