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LETTER

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

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Abstract—The stable hydrogen isotopic ratio (δD) of stem water is shown to deviate from source water values in young, nonsuberized woody stems, reflecting an isotopic enrichment associated with cuticular water loss and/or transpiration. The extent of this enrichment was greater in trees which produce a new set of leaves annually (e.g., deciduous-leaved species) than in trees which retain their leaves for two or more years (e.g., coniferous or evergreen-leaved species). Stem-water enrichment was also greater in rapidly growing stem tissues than in mature, fully suberized or lignified stems which had ceased growing and showed little or no isotopic enrichment. These results are discussed in terms of their implications for studies which use xylem-sap δD values to evaluate plant water sources and uptake. We also discuss our findings in the context of long-term investigations that use cellulose or cellulose nitrate δD values for climatic reconstruction or to infer past water-use or distributional patterns in woody vegetation.

INTRODUCTION

THE PAST FIVE TO FIFTEEN years have brought an increased interest in applying hydrogen (and oxygen) isotope ratio analyses to both short-term studies of water uptake by plants (WHITE et al., 1985; DAWSON and EHLERINGER, 1991; EHLERINGER et al., 1991; SMITH et al., 1991; EHLERINGER and DAWSON, 1992; FLANAGAN et al., 1992; THORBURN et al., 1993; DAWSON, 1993; THORBURN and WALKER, 1993) and to long-term studies of paleoclimatic reconstruction based upon the isotopic analysis of wood cellulose (EPSTEIN, 1978; YAPP and EPSTEIN, 1976, 1977, 1982a,b; BURK and STUIVER, 1981; BRENNINKMEIJER, 1983; WHITE, 1983; WHITE et al., 1985; KRISHNAMURTHY and EPSTEIN, 1985; EDWARDS and FRITZ, 1986, 1988). Water-uptake studies involve the analysis of the stable hydrogen or oxygen isotopic composition of xylem-sap, while those aimed at reconstructing past climates involve the interpretation of hydrogen (and oxygen) isotope data from tree-ring cellulose (SCHIEGL, 1974; GRAY and THOMPSON, 1976, 1977; DENIRO and EPSTEIN, 1979; DENIRO, 1981; DUBOIS, 1984; NORTHFELT et al., 1981) or leaf and stem macrofossils present in packrat middens (reviewed in LONG et al., 1990). The accuracy of short-term observations depends upon the ability to distinguish among possible water sources available to plants. Precision in reconstructing long-term climatic patterns (e.g., temperature, humidity, carbon dioxide concentrations, precipitation, water-use) depends on understanding the relationship between paleoclimatological variables and the δD of leaf cellulose or nitrated cellulose from secondary xylem (tree rings; ZIEGLER, 1988; YAKIR, 1992). Since water is the only source of hydrogen for plants and there is no isotopic fractionation during water uptake, the stable hydrogen isotope ratio (δD) of water contained within the xylem-sap should reflect the water source that a particular individual is using (WERSHAW et al., 1966; WHITE et al., 1985; DAWSON and EHLERINGER, 1991; reviewed in EHLERINGER and DAWSON, 1992, and DAWSON, 1993).

If there is (1) complete equilibrium between the hydrogen atoms of initial organic compounds (e.g., sugars) and the xylem-sap during cellulose formation and/or (2) no fractionation occurs during the biochemical reactions leading to cellulose synthesis in stems, then the δD of nitrated cellulose obtained from tree rings should reflect a weighted estimate of water sources used by the tree during the growing period when those tree rings were being formed. Evidence in support of this pattern has been reported by several investigators (YAPP and EPSTEIN, 1982a; WHITE, 1983; RAMESH et al., 1985; EDWARDS and FRITZ, 1986, 1988), although the slope of the "water source" and nitrated cellulose δD relationship did not always follow a 1:1 line. YAPP and EPSTEIN (1982a,b) and EDWARDS and FRITZ (1986, 1988) proposed that humidity may play a role in modifying the source-water signal that otherwise was expected to determine nitrated cellulose δD values in tree rings. This would require that a portion of the enriched D/H signal originating in the leaves was directly or indirectly retained in sugars transported between the leaf and the site where xylem (wood) tissues are synthesized. Modification of the original source water D/H value can arise from evaporative enrichment of leaf water. Subsequent exchange of these hydrogens in leaf water with sugars within the leaf, fractionation events during cellulose synthesis, or both (ESTEP and HOERING, 1980, 1981; ALLISON et al., 1985; YAKIR et al., 1989, 1990; YAKIR, 1992) could lead to marked differences in the δD value obtained from wood cellulose and the isotopic value of the source water that was being used by the plant at the time when wood formation was taking place. In addition, sample collection protocols may further com-

plicate the issue if stem tissues exhibited any evaporative enrichment (see EDWARDS, 1990; DENIRO and COOPER, 1989, 1990; and the following text). Any of these possibilities could account for the deviation in the 1:1 line between source water and leaf cellulose or in the nitrated cellulose δD of tree-rings observed by several investigators (YAKIR, 1992). In this study we show that actively growing nonsuberized or "green" stems can exhibit significant evaporative stem-water enrichment and for this reason can cause problems of interpretation for short-term studies of water uptake and water sources in plants. We also discuss our findings in light of their implications for long-term paleoenvironmental studies which use the hydrogen isotopic analysis of leaf or twig cellulose obtained from macrofossils, where the influence of evaporative stem-water enrichment on data interpretation is obvious and for tree ring studies which use the analysis of cellulose nitrate δD where the implications of our findings are less obvious. For all these studies we recommend that some stem materials be avoided when sampling so that problems caused by evaporative enrichment are minimized.

MATERIALS AND METHODS

Stems were excised at mid-day from mid-canopy lateral branches of a variety of deciduous and coniferous (evergreen) trees ($n = 7$ trees per species) during the most active, leaf expansion, growth phase and later after leaf maturation when stem growth had slowed or ceased and the woody tissues had formed a fully suberized or lignified layer between the outer bark and inner xylem tissues. Mid-day sample collection was used because any isotopic enrichment that might occur in the stems is likely to vary diurnally due to changes in the water vapor pressure gradient between the stem and the air. If isotopic enrichment occurs in stem water, our sampling strategy will reflect the maximum daily enrichment. For one deciduous tree species, *Acer negundo* (box elder), both pre-dawn and mid-day stem collections were made to document the daily minimum and maximum stem water enrichment, respectively. During each sample period, stems were divided into sections from tip to base (stem tips were the youngest tissues). Both stem samples and source-water samples were collected in glass vials and sealed with Parafilm. Samples were kept frozen until the water was extracted.

Water was extracted for 1 h from tissues by cryogenic vacuum distillation. The water samples were reduced to diatomic hydrogen using a reduction reaction with zinc (COLEMAN et al., 1982). A 5 μL subsample and the zinc alloy (obtained from J. Hayes, Indiana University) were sealed in an evacuated Vycor glass tube and then baked at 500°C for 60 min. Raising the reaction temperature to 500°C over the originally described 420°C improved sample precision to ±0.8‰. The diatomic hydrogen resulting from the combustion was analyzed on a Finnagan MAT delta S gas isotope ratioing mass spectrometer (San Jose, CA., USA).

Isotopic composition is expressed in delta notation (‰) as,

$$\delta D = \left[\frac{D/H_{sample}}{D/H_{standard}} - 1\right] \times 1000$$

with SMOW as the standard.

RESULTS AND DISCUSSION

In principle, the isotopic composition of xylem sap should remain constant as water moves into the roots and up through the plant, until the point at which that water is exposed to evaporative enrichment. In woody, perennial plants, the majority of this water-movement pathway through the tree trunk and stems is enclosed by thick, suberized and/or lignified tissues that do not exchange gases with the external atmosphere (STÅLFELT, 1972; KOLATTUKUDY, 1981; SCHÖNHERR, 1982). Isotopic enrichment of xylem-sap should therefore not occur in leaf tissues until there is evaporative gas exchange between the inside of the leaf, at essentially 100% relative humidity, and the atmosphere (less than 100% relative humidity; see DONGMANN and NÜRNBERG, 1974; and FERHI and LETOLLE, 1977). Diffusion of isotopically enriched leaf water in a backward direction towards stem tissues is low or disappears with distance and as such its effect will be minimal primarily because of the relatively long distances involved between leaf blade and stem tissues. The possible influence of advection or diffusion of isotopically enriched water between the phloem, which would also be exposed to evaporation, and the xylem is unknown.

There was a progressive and consistent enrichment of the δD of xylem-sap above the source water values in the most distal stem segments (Fig. 1). The isotopic enrichment above source water values was greater in the most rapidly growing stems (the youngest tissues). Older, mature stems showed little or no isotopic enrichment above source water values (Fig. 1). Stem-water enrichment was also greater in deciduous-leaved species than in evergreen-leaved (coniferous) species. These data clearly demonstrate the possibility of misinterpreting plant water sources if only distal stem segments are sampled, because these stems tissues are not sufficiently suberized to prevent evaporative enrichment. These observations are consistent with the findings of STÅLFELT (1972) who characterized cuticular conductance to water vapor loss from stems of deciduous- and evergreen-leaved species and found that water loss was greater in the deciduous-leaved species, presumably indicating that suberization occurred later in these species when compared to coniferous species. This result was confirmed by HOOK and BROWN (1972). FOOTE and SCHAEDLE (1976) have shown that there can be significant gas exchange in the green bark of 5- to 7-year-old aspen stems, a deciduous-leaved tree (also, see COE and MCLAUGHLIN, 1980). On the other hand, stem gas exchange was much lower in *Pinus radiata*, an evergreen coniferous tree, and those rates decreased as a function of stem distance from the tip (ROOK and CORSON, 1978). Lastly, it is entirely possible that the differences observed between the deciduous- and evergreen-leaved species in the magnitude of isotopic enrichment might be related to differences in the conductive tissues in the xylem; deciduous-leaved species generally have more direct flow paths and higher xylem-sap flow rates that do the coniferous trees used in the present study (ZIMMERMANN and BROWN, 1971).

While the stem-water enrichment can extend as far back from the stem tip as ten leaf nodes (Figs. 1 and 2), the actual stem segment length may be relatively short, on the order of 2–10 cm, depending on the particular species and its growth characteristics. Leaf internode distances tend to be much longer in deciduous-leaved tree species, however, and so stem water enrichment may occur as far back as 1 m from the active growing tip. Rapidly expanding and young stem tissues are known to achieve significant gas exchange (both CO_2 and H_2O; see COMSTOCK and EHLERINGER, 1990, and references therein). Frequently these stems are green and actively involved in photosynthesis. It is only after stem tissues have

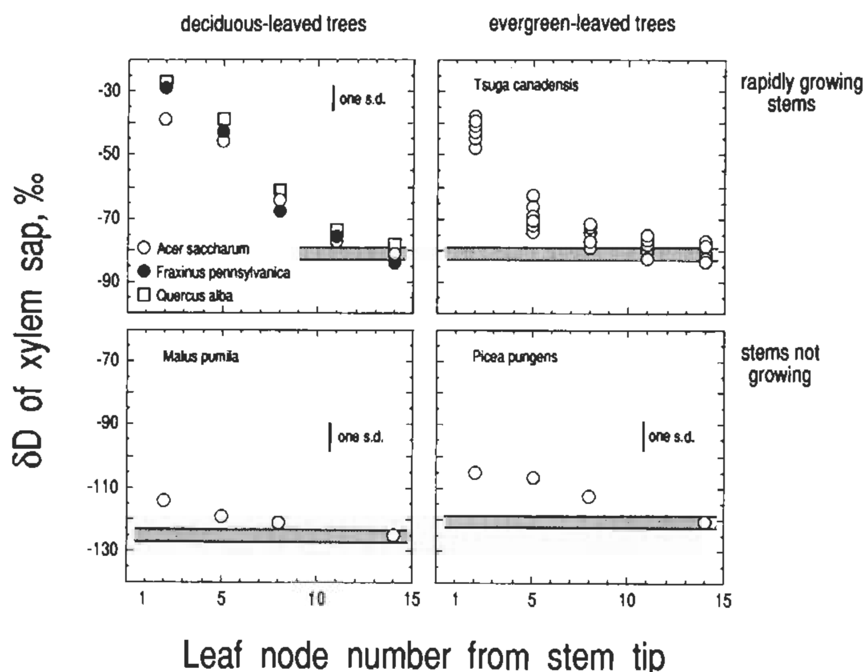

FIG. 1. The stable hydrogen isotopic ratio (δD) of xylem sap (‰) as a function of the number of nodes away from the stem tip in deciduous and coniferous tree species when those stems were growing rapidly (upper two panels) and when these same stems were not undergoing any stem growth or were dormant. The stippled bar on each panel is the δD of source water used by these tree species. Small leaf node numbers are the nonsuberized portions of the stems. Seven trees per species were used. For *Tsuga canadensis* (hemlock) each point represents a single tree. For *Acer saccharum* (sugar maple), *Fraxinus pennsylvanica* (ash), *Quercus alba* (oak), *Malus pumila* (apple), and *Picea pungens* (spruce), points represent the mean for the seven trees sampled; the vertical bar in three of the four panels shows one standard deviation (s.d.) from the sample mean.

matured that epidermal and subepidermal suberization results in stem segments that are no longer exchanging gases with the atmosphere, and therefore its xylem-sap no longer subject to evaporative enrichment (Fig. 1).

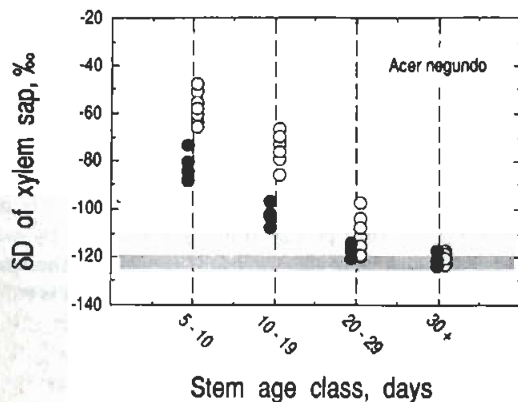

FIG. 2. The stable hydrogen isotopic ratio (δD) of xylem sap (‰) as a function of the stem age class (days old) in the deciduous tree species, *Acer negundo* (box elder). The stippled is the δD of source water used by this species. Young (0–25 days old), actively growing, stems are green and have not yet formed bark or a suberized layer that would restrict water loss and hence isotopic enrichment. Open circles represent mid-day collections, closed circles are pre-dawn collections. The difference between the two symbol types represents the daily variation in stem-water enrichment seen in this species. Symbols have been slightly offset so that they can be more easily seen in areas of overlap.

The developmental maturation of stem tissues is shown in Fig. 2 for *Acer negundo*, a widespread deciduous tree species in the United States. Young, rapidly growing portions of the stem exhibited significant isotopic enrichment of xylem-sap over source water values. The evaporative enrichment decreased with distance away from the proximal tip so that water in stem tissues older than 30 days was not enriched above source water values. This was especially pronounced in stems collected at mid-day (open symbols in Fig. 2). However, in stems collected at pre-dawn, when evaporative enrichment would be expected to be less (because the evaporative gradient is less), significant isotopic enrichment of the stem water was still observed, though it was less than that observed for mid-day stem collections. These data suggest that collecting older stems (with bark) at pre-dawn is the best collection protocol. Presumably, samples collected at mid-day from stem tips not only have a smaller volume of wood (and water) per unit of evaporative surface area, but the flux of water vapor directly out of the stems would be greatest at this time of the day. Details of how stems were sampled for xylem-sap analysis are rarely presented in experimental protocols, yet the data shown here clearly demonstrate that they are likely to have a significant influence on data interpretation. The data in Figs. 1 and 2 indicate that substantial variation in isotopic enrichment of xylem-sap will occur among species, among segments at various distances from the stem tips, and among different aged tissues.

One obvious conclusion that can be drawn from these data is that it is critical to obtain only mature, suberized stem

tissues when sampling stem material for water uptake and/or source water studies. The data in Figs. 1 and 2 were obtained from tree species that typically have several years of growth on a given stem or branch, making it likely that if stems are sampled far enough from the distal end that there will be no isotopic enrichment of the xylem-sap. This is typically not found in herbaceous species, where stem production is commonly an annual phenomenon and those stems may always exhibit some evaporative-based isotopic enrichment. Sampling strategies for herbaceous plants are discussed by DAWSON (1993) and THORBURN and MENSFORTH (1993).

To date, it is not known what the influence of isotopic enrichment in stem water might be on the δD of cellulose, especially the cellulose composing tree rings. The variation that arises in the stable hydrogen isotopic composition of plant carbohydrates such as cellulose is complex and poorly understood (YAKIR, 1992) from either a biochemical or biophysical perspective. Understanding what the final stable hydrogen isotopic composition of cellulose will be should require knowledge of three processes. First, the magnitude of evaporative enrichment in leaf water, the water source directly involved in the process of carbohydrate synthesis (photosynthesis). At this first step, if leaf water enrichment has occurred, we would expect any carbohydrates that were formed also to be enriched in D relative to the water source taken up by plant roots. Secondly, the process of photosynthesis itself can produce carbohydrates with carbon-bound hydrogens depleted in D by 150–200‰ relative to source water (YAKIR and DENIRO, 1990; LUO and STERNBERG, 1991; YAKIR, 1992). Thirdly, postphotosynthetic metabolism, such as when glucose is β-linked into cellulose, can again cause an enrichment in D by 140–180‰ relative to the source water. As such, the final δD of cellulose will depend upon an interaction of these three effects and the balance between biophysical enrichment processes and biochemical fractionation processes caused enzyme-mediated reactions of carbohydrate metabolism. Interestingly, and perhaps fortuitously, there appears to be a "balance" between enrichment and depletion which results in a very good correlation between the δD of cellulose nitrate contained in tree rings and that of the source water being utilized by the tree (YAPP and EPSTEIN 1976, 1977, 1982a,b; BRENNINKMEIJER, 1983; WHITE, 1983; WHITE et al., 1985; KRISHNAMURTHY and EPSTEIN, 1985; EDWARDS and FRITZ, 1986, 1988; T. E. Dawson and J. R. Ehleringer, unpubl. data).

Alternatively, the good correlation between the δD of cellulose nitrate contained in tree rings and that of the source water being utilized by the tree may result because the sugars which are translocated to the sites of cellulose synthesis in the tree trunk are being linked in the presence of the source water that has not yet been exposed to biophysical or biological fractionation processes. DENIRO and COOPER (1989) evaluated cellulose $\delta^{18}O$ in stems newly formed from potato tubers (see also COOPER and DENIRO, 1989a,b). While they observed a 1:1 correspondence between stem cellulose and tuber water $\delta^{18}O$ values, the water/cellulose fractionation factor they observed (30.5‰) was greater than that expected (27‰) (STERNBERG et al., 1986, 1984a,b). They concluded that the difference between the observed and expected isotopic values was likely due to the fact that a portion of the tuber starch $\delta^{18}O$ signal may have been retained and then incorporated into stem cellulose during its formation. In nature, similar water/cellulose fractionation could also occur for either oxygen or hydrogen stable isotopes, or it is plausible that water in stems could have become enriched by the process of evaporative water loss through nonsuberized tissues such as shown here (also see DENIRO and COOPER, 1990, and EDWARDS, 1990).

With regards to other types of long-term studies other than those which use tree ring analyses, the findings we present here may have very important ramifications, especially for paleoenvironmental or past vegetational reconstructions. For example, our data suggests that the use of fossil leaf and stem materials (macrofossils) contained in packrat middens must be evaluated with caution. A recent review by LONG et al. (1990) has advocated the analysis of δD in fossil twigs and leaves obtained from packrat middens as a method for reconstructing mean annual or mean growing season temperatures in the Southwestern United States during the past 8,000 to 30,000 years. This information is then correlated with other macrofossil changes preserved in the middens (e.g., species presents and abundance) as a means for determining the speed and nature of vegetational change in relation to climate (temperature) change. A potential source of error not considered in studies of fossil leaves and twigs is that which could arise from evaporative enrichment of water (such as shown here) in these tissues that is then incorporated and "recorded" in the cellulose. Our data show that isotopic enrichment of 40–80‰ is not uncommon in small twigs or leaves (also see YAKIR et al., 1989, 1990). If we use DANSGAARD's (1964) estimate of a 5.6‰ change per °C (from the δD of modern precipitation), this would mean a 7–14°C error in the temperature estimated from fossil leaf or twig materials with cellulose having been exposed to evaporative enrichment processes. LONG et al. (1990) do advocate collecting modern plant samples to correct for possible sources of error associated with past changes in air-mass trajectories, atmospheric humidity (which would influence the degree of evaporative enrichment in stem water), or other atmospheric variables. We would add to this list and advocate the collection of older, suberized, stems and *not* leaf material. Suberized stem samples are discernible if macrofossil twigs where thin sectioned (ESAU, 1977).

In conclusion, our data show that one must use only suberized stems to prevent potential problems caused by evaporative enrichment and also measure the δD of the water contained in the xylem-sap rather than assuming it is equivalent to that of the incoming precipitation or other surface water sources. The implications of not employing such a protocol will have important ramifications for water plant–water source studies. The implications of stem-water enrichment processes for long-term studies which use tree-ring $\delta^{18}O$ or δD analyses from cellulose is unknown. Our data suggest that a detailed analysis is warranted of how stem-water enrichment influences the isotopic value of carbohydrates during transport and at the sites of cellulose synthesis.

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