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# Atmospheric $CO_2$ enrichment can increase the $^{18}O$ content of leaf water and cellulose: paleoclimatic and ecophysiological implications

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ABSTRACT: The $\delta^{18}O$ values of daytime total leaf water and leaf cellulose in yellow-poplar *Liriodendron tulipifera* (L.) (Magnoliaceae) were elevated approximately 2‰ when exposed to ambient + 300 µmol $mol^{-1}$ $CO_2$ in outdoor open-top chambers relative to ambient $CO_2$ concentrations. In growth chambers, $\delta^{18}O$ values of total leaf water during the light cycle were also enriched in a drought-stressed, 700 µmol $mol^{-1}$ $CO_2$ atmosphere treatment with limited changes in humidity. In both experiments, no significant differences were observed among treatments in δD values of leaf water in ambient or elevated $CO_2$. Simultaneous isotopic measurements made of *Quercus alba* (L.), also growing in the same open-top chambers, did not show any significant difference among treatments in either $\delta^{18}O$ or δD values in leaf water for ambient, ambient + 150 or ambient + 300 µmol $mol^{-1}$ $CO_2$ treatments. Differences between the species that may be important to this variable response include apparent longer water-turnover times in *L. tulipifera* that were inferred from stomatal conductance and leaf water content measurements. The increases in $\delta^{18}O$ values of leaf water in *L. tulipifera* without parallel increases in δD values indicate that elevated $CO_2$ treatments can affect the kinetics of leaf water evapotranspiration, for which $H_2{}^{18}O$ evaporation is more sensitive than $DH^{16}O$. The apparent long-term incorporation of this increase in $\delta^{18}O$ values of daytime total leaf water into leaf cellulose of *L. tulipifera* may introduce a new complication into the use of stable isotopes of fossilized plant fibers as paleoclimatic indicators because of the long-term variation in atmospheric $CO_2$ content.

KEY WORDS: Oxygen isotopes · Carbon dioxide enrichment · Hydrogen isotopes · Isotopic content of leaf water · Isotopic content of cellulose · Paleoclimate

## INTRODUCTION

The isotopic fractionation of water by plants during transpiration was among the first biological isotopic effects observed (Washburn & Smith 1934). This increase in the concentration of the rarer, stable isotopes of deuterium (D) and $^{18}O$ in leaf water is important to climate change analyses because of the growing efforts to use stable oxygen and hydrogen isotopes of plant organic matter as proxy paleoclimatic indicators (Edwards et al. 1985, Luckman et al. 1985, Ramesh et al. 1986, 1989, Edwards & Fritz 1988, Van Geel & Middledorf 1988, Betancourt et al. 1990, Epstein & Krishnamurthy 1990, Lipp et al. 1991, Swart 1992, MacDonald et al. 1993). In addition it has been suggested that the isotopic content of leaf water modifies the stable isotope composition of continental water vapor and subsequent precipitation (Bariac 1988, Bariac et al. 1990, 1991) and that leaf water oxygen isotope content specifically influences the oxygen isotope content of atmospheric carbon dioxide (Francey & Tans 1987, Yakir et al. 1989, Berry 1992, Farquhar et al. 1993) and atmospheric oxygen (Dongmann et al. 1972, Sowers et al. 1991 and references therein). Consequently the processes influencing the stable isotope composition of leaf water may be important for understanding global water, carbon dioxide and oxygen cycles.

Although it has been shown that the oxygen isotopic content of atmospheric carbon dioxide has little direct influence on the oxygen isotopic composition of either leaf water or cellulose (DeNiro & Epstein 1979), increasing atmospheric carbon dioxide concentrations may have significant indirect effects on heavy stable

isotope enrichment in leaf water. This would result from expected decreases in transpiration and increases in water use efficiency by plants as $CO_2$ levels increase (Eamus & Jarvis 1989). Despite speculation that there will be no significant change in deuterium content of leaf water as $CO_2$ levels increase (Epstein & Krishnamurthy 1990), this issue has been only peripherally studied so far (Yakir & DeNiro 1990).

The processes leading to concentration of the heavy isotopes $^{18}O$ and D in leaf water are similar in many respects to evaporation that alters the isotopic composition of terrestrial surface waters, but surface water evaporation models do not predict accurately isotopic enrichment for either $^{18}O$ or D in leaf water (Farris & Strain 1978, Leaney et al. 1985, Bariac et al. 1987, Flanagan et al. 1991a, b, Walker & Lance 1991). This is a consequence of at least 2 elements of evaporation from plants that prevent simple application of hydrological models of evaporation, and both of these factors may be influenced by increased $CO_2$ concentrations. First, unlike evaporating surface water, the total water pool within plants is thought to be compartmentalized, and consists of relatively unfractionated vascular water as well as water influenced by transpiration (Lesaint et al. 1974, Ferhi et al. 1983, Leaney et al. 1985, Yakir et al. 1989, Flanagan et al. 1991a). Second, the kinetic isotopic fractionation controlling the irreversible loss of water from sites of evaporation in the leaf and through the boundary layer surrounding the leaf is highly variable spatially and temporally. Increased $CO_2$ concentrations could change proportions of water subject to evapotranspiration, and through decreased stomatal conductance, increase the effective kinetic path controlling irreversible loss of water from sites of evaporation in the leaf. Lower stomatal densities with increasing $CO_2$ concentrations might also lead to the same general result.

Both water compartmentalization and kinetic isotopic fractionation potentially influence total leaf concentrations of $H_2{}^{18}O$ and $DH^{16}O$. Meteoric water taken up by plants is derived from precipitation condensed under isotopic exchange conditions that approach equilibrium, and most terrestrial precipitation clusters along a least square fit line described by the equation $\delta D = 8\,\delta^{18}O + 10‰$ (Craig 1961), where $\delta^{18}O$ or $\delta D = [(R_{sample}/R_{SMOW}) - 1] \times 1000$ per mil; R is $^{18}O/^{16}O$ or D/H; and SMOW is Standard Mean Ocean Water (Gonfiantini 1961) (distributed by the International Atomic Energy Agency).

Total leaf water includes an uncertain fraction of this meteoric water as well as other waters that have been subject to evapotranspiration under non-equilibrium conditions. During evapotranspiration, kinetic processes are also significant. The eddy and molecular diffusion coefficients affecting evaporative enrichment of water mixtures containing $DH^{16}O$ are small relative to equilibrium controlled enrichment of deuterium. By contrast heavy isotope enrichment of $H_2{}^{18}O$ is more sensitive to kinetic processes (Gat 1971). As a result, in plant waters subject to transpiration, the ratio of $H_2{}^{18}O$ to $DH^{16}O$ increases and the slope $m$, in the relationship $\delta D = m\,\delta^{18}O + b$, declines from the 8 of meteoric water to slopes ranging from 1.3 to 4.9 (Bricout et al. 1972, Lesaint et al. 1974, Allison et al. 1985, Sternberg et al. 1986, Cooper & DeNiro 1989, Cooper et al. 1991). There is disagreement over the relative importance of kinetic isotopic fractionation and water compartmentalization in total heavy isotopic enrichment. For example, it has been estimated that the fraction of wheat leaf water subject to heavy isotope enrichment can be as low as 40 to 60%, depending upon stress treatment (Walker et al. 1989). Although mixing of isotopically unfractionated waters with plant water subject to evapotranspiration could result in significant modification of coefficients in the relationship $\delta D = m\,\delta^{18}O + b$ for total leaf water, these estimates of isotopically fractionated water have been criticized as unreasonably low (Flanagan et al. 1991b). This criticism is based upon the structural volume of leaf vascular tissue carrying presumably isotopically unfractionated water (~5%). Nevertheless, as pointed out previously, heavy isotopic enrichment in leaf water still cannot be satisfactorily explained by our current estimates of the magnitude of kinetic processes either.

Therefore, in planning our studies of the response of leaf water heavy isotope enrichment to elevated carbon dioxide, we anticipated that controlled experiments could provide information for applications of stable isotope methodologies in paleoclimatology and biogeochemical cycling, as well as the more basic plant physiological questions associated with fractionation of the isotopes of hydrogen and oxygen during transpiration.

Following completion of these measurements, we analyzed a subset of leaf cellulose samples to see if the short-term patterns of leaf water heavy isotope enrichment patterns we observed influenced the oxygen isotope composition of leaf cellulose synthesized over the growing season. This work was pursued because initial results indicated that in some circumstances, oxygen isotope ratios in leaf water were affected by $CO_2$ concentrations. These findings, while useful for plant physiological studies, are most directly important for interpretation of stable oxygen isotope variability in preserved plant materials such as cellulose.

## METHODS

**Growth chamber experiment.** Twelve dormant *Liriodendron tulipifera* (L.) seedlings (yellow-poplar,

family: Magnoliaceae, approximately 15 cm height) were planted in pots (15 cm diameter by 18 cm) 2 mo before the experimental sampling. After buds opened, the seedlings were placed on 23 March 1990 in 2 separate, temperature- and humidity-controlled growth chambers, one chamber with ambient and the other flushed continually with 700 µmol $mol^{-1}$ concentrations of $CO_2$. A 12 h photoperiod was used during each 24 h period; photosynthetic photon flux density at the top of the plant canopy was 675 µmol $m^{-2}$ $s^{-1}$. A drought treatment was initiated on half of the seedlings in each chamber on 23 April 1990; after this date, they were not watered, while the wet treatment plants were watered 3 times per week. The water used for irrigation was from a homogeneous source and had the following isotopic composition: $\delta D = -40‰$ and $\delta^{18}O = -8.7‰$, which is within the observed range of local meteoric groundwater (Dreier et al. 1989, Shevenell 1993, L. W. Cooper unpubl. data).

Leaves were sampled over a 24 h period 8 times, including day and night cycles on 3–4 May 1990. Each sample consisted of 1, 2, or most commonly 3 leaves (~5 g fresh weight) from separate seedlings.

**Open-top field chamber experiment.** The saplings we used for both ambient and elevated $CO_2$ treatments were being grown directly in the ground as part of a longer-term study of productivity and compensatory responses of *Liriodendron tulipifera* and white oak *Quercus alba* L. to elevated $CO_2$ (Norby et al. 1992, Gunderson et al. 1993). Our sampling was accomplished on 30–31 August 1990, towards the end of the second growing season in which the trees had been exposed day and night during the growing season to 3 $CO_2$ concentrations: ambient (354.5 µmol $mol^{-1}$), ambient + 150 (502.9 µmol $mol^{-1}$), and ambient + 300 (655.6 µmol $mol^{-1}$). The trees, germinated from seed under the experimental $CO_2$ levels, were growing in soil within open-top chambers, exposed to essentially the same ambient light, temperature, humidity, precipitation, and soil conditions, with no fertilizer or supplemental water. The open-top chambers used were 3 m diameter aluminum-framed cylinders, covered with PVC film (Rogers et al. 1983). Axial fans (560 W) provided a flow rate of 70 $m^3$ $min^{-1}$, or 4 complete air changes per minute. The $CO_2$ concentration in each chamber was monitored continuously (AR-8000 multistation; Anarad, Inc., Santa Barbara, CA, USA) using a non-dispersive infrared gas analyzer, with microprocessor controlled signal processing and data storage. Calibration of the gas analyzer was accomplished as described in Gunderson et al. (1993).

Leaves were sampled over a 24 h period 7 times, from 09:00 h local time on 30 August 1990 to 09:00 h the following morning, with 2 or 3 leaves (~5 g fresh weight) selected for each isotopic analysis. Each separate, fully expanded leaf was selected from a separate tree growing in each treatment. For the *Liriodendron tulipifera* saplings, leaves selected were from branches, 5 or 6 leaves from the meristem, so all of the leaves sampled were mature and approximately the same physiological age. For *Quercus alba* saplings, which produce leaves in recurrent flushes, only new flushes of leaves of similar age were sampled.

**Sample treatment.** For each leaf sampled for leaf water isotopic content, in both experiments, leaf fresh weight and leaf area were measured quickly using an electronic balance and area meter (LI-3100 Area Meter; Li-Cor, Inc.). Leaves were then sealed within 25 mm o.d. test tubes with a rubber septum wrapped in Parafilm™ and frozen prior to cryogenic distillation (Cooper & DeNiro 1989). Following cryogenic distillation of leaf water for isotopic analyses, dry weights of leaves were determined. A soil-saturating rainfall (16 mm) fell on the evening of 29 August, and served as a likely partial or predominant source water for the leaves sampled. This rainfall had an isotopic composition of −15‰ (δD) and −3.9‰ ($\delta^{18}O$), which is somewhat more enriched than average local groundwater (δD ca −30‰ and $\delta^{18}O$ ca −7‰; Dreier et al. 1989, Shevenell 1993, L. W. Cooper unpubl. data).

**Physiological measurements.** Concurrent environmental and physiological measurements in both experiments included photosynthetic photon flux density, leaf and air temperature, relative humidity, transpiration rate, and stomatal conductance using a steady-state porometer (LI 1600, Li-Cor, Inc.).

**Isotopic analyses.** Leaf water oxygen isotope ratios were determined by equilibrating 0.5 to 1.0 ml of cryogenically distilled plant water samples with approximately 300 µmol $CO_2$ for 48 h, purifying the equilibrated $CO_2$ cryogenically, analyzing the $CO_2$ mass spectrometrically, and using mass balance considerations to calculate the original oxygen isotope composition of the sampled water (Epstein & Mayeda 1953). Hydrogen isotope ratios were determined using 2 methods: for the growth chamber studies, by passing approximately 10 µl of water over uranium metal heated to 700 °C, which released hydrogen gas that was collected with a uranium hydride pump (Bigeleisen et al. 1953, Friedman & Hardcastle 1970); for the open-top chamber studies, by reacting 3 µl of water with zinc alloy (source: Geochemistry Laboratories, Indiana University) loaded within a sealed manifold under an argon atmosphere. These water samples were reacted in a sealed Pyrex tube at 500 °C for 0.5 h, followed directly by mass spectrometric measurement of the hydrogen gas generated. Collections for isotopic analyses were also made of water vapor in both the growth chamber and open-top chamber experiments within 1 cm of leaf surfaces using a molecular sieve

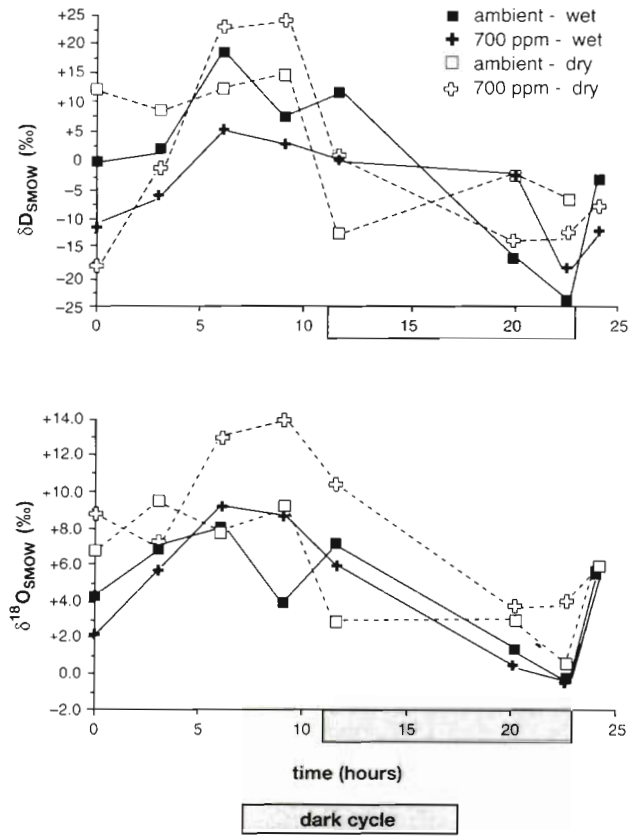


Fig. 1. *Liriodendron tulipifera*. Stable isotope composition of leaf water of growth chamber-grown samples, 4–5 May 1990, under wet and dry conditions and ambient and 700 µmol $mol^{-1}$ $CO_2$

pump activated by liquid nitrogen (Cooper et al. 1991). Corrections to $\delta^{18}O$ values were made for the small volumes of water analyzed (100 µl), by using matched-volume machine standards (Cooper et al. 1991).

Following completion of the analyses of the leaf water samples, we chose to assay a subset of dried leaf samples for organic $^{18}O$ content to see if short-term $^{18}O$ enrichment patterns observed over the diurnal period of the experiments were integrated into the $\delta^{18}O$ values of leaf cellulose synthesized over the growing season. The leaves of *Liriodendron tulipifera* saplings (ambient and + 300 µmol $mol^{-1}$ treatment) collected 7 times over the 24 h period of 30–31 August 1990 were used as independent samples of leaf organic matter. Leaf tissue without major veins was used. Cellulose was extracted using a sodium chlorite-acetic acid oxidation procedure (Wise 1944). Vacuum-dried samples were then pyrolized in the presence of mercuric chloride at 520 °C for 5 h to form CO, $CO_2$, and HCl. CO was converted to $CO_2$ by high voltage discharge and HCl was removed by reaction with isoquinoline, followed by mass spectrometric measurement of the purified $CO_2$ (Epstein et al. 1977).

Precisions (± SD), based upon repeated mass spectrometric determinations of secondary water standards analyzed concurrently with the samples, were found to be ± 0.1‰ for leaf water $\delta^{18}O$ values and ± 2‰ for δD values determined using the uranium method and ± 1‰ for samples determined using the zinc method. Precision for the the cellulose $\delta^{18}O$ values, based upon concurrent pyrolysis of a secondary cellulose standard, was found to be ± 0.3‰. Our laboratory utilizes a Fisons Instruments/VG SIRA Series II dual inlet, triple beam collector mass spectrometer. Laboratory gas samples calibrated to SMOW were used as reference gases.

## RESULTS

### Growth chamber experiment

**Isotopic measurements.** Leaf water of *Liriodendron tulipifera* seedlings showed diurnal enrichment in both $^{18}O$ and D (Fig. 1). Heavy isotope enrichment was greatest for $^{18}O$ and D in the drought-stressed, 700 µmol $mol^{-1}$ treatments, although it should be noted that δD values were only slightly higher than in the 2 well-watered treatments and the $\delta^{18}O$ values of leaf water in the drought-stressed treatment were initially higher than all other treatments as the experiment started.

**Physiological measurements.** Leaf conductance was lower in the droughted seedlings, especially in elevated $CO_2$ (Fig. 2). This trend was reflected in the integrated whole-plant water use over the course of the experiment (Table 1). Leaf water content did not differ

Table 1 *Liriodendron tulipifera*. Water use characteristics of seedlings in growth chambers. Leaf conductance data are means (± SD) of measurements plotted in Fig. 2. Whole-plant water use data are means (± SD) of 3 plants per treatment over a 24 h period. Leaf water content data are means (± SD) of 8 leaf collections over the 24 h experiment

| Treatment | Leaf conductance ($cm\ s^{-1}$) | Whole-plant water use ($g\ m^{-2}$) | Leaf water content ($g\ H_2O\ g^{-1}$ dry wt) |
|---|---|---|---|
| **Well-watered** | | | |
| Ambient $CO_2$ | 0.39 ± 0.18 | 782 ± 118 | 2.53 ± 0.51 |
| Elevated $CO_2$ | 0.45 ± 0.10 | 767 ± 66 | 3.16 ± 1.26 |
| **Droughted** | | | |
| Ambient $CO_2$ | 0.39 ± 0.10 | 687 ± 85 | 2.93 ± 0.32 |
| Elevated $CO_2$ | 0.20 ± 0.09 | 395 ± 88 | 3.07 ± 1.08 |

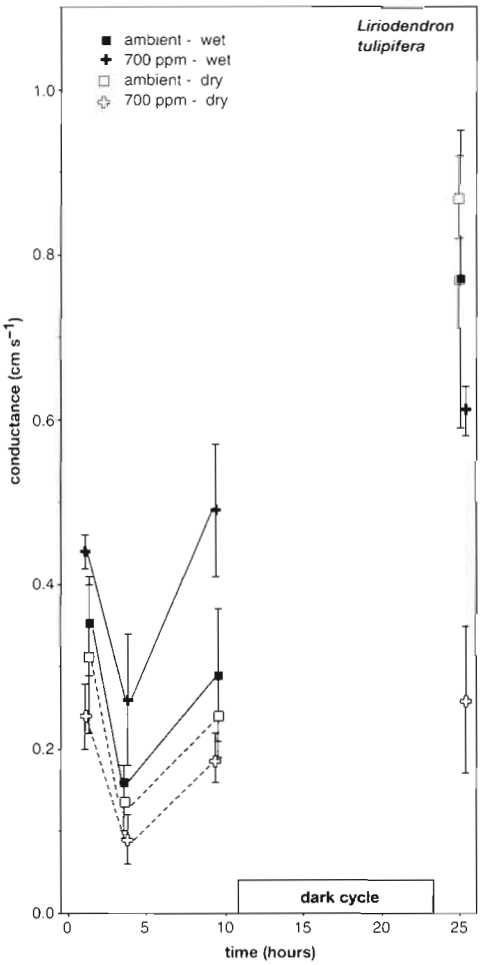


Fig. 2. *Liriodendron tulipifera*. Leaf conductances for growth chamber-grown samples, 4–5 May 1990, under wet and dry conditions and ambient and 700 µmol mol$^{-1}$ $CO_2$

significantly between treatments (Table 1), implying a longer average residence time for water in the droughted, $CO_2$-enriched seedlings. There were no differences in leaf water potential, measured with a Schollander pressure chamber (data not shown). Physical conditions during the experiment are outlined in Table 2.

### Open-top field chamber experiment

**Water isotopic measurements.** Leaf water of *Liriodendron tulipifera* saplings showed strong diurnal enrichment in both $^{18}O$ and D (Fig. 3). Enrichment of $^{18}O$ in the ambient + 300 µmol mol$^{-1}$ treatment was consistently higher than other treatments, although there was no consistent pattern for δD values of leaf water among the 3 treatments. Patterns of leaf water heavy isotope enrichment in *Quercus alba* resembled patterns in *L. tulipifera*, including the extent of daily change in isotopic composition, as well as the actual isotopic composition measured (Fig. 4), but no treatment resulted in consistently greater heavy isotope enrichment.

**Cellulose isotopic measurements.** Cellulose extracted from the dried leaves of *Liriodendron tulipifera* (+300 µmol mol$^{-1}$ treatment) collected 7 times over the 24 h period of 30–31 August 1990 had $\delta^{18}O$ values significantly higher than cellulose extracted from the corresponding 7 ambient samples (Table 3; 1-tailed *t*-test; $p < 0.01$). Mean $\delta^{18}O$ values were 2‰ enriched in the ambient + 300 µmol mol$^{-1}$ treatment ($\delta^{18}O$ = +27.5‰) relative to the ambient treatment ($\delta^{18}O$ = +25.5‰), which was similar to the mid-day short-term difference we observed in leaf water isotopic content between the 2 treatments.

**Physiological measurements.** Leaf conductance declined with increasing $CO_2$ concentration in both species (Fig. 5). Leaf water content was lower in *Quercus alba* than in *Liriodendron tulipifera* and tended to decrease with increasing $CO_2$ concentration in both species (Table 4). However, water content declined proportionally less than conductance, and assuming that transpiration was proportional to conductance, this indicates that the average residence time of leaf water was longer in *L. tulipifera* than in *Q. alba*, and longer in elevated $CO_2$ than in ambient $CO_2$ treat-

Table 2. Growth chamber physical conditions during experiment, 4–5 May 1990

| | |
|---|---|
| Light cycle air temperature range (observed in both chambers) | 24.0–26.6 °C |
| Light cycle leaf temperature range (observed in both chambers) | 25.0–27.4 °C |
| Night cycle air temperature range (observed in both chambers) | 16.2–17.6 °C |
| Night cycle leaf temperature range (observed in both chambers) | 16.1–17.7 °C |
| Light cycle relative humidity range (observed in both chambers) | 80–90 % |
| Night cycle relative humidity (observed in both chambers) | 86–96 % |
| Day air vapor δ values, ambient, 3:45 to 5:45 h after experiment start | −83‰ (δD)<br>−14.4‰ ($\delta^{18}O$) |
| Day air vapor δ values, 700 µmol $CO_2$ mol$^{-1}$, 7:40 to 9:00 h after experiment start | −67‰ (δD)<br>−11.3‰ ($\delta^{18}O$) |
| Night air vapor δ values, ambient, 22:00 to 23:00 h after experiment start | −86‰ (δD)<br>−10.6‰ ($\delta^{18}O$) |
| Night air vapor δ values, 700 µmol $CO_2$ mol$^{-1}$, 20:00 to 22:00 h after experi ment start | −80‰ (δD)<br>−14.9‰ ($\delta^{18}O$) |

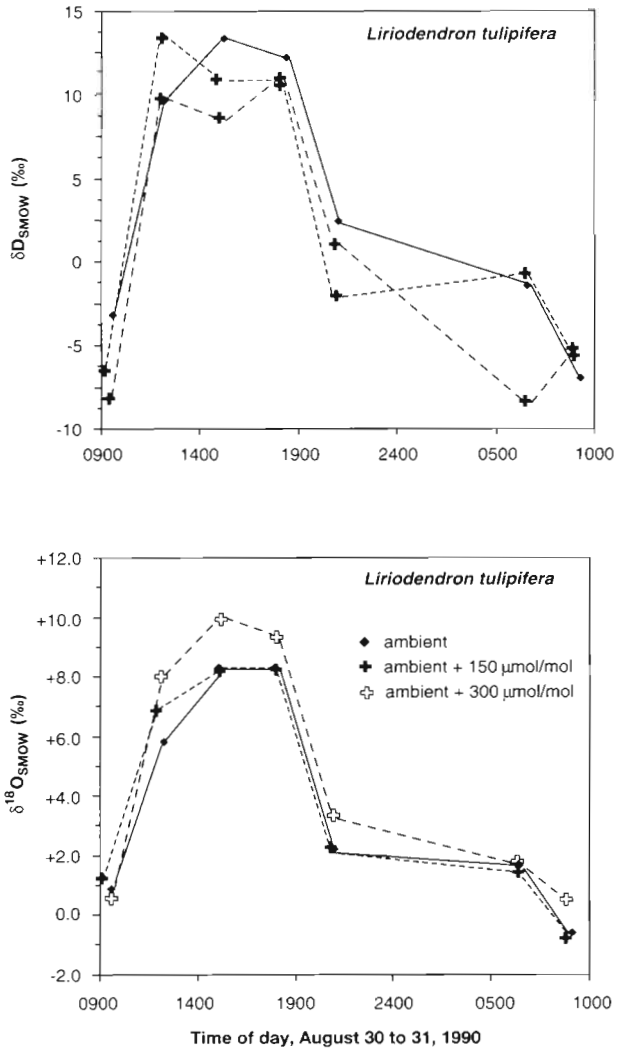


Fig. 3. *Liriodendron tulipifera*. Stable isotope composition of leaf water of samples, 30–31 August 1990, grown in the ground within open-top chambers under ambient, ambient + 150 and ambient + 300 µmol mol$^{-1}$ $CO_2$

Table 3. *Liriodendron tulipifera*. $\delta^{18}O$ values (‰) of cellulose extracted from de-veined leaves (ambient $CO_2$ and ambient + 300 µmol mol$^{-1}$ $CO_2$ treatments) used for leaf water isotopic measurements, open-top experiment, 30–31 August 1990

| | Ambient $CO_2$ treatment | + 300 µmol mol$^{-1}$ treatment |
|---|---|---|
| | +26.83 | +25.76 |
| | +26.11 | +27.76 |
| | +23.64 | +28.84 |
| | +25.22 | +28.82 |
| | +26.53 | +25.47 |
| | +24.07 | +27.59 |
| | +26.27 | +27.92 |
| mean ± SD: | +25.5 ± 1.3 | +27.5 ± 1.3 |

ments. Leaf water potential (pre-dawn and midday) did not differ significantly between species (not shown). Physical conditions during the experiment are outlined in Table 5.

## DISCUSSION

Plant physiological responses to increased $CO_2$ concentration are a complex of direct, indirect, and interacting effects. A separate study of the growth response of the *Liriodendron tulipifera* saplings used in our open-top chamber experiment has shown that despite

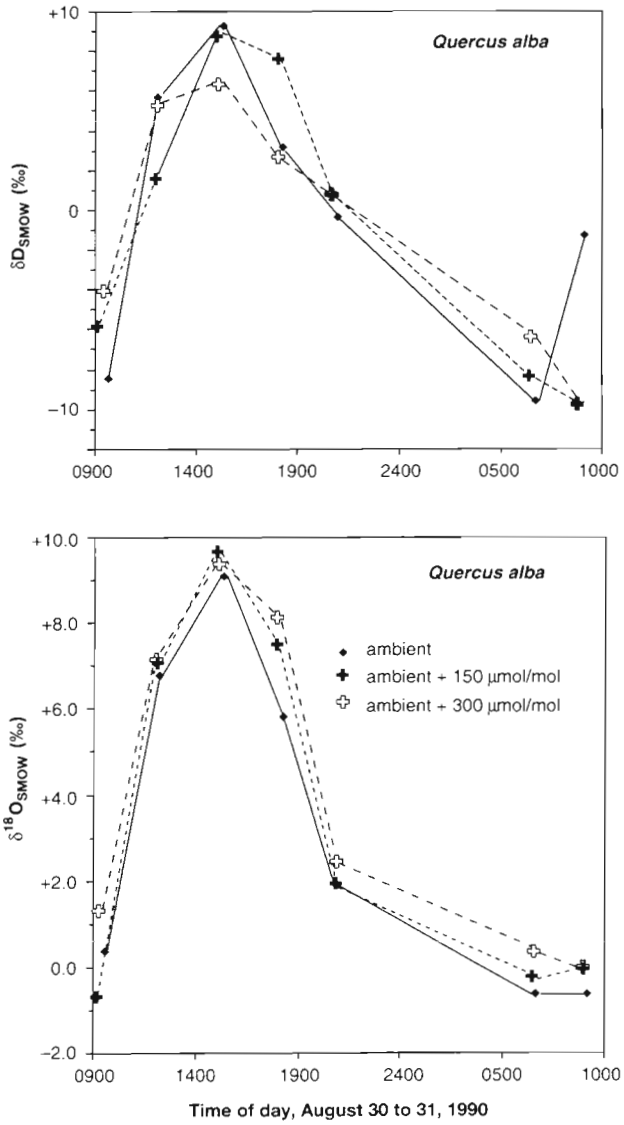


Fig. 4. *Quercus alba*. Stable isotope composition of leaf water of samples, 30–31 August 1990, grown in the ground within open-top chambers under ambient, ambient + 150 and ambient + 300 µmol mol$^{-1}$ $CO_2$

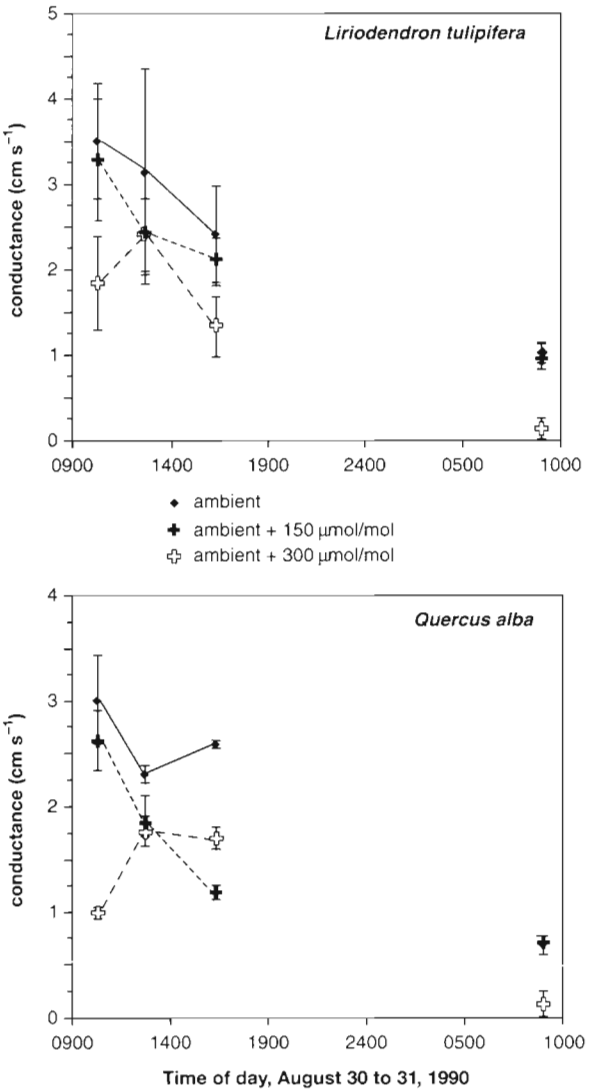


Fig. 5. *Liriodendron tulipifera* and *Quercus alba*. Leaf conductances of samples, 30–31 August 1990, grown in the ground within open-top chambers under ambient, ambient + 150 and ambient + 300 µmol mol$^{-1}$ $CO_2$

Table 4. *Liriodendron tulipifera* and *Quercus alba*. Water use characteristics of saplings growing within open-top field chambers. Leaf conductance data are the means (± SD) of measurements of 3 leaves per treatment. Leaf water content data was measured at 7 times during the 24 h experiment

| Species | $CO_2$ enrichment (µmol mol$^{-1}$) | Leaf conductance (cm s$^{-1}$) | Leaf water content (g $H_2O$ g$^{-1}$ dry wt) |
|---|---|---|---|
| *L. tulipifera* | Ambient | 2.52 ± 1.10 | 2.11 ± 0.18 |
| | +150 | 2.22 ± 0.96 | 2.07 ± 0.17 |
| | +300 | 1.49 ± 0.86 | 3.16 ± 1.26 |
| *Q. alba* | Ambient | 2.15 ± 1.02 | 1.18 ± 0.11 |
| | +150 | 1.60 ± 0.84 | 1.17 ± 0.06 |
| | +300 | 1.23 ± 0.64 | 1.02 ± 0.08 |

Table 5. Open-top chamber physical conditions during experiment, 30–31 August 1990

| | |
|---|---|
| Air temperature range (observed in all 3 chambers) | 21.4–35.8 °C |
| Leaf temperature range (observed in all 3 chambers) | 21.5–35.6 °C |
| Relative humidity range (observed in all 3 chambers) | 53–92 % |
| Day air vapor δ value (10:30 to 13:30 in ambient chamber) | −64‰ (δD)<br>−11.6‰ ($\delta^{18}O$) |
| Night air vapor δ value (23:00 to 05:30 in ambient chamber) | −85‰ (δD)<br>−9.2‰ ($\delta^{18}O$) |

a sustained increase in leaf-level photosynthesis in the elevated $CO_2$ treatments, whole plant carbon storage did not increase significantly (Norby et al. 1992). Instead, a relative decrease in leaf production was balanced by increased carbon allocation to fine root production.

The patterns of leaf-water heavy-isotope enrichment we observed in response to elevated $CO_2$ also appear to share this complexity. No significant differences in heavy-isotope enrichment patterns in open-top chamber *Quercus alba* were observed regardless of $CO_2$ concentration. By contrast, for *Liriodendron tulipifera* in both open-top and drought-stressed chamber experiments, $^{18}O$ content of leaf water was elevated. The increase in both deuterium and $^{18}O$ enrichment in the drought stressed treatment in the chamber experiment is not surprising, but the difference in $\delta^{18}O$ values (but not δD values) in the outdoor open-top chambers exposed to identical physical conditions cannot be explained by different levels of drought stress. The most likely explanation for this effect is an increase in overall kinetic isotopic fractionation effects caused by greater stomatal resistance to water loss from sites of evaporation within the leaf into the atmosphere as $CO_2$ concentration increased. As discussed in the introduction, $^{18}O$ enrichment is more sensitive to this effect than deuterium. Of course at some high concentration of $CO_2$, δD values should also increase in response to these changes, although only the additional $^{18}O$ enrichment was apparent in the open-top chamber experiment. Another alternate, but not necessarily mutually exclusive way of describing this physiological change would be an increase in the proportion of stomatal resistance relative to total resistance. This effect is consistent with our measurements of leaf conductance in

*L. tulipifera*, which declined with increasing $CO_2$ concentrations.

Following these observations of increases in $\delta^{18}O$ values in leaf water, we also observed significantly higher $\delta^{18}O$ values of leaf cellulose for *Liriodendron tulipifera* leaves subjected to the ambient + 300 µmol $mol^{-1}$ treatment (Table 3). These results may have implications for paleoclimatic studies using the stable hydrogen and oxygen isotope compositions of cellulose or other preserved plant materials. In particular it appears that the oxygen isotope composition of leaf water and any organic material synthesized in relation to it can have an elevated heavy isotope composition in $CO_2$-enriched atmospheres as an indirect effect of altered stomatal conductance and transpiration. Models indicate that atmospheric carbon dioxide levels have varied greatly over geological time, with an order of magnitude greater concentration present during the early Paleozoic (Berner 1990). More relevant for most applications of the stable hydrogen and oxygen isotope composition of organic matter is that no stable isotope measurements of leaf water have been made using plants grown exposed to $CO_2$ concentrations as low as those associated with the last glacial maximum (180 to 200 µmol $mol^{-1}$). $\delta^{18}O$ values of fossil wood cellulose from southern Ontario, Canada, may have decreased as much as 10‰, over the past 11 000 yr, as a result of increases in average humidity, although this decrease has likely been partly offset by increases in the $^{18}O$ content of meteoric water (Edwards & Fritz 1986). $\delta^{18}O$ variability in cellulose of a smaller magnitude has also been observed for cellulose extracted from peat (Brennikmeijer et al. 1982). The decrease in fossil wood cellulose $\delta^{18}O$ values during a period of increasing $CO_2$ concentration shows a trend opposite to what we observed for the isotopic composition of leaf water of *L. tulipifera* for the +300 µmol $mol^{-1}$ treatment (higher $\delta^{18}O$ values associated with highest $CO_2$ treatment). This indicates that any effect from $CO_2$ increases on heavy isotope enrichment due to changes in stomatal conductance may be smaller than other changes caused by humidity and other climatic factors. Similarly, the effects of $CO_2$ concentration on leaf conductance are smaller than seasonal variation in conductance (Gunderson et al. 1993). However, the increase in humidity over time inferred from the Ontario wood samples could be an underestimate if the effects observed in our experiment are significant relative to all other factors influencing the oxygen isotope composition of cellulose. Although data on natural variations in oxygen isotope composition of organic matter are not extensive, the importance of relative humidity relative to $CO_2$ concentration is also suggested by the work of Sternberg et al. (1989), who observed lower, rather than higher, leaf cellulose $\delta^{18}O$ values in a tropical forest understory, where $CO_2$ concentrations and humidity are both higher than in the canopy. Here, as in the fossil cellulose samples, it appears that any heavy isotope enrichment due to higher $CO_2$ concentrations is counteracted and possibly masked by humidity effects.

Although the heavy enrichment patterns in leaf water may have implications for paleoclimatic studies, it is not clear why the leaf water of *Liriodendron tulipifera* showed an apparently kinetically driven response to increasing $CO_2$ concentrations while *Quercus alba* did not. Leaf water content of *L. tulipifera* was significantly higher than in *Q. alba* (Table 4), while typical leaf conductances were somewhat lower for *Q. alba* than for *L. tulipifera* (Table 4, Fig. 5). This suggests that water turnover time was longer in *L. tulipifera* and longer at higher $CO_2$ concentrations. Longer water turnover time would lead to less dilution of heavy isotope-enriched leaf water by soil water sources, but this would affect both $\delta D$ and $\delta^{18}O$ values, so this lowered dilution was not apparently significant in our experiment. Consistent with physical models of water evaporation (e.g. Craig & Gordon 1965, Dongmann et al. 1974), the range of heavy-isotope $\delta D$ and $\delta^{18}O$ values observed in each species were similar. For example, $\delta D$ values of *Q. alba* (regardless of treatment) ranged from −10 to +9‰. By comparison, $\delta D$ values of *L. tulipifera* ranged from −9 to +14‰. The difference between species in $\delta^{18}O$ value ranges was even closer to analytical error: −0.8 to +9.7‰ for *Q. alba* and −0.8 to +10.0‰ for *L. tulipifera* (Fig. 6). It is possible, however, that a longer water turnover time, when combined with lowered leaf conductance at higher $CO_2$ concentrations, could lead to proportionally greater $^{18}O$ enrichment relative to deuterium, as $H_2{}^{18}O$ remained within the leaf over a longer period, while proportionally more $DH_2O$ evaporated. The apparent longer residence time for water within *L. tulipifera* leaves in the ambient + 300 µmol $mol^{-1}$ treatment could therefore lead to such a kinetic isotopic fractionation effect that would not be apparent during the shorter turnover period in *Q. alba*. Our observations of similar magnitudes and absolute degrees of heavy isotope enrichment in leaves with different water content do not support our use of the explanation that significantly different volumes of water are subject to heavy isotope enrichment in the 2 different species studied (Leaney et al. 1985, Walker et al. 1989).

Although there were statistically significant differences in $\delta^{18}O$ values of *Liriodendron tulipifera* leaf cellulose between ambient and ambient + 300 µmol $mol^{-1}$ treatments (Table 3), internal variability was high within sample groups. This suggests that the oxygen isotope composition of cellulose could be dependent upon the fine-scale isotopic content of leaf water

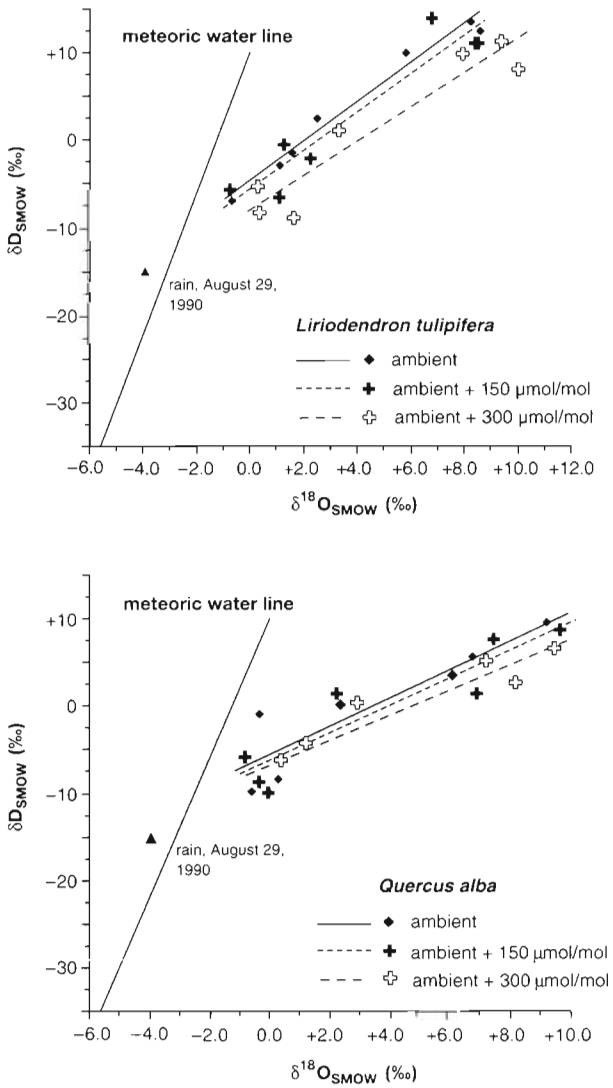


Fig. 6. *Liriodendron tulipifera* and *Quercus alba*. $\delta^{18}O$ and $\delta D$ values of leaf waters for all samples grown in the ground within open-top chambers, 30–31 August 1990, together with isotopic composition of 16 mm rain, 29 August 1990. Meteoric water line corresponds to $\delta D = 8\delta^{18}O + 10‰$, described by Craig (1961). Individual regression lines for *L. tulipifera*: ambient $CO_2$: $\delta D = 2.19\delta^{18}O - 5.64$, $r^2 = 0.91$, n = 7; ambient + 150 µmol $mol^{-1}$ $CO_2$: $\delta D = 2.20\delta^{18}O - 4.71$, $r^2 = 0.97$, n = 7; ambient + 300 µmol $mol^{-1}$ $CO_2$: $\delta D = 1.94\delta^{18}O - 7.96$, $r^2 = 0.90$, n = 7. For *Q. alba*: ambient $CO_2$: $\delta D = 1.61\delta^{18}O - 6.46$, $r^2 = 0.86$, n = 7; ambient + 150 µmol $mol^{-1}$ $CO_2$, $\delta D = 1.60\delta^{18}O - 5.37$, $r^2 = 0.82$, n = 7; ambient + 300 µmol $mol^{-1}$ $CO_2$: $\delta D = 1.44\delta^{18}O - 6.82$, $r^2 = 0.89$, n = 7

at the site of cellulose synthesis, a concept that has been advanced elsewhere (DeNiro & Cooper 1989).

While the more widely recognized factors of humidity, temperature, soil water, and water vapor clearly are the most important in governing the stable oxygen and hydrogen isotope composition of leaf water and cellulose, this work demonstrates that significant changes in the oxygen isotope composition of *Liriodendron tulipifera* can be solely attributed to doubling of the current ambient $CO_2$ concentration. It is possible that at some point, increases in $CO_2$ concentration would also affect the deuterium content of leaf water independent of such water stress induced increases observed in the chamber experiment. This work indicates, as has been indicated by previous studies, that caution should be exercised in interpreting paleoclimate through empirical use of proxy stable isotope indicators, such as preserved cellulose, until the factors influencing interspecific stable isotope variation in leaf water and cellulose are better understood.

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