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Separating soil and leaf water ^{18}O isotopic signals in plant stem cellulose

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Abstract—The oxygen-18 signal of soil and leaf water are both recorded in heterotrophically synthesized plant stem cellulose. Presently, these signals can only be teased apart with modeling and assumptions on the nature of the isotopic enrichment of leaf water. A method by which these two signals are chemically separated and analyzed is tested here. Heterotrophically synthesized cellulose from germinating seeds having a mixture of isotopic signals from the reserve carbohydrate (starch) and that of the water during cellulose synthesis was hydrolyzed and the resulting glucose converted to glucose phenylosazone. The analysis of the $^{18}O/^{16}O$ ratios of cellulose and of glucose phenylosazone were used to calculate the oxygen isotope ratio of the oxygen attached to the second carbon of the glucose moieties of the cellulose molecule. The calculated $\delta^{18}O$ value of this oxygen was highly correlated with that of the water available for cellulose synthesis showing a nearly one-to-one relationship (slope = 1.027) and leading to the conclusion that it completely exchanges with water during heterotrophic cellulose synthesis. Once this method is refined so as to increase precision, it will be possible to derive the $\delta^{18}O$ values of soil water available to plants from the oxygen isotope analysis of stem cellulose and its derivative.

1. INTRODUCTION

Stable oxygen isotope analysis of plant celluose has the potential of providing a valuable tool in the study of paleoclimate, ecology, hydrology and other fields. Several investigators have used $\delta^{18}O$ values of fossil wood cellulose to infer past climate (Epstein et al., 1977; Gray and Thompson 1977; Edwards et al., 1985; Jahren and Sternberg 2002). The basic principle behind the utility of this technique is that the oxygen isotope ratio of precipitation is related to temperature and atmospheric circulation patterns (Dansgaard 1964). It was first proposed that the isotopic signal of plant cellulose could be correlated with the isotopic ratios of soil water which is ultimately derived from precipitation, hence the utility of $\delta^{18}O$ analysis of cellulose (Epstein et al., 1977). However, it was quickly realized that there are several unknown biochemical and physiochemical processes occurring during transpiration, biochemical fixation of carbon dioxide, and post photosynthetic biochemical reactions during cellulose synthesis which alter the soil water isotopic signal in the cellulose molecule. Therefore, inference of climate based on oxygen isotope ratios of tree ring cellulose has mostly been of an empirical nature (Saurer et al., 1997; Anderson et al., 1998, Anderson et al. 2002 and several others).

Physiologists and paleoclimatologists are attempting to derive mechanistic models to explain the process of ^{18}O incorporation in stem cellulose (Epstein et al., 1977; Burk and Stuiver 1981; Roden et al., 2000). These models should consider all the biophysical and biochemical processes occurring during transpiration, photosynthesis and heterotrophic cellulose synthesis in non-photosynthetic tissues. The long range goal is that once all of these processes are understood, isotopic ratios of soil water and ambient relative humidity can be "back-calculated" from the $\delta^{18}O$ values of stem cellulose. Much progress has been made and the major observations relevant to these models are:

1. There is no oxygen isotopic fractionation during water uptake by plants; i.e., the isotopic composition of stem water is the same as that of the water in the soil (Gonfiantini et al., 1965; Dawson and Ehleringer 1993; Lin and Sternberg 1993). Presumably the isotopic composition of this water is the closest to the meteoric water and contains global or regional climate information (Burns and McDonnell 1998; Tang and Feng 2001).
2. There is isotopic enrichment of leaf water relative to stem water for leaves undergoing transpiration. This enrichment is complicated by several factors such as the isotopic composition of atmospheric vapor, relative humidity, and back diffusion of $H_2^{18}O$ from the leaf mesophyll to vascular tissue (Farquhar et al., 1998).
3. $\delta^{18}O$ values of cellulose synthesized autotrophically is approximately 27‰ more enriched than the $\delta^{18}O$ of water available during photosynthesis. Autotrophic cellulose synthesis is defined as the cellulose synthesized in photosynthetic tissues (Roden et al., 2000). This finding is based on the observations of $\delta^{18}O$ values of cellulose from submerged photosynthesizing aquatic plants which do not transpire (DeNiro and Epstein 1981; Sternberg et al., 1984). Evidence that sucrose translocated from leaves also has $\delta^{18}O$ values 27‰ above that of modeled leaf water was presented in an elegant experiment by Barbour et al. (2000) where the $\delta^{18}O$ values of sucrose from phloem of photosynthesizing leaves were compared with the $\delta^{18}O$ values of leaf water.
4. About 40% of the sucrose oxygen atoms that are incorporated in the cellulose molecule will exchange with water during heterotrophic cellulose synthesis. Heterotrophic cel-

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lulose synthesis is defined as cellulose synthesized in non-photoysnthetic tissues such as plant stems, roots, and tree trunks (Roden et al., 2000). This conclusion is based on several experiments done with tissue cultures (Sternberg et al., 1986), germinating seeds (Luo and Sternberg 1992) and aquatic plants grown in the dark supplied with sucrose (Yakir and DeNiro 1990). Evidence that these in vitro systems are realistic simulators of processes occurring during the translocation of sucrose from the leaf to the trunk for heterotrophic cellulose synthesis was presented by Roden et al. (2000) showing a good agreement between experiments with hydroponic tree cultures and previously presented in vitro studies. Therefore, plant stem cellulose contains a mixture of isotopic signals from leaf (autotrophic) and stem (heterotrophic) cellulose synthesis.

As such, the ambiguity of ^{18}O analysis of plant stem cellulose is whether a high $\delta^{18}O$ value of the cellulose is caused by either an increase in the $\delta^{18}O$ value of precipitation (making stem water isotopically more enriched), a decrease in relative humidity or an increase in the isotopic composition of atmospheric vapor during photosynthesis (making leaf water isotopically more enriched). Using seed germination experiments we demonstrate here a method which can potentially dispose of this ambiguity. Heterotrophically synthesized cellulose is hydrolyzed and converted to glucose phenylosazone. The $\delta^{18}O$ values of cellulose and its derivative are used to calculate the $\delta^{18}O$ value of one of the oxygen atoms in cellulose that completely exchanges with water during heterotrophic cellulose synthesis. Since there is no discrimination during plant water uptake it will be possible to calculate the $\delta^{18}O$ value of soil water using this technique.

2. THEORY

Cellulose is a long polymer of glucose moieties. The oxygen attached to the first carbon of glucose is lost during the polymerization of glucose by condensation reactions (Farquhar et al., 1998). Therefore oxygen atoms present in a cellulose molecule are attached to carbon 2, 3, 4, 5 and 6 of the glucose moieties (Fig. 1). The $\delta^{18}O$ value of a cellulose molecule ($\delta^{18}O_{Cellulose}$) is the average of the $\delta^{18}O$ values of the oxygen atoms attached to these carbons and approximated by the following equation:

$$\delta^{18}O_{Cellulose} = \frac{\sum_{i=2}^{6}\delta^{18}O^{i}}{5}, \quad (1)$$

where $\delta^{18}O^{i}$ pertains to the $\delta^{18}O$ value of the oxygen attached to the i^{th} carbon. The above equation can be modified to:

$$5 \times \delta^{18}O_{Cellulose} = \delta^{18}O^{2} + \sum_{i=3}^{6}\delta^{18}O^{i}, \quad (2)$$

During hydrolysis of cellulose carbon 1 regains oxygen from the hydrolysis solution, but this oxygen as well as the oxygen attached to the second carbon is eliminated during the glucose phenylosazone reaction (Fig. 1). Therefore, the $\delta^{18}O$ value of glucose phenylosazone ($\delta^{18}O_{p\text{-}g}$) represents the average $\delta^{18}O$ value of oxygen attached to carbons 3, 4, 5, and 6 and approximated by the following equation:

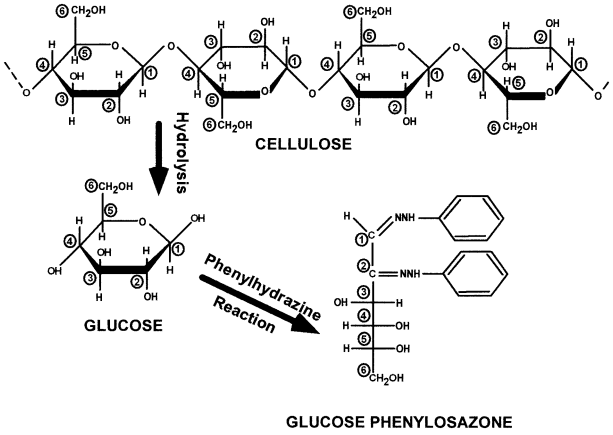

Fig. 1. Chemical derivatization of cellulose by hydrolysis and the phenylhydrazine reaction to produce glucose phenylosazone. Carbons are numbered according to standard organic chemistry notation.

$$4 \times \delta^{18}O_{p\text{-}g} = \sum_{i=3}^{6}\delta^{18}O^{i}. \quad (3)$$

Equations 2 and 3 can be merged and the following equation derived:

$$\delta^{18}O^{2} = (5 \times \delta^{18}O_{Cellulose}) - (4 \times \delta^{18}O_{p\text{-}g}), \quad (4)$$

where $\delta^{18}O^{2}$ represents the $\delta^{18}O$ value of the oxygen attached to the second carbon of cellulose's glucose moieties. In other words: 4 times the $\delta^{18}O$ value of glucose phenylosazone subtracted from 5 times the $\delta^{18}O$ value of cellulose will yield the calculated $\delta^{18}O$ value of the oxygen attached to the second carbon in a cellulose molecule. We propose that this oxygen, being a carbonyl oxygen in fructose and easily exchangeable with water (Sternberg et al., 1986) has the highest probability of exchanging with water during heterotrophic cellulose synthesis since even if the hexose precursor (glucose and/or fructose) does not undergo cycling into the triose sugars (Hill et al., 1995), this oxygen can still exchange with water.

3. MATERIALS AND METHODS

3.1. Growing Cellulose Heterotrophically

The culture media solution consisted of 200 mL of water having a specific $\delta^{18}O$ value, 4 g of agar and 0.4 mL of an antifungal agent (PPM™, Plant Cell Technology). This solution was placed in 1L Erlenmeyer flasks, plugged with cotton plugs and autoclaved at 120°C for 20 min. Water enriched in ^{18}O (Icon Services) was used to create culture solutions having different $\delta^{18}O$ values (−1.45‰, +5.8‰, +91.42‰, +97.2‰, +223.2‰ and +326.5‰). We used a large range of $\delta^{18}O$ values to avoid any ambiguity that could be brought about by the precision of the analytical methods. Approximately 100 mL volume of wheat seeds (*Triticum aestivum*) acquired from the local health food store was sterilized by soaking in a 20% solution of commercial unscented bleach for a period of one hour. After soaking, seeds were rinsed with 1L of sterilized distilled water in a laminar flow hood and soaked overnight in 200 mL of 0.2% PPM™ solution having the same $\delta^{18}O$ values of the culture medium solution. The following day the seeds were strained and placed in culture media under sterile conditions

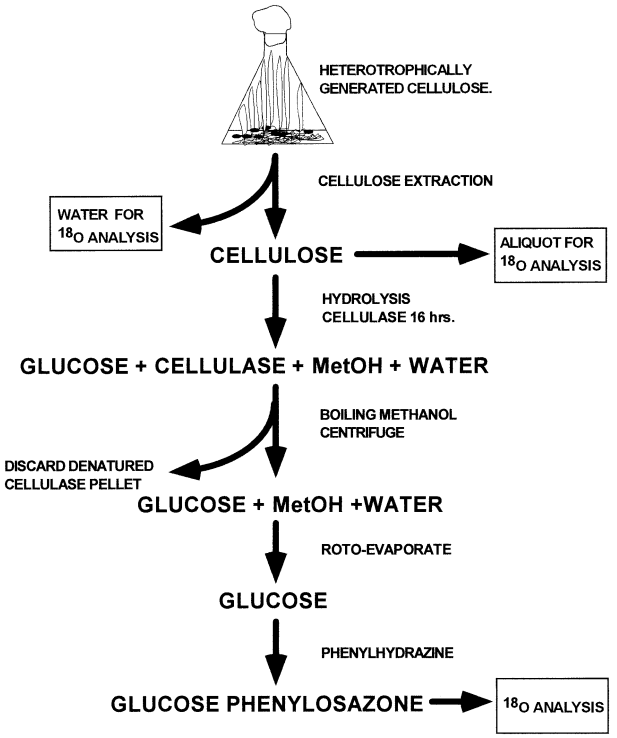

Fig. 2. Procedure for the chemical reactions shown in Figure 1, starting from heterotrophically synthesized cellulose to the synthesis of glucose phenylosazone.

in a laminar flow hood. All culture flasks were kept in the dark at 25°C to force cellulose synthesis to be solely heterotrophic; i.e., the sole source of glucose moieties are from starch reserves in the seed endosperm. Seedlings were allowed to grow for approximately 8 d until sufficient cellulose was heterotrophically synthesized (approximately 1.5 g).

3.2. Cellulose Extraction

A small aliquot of the agar medium was removed from each culture after 8 d and stored in sealed glass tubes for water extraction and isotopic analysis (Fig. 2). Seedlings were separated from agar and endosperms containing old seed coat removed. Leaves, stems and roots were washed several times with hot distilled water and dried at 60°C for 1 week. Seedlings from each culture were ground in a Wiley mill (Mesh #40) and cellulose extracted according to previous methods (Sternberg 1989). A small aliquot ($\sim$ 20 mg.) of the extracted cellulose was saved for oxygen isotope analysis of cellulose (Fig. 2).

3.3. Cellulose Hydrolysis

The rest of the cellulose was autoclaved with 25 mL of distilled water at 120°C for 20 min to soften the cellulose micro-fibrils. After cooling, 0.5 g of cellulase, an enzyme which hydrolyzes cellulose (EC 3.2.1.4 from *Penicillium funicolosum*, 9.4 U/mg solid, Lot 58H3291, Sigma Chemicals), was added to the flask and incubated overnight at 36°C with a constantly stirring magnetic stir-bar (Fig. 2). After hydrolysis, 75ml of 95% methyl alcohol (MetOH) was added to the hydrolysis solution and the solution boiled for 15 min (Fig. 2). The hot alcohol caused the cellulase enzyme to denature and precipitate. The solution containing glucose from hydrolysis, alcohol, water and coagulated cellulase was centrifuged, the supernatant decanted into a 200 mL round bottom flask, and the cellulase pellet discarded. The glucose solution in the round bottom flask was roto-evaporated to a syrup and freeze dried to solid glucose for several days. The only oxygen of the glucose molecule that can exchange with water is the carbonyl oxygen attached to the first carbon, whereas all other oxygens, which are hydroxide oxygens, do not exchange with water (Sternberg et al., 1986). Since the oxygen in the first carbon is eliminated by the glucose phenylosazone reaction, it was not necessary to monitor the oxygen isotope ratios of the hydrolysis solution or that of any other subsequent reaction.

3.4. Glucose Phenylosazone Reaction

The glucose in the 200 mL round bottom flask was diluted with 8.5 mL of distilled water and heated to *circa* 80°C in a water bath to which 1 g. of sodium acetate and 0.33 mL of 12N HCl was added. 1.83 g of phenylhydrazine hydrochloride was diluted in 15 mL of hot distilled water and added to the glucose solution. This mixture was allowed to react for 20–30 min after which the glucose phenylosazone formed a precipitate. The reaction mixture was vacuum filtrated in a scintered glass filter funnel (Pyrex, 40–60 C) and the glucose phenylosazone precipitate washed several times with water. The glass funnel was plugged with a small septum and the precipitate soaked in MetOh for a few minutes and refiltered. This washing with MetOH was repeated once more. The filter funnel was covered with a perforated aluminium foil and freeze dried. Dried filtrate was scraped from funnels and used for isotopic analysis (Fig. 2).

3.5. Isotope Analysis

Oxygen isotope analysis of culture media water was done by the CO_2 equilibration method (Epstein and Mayeda 1953). $\delta^{18}O$ value of purified CO_2 from the equilibration was measured in a dual inlet isotope ratio mass spectrometer (Prism, Micromass) at the University of Miami.

Isotope ratios of $^{18}O/^{16}O$ in cellulose and glucose phenylosazone were measured on CO gas produced via a high temperature conversion/elemental analyzer system coupled to a stable isotope ratio mass spectrometer (TC/EA, manufactured by ThermoFinnigan®), similar in approach to the methods of Farquhar et al. (1997) and of Saurer et al. (1998). Cellulose or glucose phenylosazone (1.3–1 mg) enclosed in a silver cup was introduced into carbon lined ceramic reaction oven filled half-way with glassy carbon. The reaction furnace was held at a temperature of 1350°C under a continuous flow of ultra pure He gas at 120 mL/min (TriGas ultra-pure carrier grade), where the introduced sample undergoes thermal decomposition forming CO, the thermodynamically preferred molecule (Santrock and Hayes, 1987). CO produced in the reaction tube was then transferred to the mass spectrometer after passing through a 1 m long Molecular Sieve 5Å packed gas chromatograph column (70°C) under a continuous flow via an open split valve (this separates from the main CO peak any trace amounts of N_2, which has the same mass as CO). The relative isotopic ratio of the sample was measured against a reference gas pulse of CO (Messer CO 4.7) from a gas injector system (ConFlow II). All isotopic measurements are reported in the standard delta notation:

$$\delta^{18}O\ (‰) = \{(R_{sample}/R_{standard}) - 1\} \times 1000 \qquad (5)$$

The $\delta^{18}O$ value for each sample-run was standardized to Vienna SMOW (VSMOW) using three previously used cellulose standards: Sigma Cellulose, Merck standard and IAEA C-3 (see Saurer et al., 1998). Internal standards (Sigma Cellulose and Merck standard) were measured with a standard off-line CO_2 method and another CO on-line method, respectively measured at the University of Cambridge and the Paul Scherrer Institute (Saurer et al., 1998). All samples for this study were measured on a ThermoFinnigan Delta C IRMS at the Southeast Environmental Research Center at Florida International University. The $\delta^{18}O$ value of IAEA CH-3 (formerly IAEA C3) from this study was determined to be 32.8 $\pm$ 0.74‰ (n=7), which falls within the range of previously published values from both on-line and off-line methods (Buhay et al. 1995; Saurer et al., 1998). Analytical reproducibility for this method is about $\pm$0.36‰ (1σ) based on n=21 measurements of the Merck cellulose standard.

4. RESULTS

$\delta^{18}O$ values of heterotrophically synthesized cellulose and its derivative, glucose phenylosazone were highly correlated

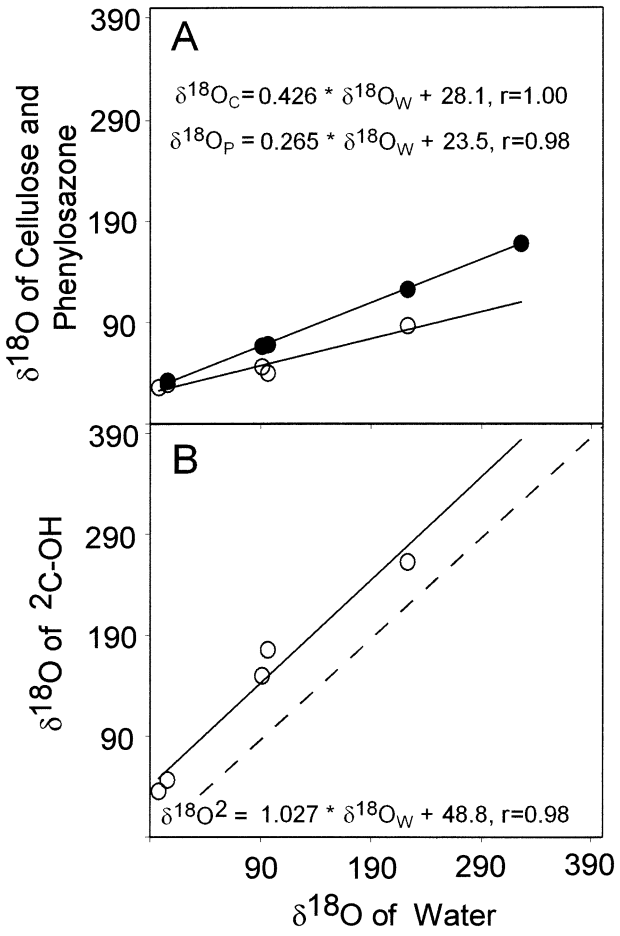

Fig. 3. A—$\delta^{18}O$ values of cellulose (full circles) and glucose phenylosazone (empty circles) versus the $\delta^{18}O$ values of water available during heterotrophically synthesized cellulose. One sample of glucose phenylosazone could not be recovered in sufficient quantities to analyze its oxygen isotope ratios. B—Calculated $\delta^{18}O$ values of the oxygen atoms attached to the second carbon of glucose moieties in the cellulose molecule versus the $\delta^{18}O$ value of the water available for heterotrophic cellulose synthesis. One extrapolated value of cellulose was used to calculate the $\delta^{18}O^2$ value of the seed culture having water with a $\delta^{18}O$ value of $-1.45‰$. Dashed line shows a relationship having a slope of 1 and intercept of 0.

with the $\delta^{18}O$ values of water available during cellulose synthesis, showing a correlation coefficient of 1.00 ($P<0.01$) and 0.98 ($P<0.01$) respectively (Fig. 3A). The percentage of oxygen labeled by water in the cellulose molecule is approximately twice as much as that of the glucose phenylosazone as evidenced by the slopes of the linear regressions (0.426 *vs* 0.265) respectively. The calculated $\delta^{18}O$ values of the oxygen in the second carbon of the heterotrophically synthesized cellulose are highly correlated with the $\delta^{18}O$ values of the water available for cellulose synthesis ($r=0.98$, $P<0.01$) having a slope nearly equal to 1 (slope = 1.027) and an intercept of $+48.8 \pm 20‰$ (standard error of the estimate, Fig. 3B).

5. DISCUSSION

The results presented here have implications in two important areas of biogeochemistry. The first is in the understanding of mechanisms whereby organic matter incorporates ^{18}O during its synthesis. The second area is the potential application of the analytical techniques presented here in paleoclimatology, hydrology, and other studies interested in integrating water and carbon cycles at the ecosystem level.

Current understanding of how cellulose in plant stems is labeled by environmental water indicates that an average of 42% of the oxygen atoms in the stem cellulose exchanges with trunk water during its synthesis (Roden et al., 2000). This value is consistent with studies using tissue cultures (Sternberg et al., 1986) and heterotrophic aquatic plant cultures using sucrose as a substrate (Yakir and DeNiro 1990), as well as germinating seeds (Sternberg et al., 1986; Luo and Sternberg 1992). Therefore all these systems, including the germination experiments shown here, can be used as model systems to study oxygen isotope labeling of plant stem cellulose during its synthesis.

The results of the experiments presented here yielded a slope of 0.42 for the linear regression between $\delta^{18}O$ values of heterotrophically synthesized cellulose and that of water which is consistent with the studies cited above. The fractionation factors for the exchange between oxygen in water and metabolites during carbohydrate metabolism are relatively small (Sternberg et al., 1986). Therefore, we interpret the slope of the relationship between $\delta^{18}O$ values of metabolites and those of water as the proportion of oxygens in the metabolite that exchanged with water (Sternberg et al., 1986, Yakir and DeNiro 1990; Luo and Sternberg 1992; Roden et al., 2000). Since there are 5 oxygen atoms per glucose moiety in cellulose molecules, one can conclude that on average 2 out of the 5 oxygen atoms in the cellulose molecule have exchanged with water during its synthesis ($2/5 \approx 0.42$). The linear regression between $\delta^{18}O$ values of glucose phenylosazone and that of water yielded a slope of 0.265. Therefore, on average 1 of the oxygens attached to carbons 3, 4, 5 and 6 of the cellulose molecule has undergone isotopic exchange with water during its synthesis ($1/5 \approx 0.265$). Although current hypothesis suggests that labeling of oxygen in cellulose occur by some of the glucose moieties cycling between fructose 1,6-bisphosphate, glyceraldehydes 3-phosphate and dihyroxyacetone phosphate (Hill et al., 1995), the results presented here suggest that very specific oxygen atoms in the cellulose molecule have exchanged with water during cellulose synthesis. Particularly interesting is the observation that the numbers of oxygen atoms in cellulose and glucose phenylosazone that exchange with water during cellulose synthesis are close to whole numbers (2 and 1 respectively). We have shown here that one of the oxygen atoms that exchanges with water during cellulose synthesis is located on the second carbon ($\delta^{18}O^2$). Since the slope of the linear regression of $\delta^{18}O^2$ versus $\delta^{18}O$ values of water is 1.027 we conclude that this oxygen completely exchanges with water during cellulose synthesis. It remains to be shown whether the second oxygen that exchanges during heterotrophic cellulose synthesis represents a specific oxygen (for example: oxygen in the 4^{th} carbon of the glucose moieties) or the average exchange for oxygen atoms attached to carbon 3, 4, 5 or 6.

Fractionation factors for the exchange between oxygens and water during heterotrophic cellulose synthesis range from $15‰$ to $27‰$ (Sternberg et al., 1986; Yakir and DeNiro 1990; Luo and Sternberg 1992). There are two possible ways in which the exchangeable oxygen atoms during heterotrophic cellulose syn-

thesis could have a particular fractionation factor relative to the $^{18}O/^{16}O$ of water. First, the fractionation factors for oxygen exchange during cellulose synthesis are all similar regardless of the location in the molecule. Second, the net fractionation factor for the exchangeable oxygen atoms of heterotrophically synthesized cellulose is an average of different fractionation factors for oxygen atoms in different locations of the molecule that have exchanged with water during cellulose synthesis. Since there is complete exchange between oxygens attached to the second carbon and water during heterotrophic cellulose synthesis, the fractionation factor for this exchange is approximated simply as the intercept of the linear regression of $\delta^{18}O^2$ on the $\delta^{18}O$ value of the water available for exchange (Fig. 3B). For the regression shown here, the intercept yields a fractionation factor for the exchange between oxygen in the second carbon of glucose moieties and water of 48.8‰, supporting the second case. Our precision of analysis, however, will have to be improved before we can firmly assert this.

The methods reported here can have important applications in the field of paleoclimatology, hydrology, and plant ecology for they potentially allow us to determine the integrated $\delta^{18}O$ values of the soil water available to plants. This cannot be done with oxygen isotope analysis of stem cellulose and currently available models. To calculate the $\delta^{18}O$ values of water available to plants based on $\delta^{18}O$ values of stem cellulose and currently available models one has to assume the following parameters: $\delta^{18}O$ values of atmospheric vapor, relative humidity, and temperature. In addition, one has to model how leaf water becomes enriched in ^{18}O when subjected to these assumed parameters. This modeling, in turn, would have to assume factors such as compartmentation and back diffusion of ^{18}O. With the method reported here, once the techniques are refined so as to increase precision, $\delta^{18}O$ values of soil water can be derived from the $\delta^{18}O$ values of stem cellulose with the simple equation:

$$\delta^{18}O_{\text{soil water}} = \delta^{18}O^2 - \Delta \quad (6)$$

where Δ represents the fractionation factor between ^{2}C-OH and water. As pointed out earlier, however, improvements in this analytical procedure will have to be made to increase precision before it can be applied. There are several steps in this analysis which could be more rigorously standardized and therefore increase the precision of measurements. First the enzymatic hydrolysis of cellulose can introduce contaminants from the enzyme preparation. Glucose from enzymatic cellulose hydrolysis can be purified from this mixture by high pressure liquid chromatography. Acid hydrolysis of cellulose may be an alternative which presents fewer contaminants. Secondly the phenylhydrazine reaction can be standardized to a more precisely controlled temperature. Thirdly, the isotopic analysis of the glucose phenylosazone can be improved since the amount of oxygen in the molecule is only 18% compared to 49% for cellulose and large samples may have interference from the nitrogen peak. Presently the analytical technique presented here is geared towards large samples (~1.5 g cellulose) and not amenable to batch analysis. Therefore, until the techniques are improved in the above aspects, it will not be applicable for the study of narrow tree rings.

Even if the analytical precision is improved, however, there is still a maximum precision limitation based on the fact that $\delta^{18}O^2$and $\delta^{18}O_{\text{soil water}}$ will be calculated from measurements having errors and these errors are propagated during the calculations. The error for the calculation of $\delta^{18}O_2$ in Eqn. 4 is given by the following equation:

$$sd_2 = \sqrt{(5 \times sd_{\text{cellulose}})^2 + (4 \times sd_{\text{p-g}})^2}, \quad (7)$$

where sd_2, $sd_{\text{cellulose}}$ and $sd_{\text{p-g}}$ represent the standard deviation of $\delta^{18}O$ values of oxygen in the second carbon of the glucose moieties, in cellulose and glucose phenylosazone respectively (Taylor 1997). With an optimistic standard deviation of $\pm$ 0.2‰ (1σ) for oxygen isotope ratio measurements of cellulose and glucose phenylosazone the best precision for $\delta^{18}O^2$ would be $\pm$ 1.28‰ (1σ). When these values are used to calculate the $\delta^{18}O$ values of water there will be the additional error associated with Δ and further propagation of errors. Therefore, at best there will be a 1 to 2‰ error in the estimation of soil water using the techniques presented here. Using the relationship between $\delta^{18}O$ values of precipitation and temperature (Burk and Stuiver 1981), and the assumption that the isotope ratios of soil water integrate the $\delta^{18}O$ values of precipitation, the above precision would translate to an error of 0.5 to 1.0°C.

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REFERENCES

Anderson W. T., Bernasconi S. M., Mckenzie J. A., and Saurer M. (1998) Oxygen and carbon isotopic record of climatic variability in tree ring cellulose (*Picea abies*): an example from central Switzerland (1913–1995). *J. Geophys. Res.* **103**(D24), 31625–31636.

Anderson W. T., Bernasconi S. M., Mckenzie J. A., Saurer M., and Schweingruber F. (2002) Model evaluation for reconstructing the oxygen isotope composition in precipitation from tree ring cellulose over the last century. *Chem. Geo.* **182,** 121–137.

Barbour M. M., Schurr U., Henry B. K., Wong S. C., and Farquhar G. D. (2000) Variation in the oxygen isotope ratio of phloem sap sucrose from castor bean: Evidence in support of the Peclet effect. *Plant Physiol.* **123,** 671–679.

Burk R. L. and Stuiver M. (1981) Oxygen isotope ratios in trees reflect mean annual temperature and humidity. *Science* **211,** 1417–1419.

Burns D. A. and McDonnell J. J. (1998) Effects of a beaver pond on runoff processes: comparison of two head water catchments. *J. Hydrol.* **122,** 253–274.

Buhay W. M., Wolfe B. B., Elgood R. J., Edwards T. W. D. (1995) A report on the $\delta^{18}O$ measurements of the IAEA cellulose intercomparison material IAEA-C3, In *Reference and intercomparison materials for stable isotopes of light elements* **IAEA-TECDOC-825,** 85–86.

Dansgaard W. (1964) Stable isotopes in precipitation. *Tellus.* **16,** 436–468.

Dawson T. E. and Ehleringer J. R. (1993) Isotopic enrichment of water in the "woody" tissues of plants: implications for plant water source, water uptake, and other studies which use the stable isotopic composition of cellulose. *Geochim. Cosmochim. Acta* **57,** 3487–3492.

DeNiro M. J. and Epstein S. (1981) Isotopic composition of cellulose from aquatic organisms. *Geochim. Cosmochim. Acta* **42,** 495–506.

Edwards J. R., Aravena R. O., Fritz P., and Morgan A. V. (1985) Interpreting paleoclimate from 18O and 2H in plant cellulose: Comparison with evidence from fossil insects and relict permafrost in southwestern Ontario. *Can. J. Earth Sci.* **22,** 1720–1726.

Epstein S. and Mayeda T. (1953) Variations of ^{18}O content of water from natural sources. *Geochim. Cosmochim. Acta* **4,** 213–224.

Epstein S., Thompson P., and Yapp C. J. (1977) Oxygen and hydrogen isotopic ratios in plants cellulose. *Science* **198,** 1209–1215.

Farquhar, G. D., Barbour, M. M., Henry, B. K. (1998) Interpretation of oxygen isotope composition of leaf material. In *Stable Isotopes: integration of biological ecological and geochemical processes* (ed. H. Griffiths) pp 27–62. BIOS Scientific Pub. Ltd.

Farquhar G. D., Henry B. K., and Styles J. M. (1997) A rapid on-line technique for determination of oxygen isotope composition of nitrogen-containing organic matter and water. *Rapid Com. Mass Spec.* **11,** 1554–1560.

Gray J. and Thompson P. (1977) Climatic information from $^{18}O/^{16}O$ analysis of cellulose lignin and whole wood from tree rings. *Nature* **270,** 708–709.

Gonfiantini, R., Gratiziu, S., Tongiorgi, S. (1965) Oxygen isotope composition of water in leaves. In *Isotope and radiation in soil-plant-nutrition studies* pp 405–410. Intern. At. Energy Agency. Vienna.

Hill S. A., Waterhouse J. S., Field E. M., Switsur V. R., and ap Rees T. (1995) Rapid recycling of triose phosphates in oak stem tissue. *Plant Cell Env.* **18,** 931–936.

Jahren A. H., and Sternberg L., da S. L. (2002) Eocene meriodinal weather patterns reflected in the oxygen isotopes of arctic fossil wood. *GSAToday* **12,** 4–9.

Lin G., Sternberg, L. S. L. (1993) Hydrogen isotopic fractionation by plant roots during water uptake in coastal wetland plants. In *Stable Isotopes and Plant Carbon/Water Relations* (eds. Ehleringer et al.) pp 497–510. Academic Press.

Luo Y. H. and Sternberg L. (1992) Hydrogen and oxygen isotope fractionation during heterotrophic cellulose synthesis. *J. Exp. Bot.* **43,** 47–50.

Roden J. S., Lin G., and Ehleringer J. R. (2000) A mechanistic model for interpretation of hydrogen and oxygen isotope ratios in tree-ring cellulose. *Geochim. Cosmochim. Acta* **64,** 21–35.

Santrock J. and Hayes J. M. (1987) Adaptation of the Unterzaucher procedure for determination of oxygen-18 in organic substances. *Anal. Chem.* **59,** 119–127.

Saurer M., Borella S., and Leuenberger M. (1997) $\delta^{18}O$ of tree rings of beech (Fagus silvatica) as a record of $\delta^{18}O$ of the growing season precipitation. *Tellus.* **49B,** 80–92.

Saurer M., Robertson I., Seigwolf R., and Leuenberger M. (1998) Oxygen isotope analysis of cellulose: an interlibrary comparison. *Anal. Chem.* **70,** 2074–2080.

Sternberg L., DeNiro M. J., and Keeley J. E. (1984) Hydrogen, Oxygen, and Carbon isotope ratios of cellulose from submerged aquatic Crassulacean acid metabolism and non-Crassulacean acid metabolism plants. *Plant Physiol.* **76,** 68–70.

Sternberg L., DeNiro M. J., and Savidge R.A. (1986) Oxygen isotope exchange between metabolites and water during biochemical reactions leading to cellulose synthesis. *Plant Physiol.* **82,** 423–427.

Sternberg, L. S. L. (1989) Oxygen and hydrogen isotope measurements in plant cellulose. In *Modern Methods in Plant Analysis* (eds. H. F. Linskens and J. F. Jackson) pp 89–98. Springer-Verlag.

Tang K. and Feng X. (2001) The effect of soil hydrology on the oxygen and hydrogen isotopic composition of plants' source water. *Earth Planet. Sci. Lett.* **185,** 355–367.

Taylor R. J. (1997) An Introduction to Error Analysis. University Science Books.

Yakir D. and DeNiro M. J. (1990) Oxygen and hydrogen isotope fractionation during cellulose metabolism in *Lemna gibba* L. *Plant Physiol.* **93,** 325–332.