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STABLE ISOTOPES OF OXYGEN IN PLANTS: A POSSIBLE PALEOHYGROMETER

by

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ABSTRACT

Ratios of oxygen-18 to oxygen-16 in cellulose of dated rings from trees grown in nature and from plants grown in controlled environments have significance for retrieving information about the environment in which they grew. Phaseolus vulgaris was grown under varying conditions of controlled temperature, humidity and $^{18}O/^{16}O$ of irrigation water. The $^{18}O/^{16}O$ in plant tissue responds mostly to different environmental relative humidity; plant tissue grown under conditions of low relative humidity produce tissue relatively high in oxygen-18. Reasons for this response are not clear to us, but the relationship may prove a useful complement to established dendroclimatologic techniques.

INTRODUCTION

The study of paleoclimates has attained a new prominence lately, especially since the winter of 1977. The knowledge and understanding of past climates should eventually enable us to predict future climates, perhaps even better than did the "Old Farmer's Almanac" for this past winter[3].

One need only glance over some recent book titles to realize that our climatic future is becoming the concern of many thoughtful people these days. "Genesis Strategy" (Schneider and Mesirow, 1976) and "The Cooling" (Ponte, 1976) are examples. The latter chillingly ominous title implies precognition, and of course, if we already knew the future climate, there would be less interest in pursuing research into proxy indicators of past climate.

ISOTOPE PALEOCLIMATE STUDIES

We have begun testing natural stable isotopes of carbon, hydrogen and oxygen in tree rings as retrospective climate indicators. The present paper focuses primarily on oxygen isotopes.

Oxygen isotopes are certainly nothing new to paleoclimate research. Their study in ice cores and marine cores over the past couple of decades have contributed enormously to our knowledge of Holocene and Pleistocene climates. Tree rings may not give us such long continuous records as ice and ocean cores, but their time resolution of a year or less simply cannot be beat.

Several papers have been published in the last couple of years (see, for example: Libby and Pandolfi, 1974; Gray and Thompson, 1976). They show empirical relationships giving a positive correlation between temperature and increasing oxygen-18 content in tree rings. But empirical relationships can be tricky, and clearly we need a better understanding of why the isotope compositions change, before confidently interpreting these values in terms of climate. There has been no satisfactory explanation of what chemical or physical step or process causes the variations in the ratio of the heavier to the lighter isotopes in plants. (Hydrogen isotopes in plant and tree-ring material have been recently discussed. See, for example: Schiegl, 1974; Epstein and Yapp, 1976.)

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[3]The present edition correctly predicted extreme cold in the Northeast and drought in the Central and Western Great Plains.

EXPERIMENTS ON PHASEOLUS VULGARIS

To try to sort out the causes of oxygen isotope variations in plants, we grew common bean plants (Phaseolus vulgaris) under controlled conditions, and analyzed the plant tissue consisting mainly of cellulose when they are young (Bonner, 1950).

The technique for extracting oxygen from dried plant tissue and placing it in carbon dioxide molecules was modified from that of Hardcastle and Friedman (1974). A few milligrams of plant matter are dried and placed in the vacuum pyrolysis tube illustrated in Figure 1. Heating to 1250°C for 1/2 hour produced a mixture of carbon

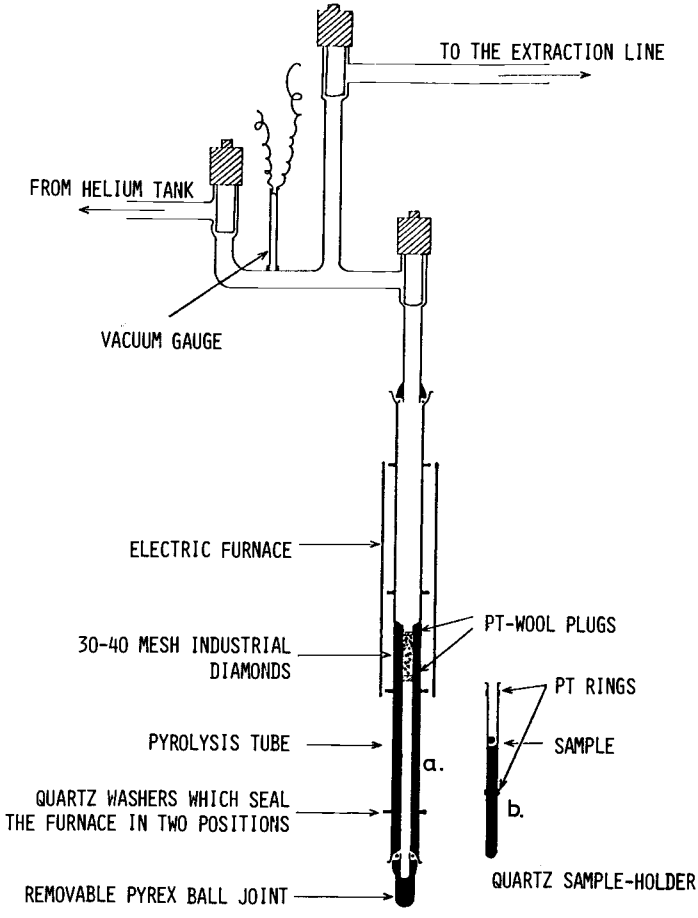

Figure 1. Scheme of the pyrolysis tube (A) used for the present experiments with detail of the sample holder (B). Further details of the extraction line and procedures have been described by Ferhi et al. (1975).

dioxide (CO_2) and carbon monoxide (CO). The CO is then converted to CO_2 in an electric discharge tube, and the combined CO_2 is thus completely labeled with the natural oxygen from plant tissue. A mass spectrometer especially designed for isotope ratio determination precisely compares the oxygen-18/oxygen-16 ratio in the sample with that in a reference CO_2 gas.

The sample's oxygen isotope value is represented by a "$\delta^{18}O$" value in per mil ($^{o}/_{oo}$) with respect to an international standard known as SMOW[4]. All values referred to here are in reference to this standard. Positive values thus contain more oxygen-18 with respect to oxygen-16 than SMOW, and negative values less.

Differences in $^{18}O/^{16}O$ from one chemical or physical phase to another arise as a result of slight differences in thermodynamic properties between, for example, $H_2{}^{16}O$ and $H_2{}^{18}O$, and between $C^{16}O_2$ and $C^{16}O^{18}O$. Reactions between phases may occur under equilibrium conditions or under non-equilibrium (kinetic) conditions. Fractionation, (enrichment or depletion of ^{18}O) is the rule rather than the exception in natural processes. Our experiments were designed to quantify the relationships between environmental conditions in plants and the $^{18}O/^{16}O$ of their photosynthetic products.

In three sets of experiments, we evaluated the effect on $\delta^{18}O$ in tissue of each of these three variables while holding the other two constant: temperature, humidity, and $\delta^{18}O$ of irrigation water. The results of the three sets are shown respectively in Figures 2a, b and c. The experiments indicate, surprisingly, that at least in very high humidity, temperature does not affect the $\delta^{18}O$ of tissue grown between 15 and $30^{o}C$. But at constant temperature, $\delta^{18}O$ is a linear function of relative humidity. Finally, if both temperature and relative humidity are kept constant and only the $\delta^{18}O$ of irrigation water is varied, the $\delta^{18}O$ in plant tissue is only slightly affected.

STABLE OXYGEN ISOTOPES IN PLANTS

Unfortunately, we know of no mechanism that satisfactorily explains all experiments. Some of the factors probably affecting the $\delta^{18}O$ values have been listed by Ferhi and Letolle (1977). Two models which may be partially operative will be discussed in this section. Water and/or carbon dioxide seem to be the most likely sources of oxygen in plant tissue. A "ruling hypothesis" in plant physiology states that the CO_2 molecule is the source of oxygen.

A dominant fact to be dealt with when considering stable isotope experiments is that CO_2 isotopically equilibrates with H_2O in a matter of hours at normal temperatures. When CO_2 is in isotopic equilibrium with water, the oxygen in CO_2 is about $40^{o}/_{oo}$ more positive than that in H_2O (O'Neil and Epstein, 1966). Thus, in a very simple model, if: (1) a plant is living in water of $\delta^{18}O = 0^{o}/_{oo}$; (2) uses only oxygen from CO_2 (which is in equilibrium with environmental water) in tissue manufacture; and (3) does not fractionate isotopic species in any step, then the tissue would have a $\delta^{18}O$ of about $+40^{o}/_{oo}$. In fact, our measurements indicate that plant tissue is about $26^{o}/_{oo}$ more positive than irrigation water, if grown at near 100% relative humidity.

A reasonable modification to the oversimplified model above would be a consideration of possible isotope fractionation of CO_2 as it diffuses into the stomata. Craig (1954) has discussed diffusion of CO_2. Re-evaluating his diffusional parameters in terms of the oxygen isotopes, a depletion of about $9^{o}/_{oo}$ seems reasonable. Figure 3 shows a highly diagramatic stomatal opening with reasonable values for diffusional fractionation of the molecules of CO_2 and H_2O. Thus, in the model with CO_2 diffusional fractionation, experimental plants grown at near 100% relative humidity are irrigated with water of $\delta^{18}O = -7.6^{o}/_{oo}$. CO_2 equilibrated with this has $\delta^{18}O = +32.8^{o}/_{oo}$ outside the leaf, but $+23.7^{o}/_{oo}$ inside due to binary diffusional fractionation. Oxygen-18 levels in experimental tissue (the variable temperature experiment) averaged $+20.2^{o}/_{oo}$.

The experiment with variable $\delta^{18}O$ of irrigation water (Fig. 2c) indicates that the irrigation water does contribute a small part of the oxygen to the plant tissue. This may happen either by direct participation of the H_2O in the reaction, or by a partial exchange of oxygen between leaf water and CO_2 or some reaction intermediate. Either way, the effect of the $\delta^{18}O$ level of the leaf water is a small one, no more

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[4] An acronym for Standard Mean Ocean Water.

$$\delta^{18}O\ (^{o}/_{oo})_{SMOW} = \left(\frac{^{18}O/^{16}O_{sample}}{^{18}O/^{16}O_{SMOW}} - 1\right) \times 1000$$

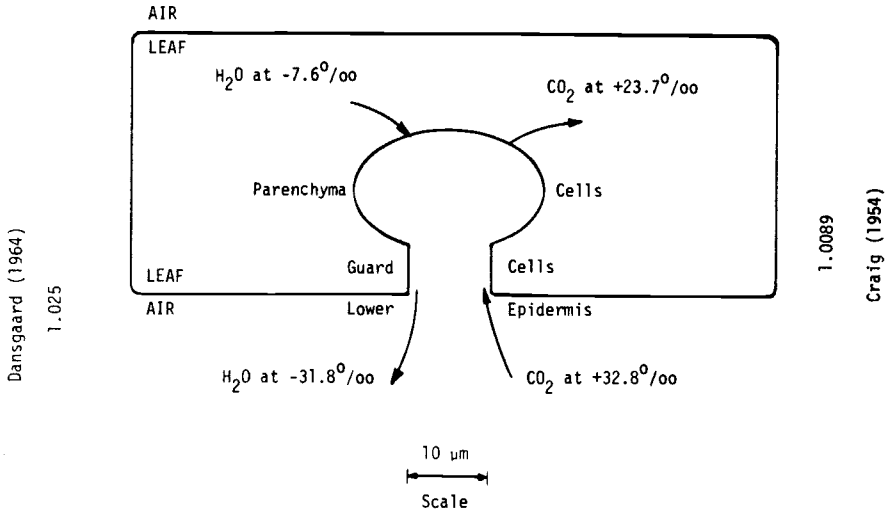

Figure 3. "Stomatal Model": It shows the $\delta^{18}O$ values of H_2O and CO_2 outside and inside the leaf and the diffusional fractionations.

than 15%, as indicated by the slope of 0.15 in Figure 2c.[5] Considering the leaf water effect to contribute 15% of the plant tissue $\delta^{18}O$, and the CO_2 the remaining 85%, the leaf tissue $\delta^{18}O$ would be:

$$(.15)\ (-7.6^{o}/oo) + (.85)\ (+23.7^{o}/oo) = 19.0^{o}/oo.$$

The result from this model is tantalizingly close to the observed average of $20.2^{o}/oo$ in the temperature experiment. Agreement between calculated and measured values using the above diffusional fractionation model is almost exact for the "98 to 100% humidity" point in Figure 2b.

Unfortunately, this diffusion model is unable to explain the humidity effect. The $\delta^{18}O$ in plant tissue ranges from $+18.6^{o}/oo$ near 100% relative humidity to $+34.2^{o}/oo$ near 20% relative humidity. The extrapolated total range is $23^{o}/oo$, assuming linearity. Since the total diffusion effect is $9^{o}/oo$, the range of $23^{o}/oo$ cannot be explained by this model.

The range can be explained, however, by an "internal equilibration" model, which allows CO_2 to equilibrate isotopically with the water inside the leaf before contributing its oxygen to plant tissue. Water in the photosynthesizing cells in leaves is not necessarily of the same isotope composition as it was when drawn from the soil into the roots. The transpiration process may enrich the leaf in water molecules composed of heavy isotopes because water vapor is isotopically lighter than the liquid from which it evaporated. This is because the lighter isotope has a higher vapor pressure. Thus, the heavier water molecules are more likely to remain in the leaf water. The extent of fractionation during evaporation depends on whether the evaporation took place by a kinetic process or an equilibrium process. Experimental values of the fractionation effects during evaporation (see, for example: Dansgaard, 1964) tell us that vapor is $10^{o}/oo$ isotopically lighter if in equilibrium with liquid, but may be as much as $25^{o}/oo$ lighter if kinetically evaporated and the vapor has no chance to re-equilibrate with liquid. These fractionation values are temperature dependent. Moreover, if the hydrogen isotopes of water are measured, it is found that the $HD^{16}O$ molecules, containing one atom of deuterium, behave differently than the $H_2{}^{18}O$ molecules in these two evaporation processes (Dansgaard, 1964). When δD (defined similarly to $\delta^{18}O$, and also using SMOW as a reference) is plotted on the ordinate and $\delta^{18}O$ on the abcissa, liquid-vapor pairs formed by the two processes are distinctive.

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[5] Previous measurements (Ferhi and Letolle, 1977) show a larger slope due to isotope fractionation during evaporation of the irrigation water. The present experiment was performed on hydroponically grown plants, with an oil seal to avoid evaporation of the water.

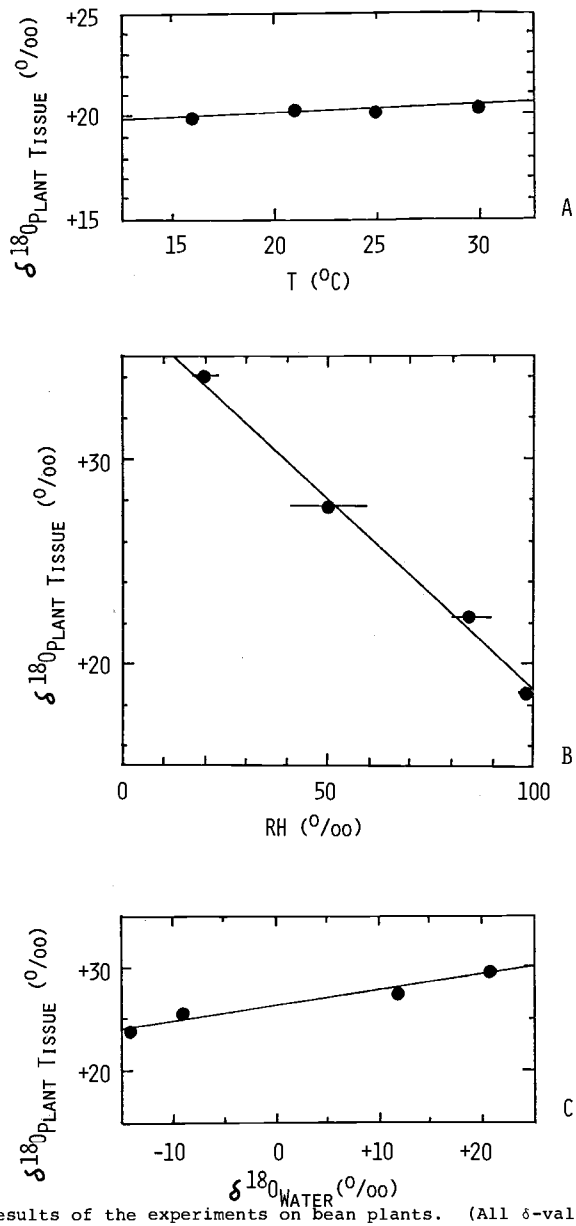

Figure 2. Results of the experiments on bean plants. (All δ-values are vs. SMOW).

a. Experiment with plants grown at constant relative humidity (90 to 100%) and varying temperatures. $\delta^{18}O$ of irrigation water = -7.6°/oo.

b. Experiment at constant temperature (21.5 ± 0.5°C) and varying relative humidity. $\delta^{18}O$ of irrigation water = -7.4°/oo.

c. Experiment at constant relative humidity (about 45%) and temperature (about 21°C) and varying isotope composition of the irrigation water.

Liquid and vapor in isotopic equilibrium fall on a line of slope 8, while liquid and vapor kinetically separated fall on a line of slope 3. Of course, a combination of the two processes would produce a slope between 3 and 8 (Dansgaard, 1964; Craig and Gordon, 1965). Measurements by Lesaint et al. (1974) of δD and $\delta^{18}O$ of water in leaves fall on a line of slope 2.9, and a maximum $\delta^{18}O$ difference between leaf water and stem water of 23°/oo. Thus, kinetic evaporation is evidently the process involved in transpiration.

This is entirely consistent with plant physiological experiments (see, for example: Zelitch, 1971) which strongly support diffusion of water vapor through stomatal openings as the dominant transpiration process. The rate of transpiration (in g cm^{-2} sec^{-2}) is thus related to humidity and diffusional resistance by "Ohm's law" (Zelitch, 1971):

$$\text{Transpiration} = \frac{X_i - X_a}{R_a + R_s}$$

where X_i = saturation water vapor concentration at the leaf temperature and X_a = vapor pressure in the surrounding air (both in g cm^{-3}); R_a and R_s are diffusional resistances of water vapor in air and stomatal openings, respectively (in sec cm^{-1}). The term ($X_i - X_a$) is proportional to [1 - (relative humidity) x 10^{-2}] if leaf temperature equals air temperature. Therefore, the lower the relative humidity, the more rapidly a plant transpires. Note also that R_s will be a function of species, light intensity, temperature and availability of water, and that R_a is a function of wind velocity, especially below 0.5 meters per second (Devlin, 1975).

The leaf water $\delta^{18}O$ values observed by Lesaint et al. (1974) can be explained by a model with a steady-state system consisting simply of a single mixed reservoir (leaf or cell within a leaf). Water flows into the reservoir with a fixed $\delta^{18}O$ and out via transpiration with a $\delta^{18}O$ which is 25°/oo lower than in the reservoir. This is illustrated in Figure 4. Water in the leaf may also undergo photolysis, but in this

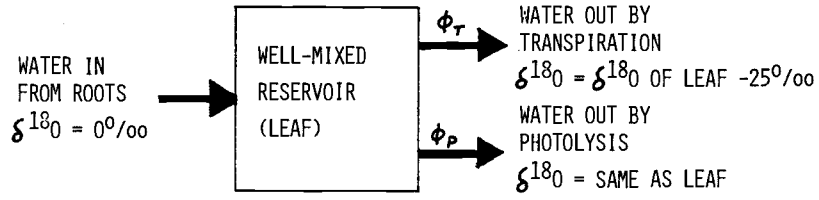

Figure 4. Scheme of the box model utilized to evaluate Figure 5 showing the dependence of $\delta^{18}O$ with ϕ_T (transpiration H_2O flux) and ϕ_P (photolysis H_2O flux).

model, we do not consider that isotope fractionation occurs in this step. The rate of transpiration is designated ϕ_T and the rate of photolysis ϕ_P. After transpiring an amount of water several times the weight of the reservoir, the system reaches an isotopic steady-state, i.e., the $\delta^{18}O$ of the reservoir water levels off. These steady-state $\delta^{18}O$ values given by the model are a function only of ϕ_T/ϕ_P, and are shown in Figure 5. Because of other variables, relative humidity cannot be placed on this scale, but the figure indicates where "high" and "low" humidity lie.

If CO_2 equilibrates isotopically with leaf H_2O, this model explains the range and direction of the humidity effect, but not its linearity. More importantly, it is not consistent with the experiments in Figure 2c which indicate little effect of irrigation H_2O and by implication leaf H_2O on the $\delta^{18}O$ of plant tissue.

We have here some laboratory empirical results in apparent contradiction with field evidence, and we are left with partial explanations, none of which may be correct. Clearly, more controlled growth experiments should be done, and they have been planned.

RELEVANCE TO PALEOCLIMATIC RESEARCH

These experiments wave a caution sign that $\delta^{18}O$ in plant tissue may be responding to temperature only in-as-much as temperature affects relative humidity. It is a long and tenuous thread that connects laboratory results obtained from plants grown under controlled conditions and field results from plants grown under the vicissitudes of nature. In nature there are diurnal variations of the temperature and humidity.

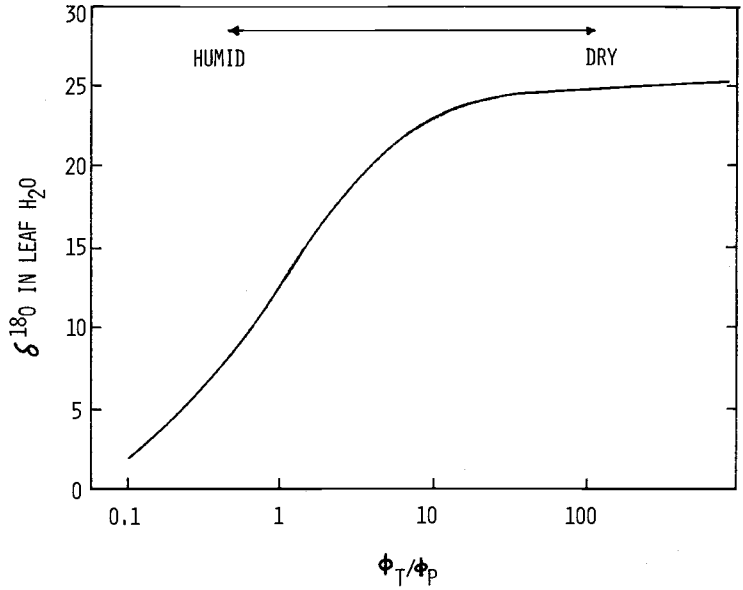

Figure 5. Calculated isotope fractionations of sap water as a function of relative humidity and of ϕ_T/ϕ_P (= transpiration flux/photolysis flux) from the model described in the text.

Also, with each precipitation event, $\delta^{18}O$ of irrigation water varies (Dansgaard, 1964; Mook, 1970). The stomata in leaf surfaces through which CO_2, O_2 and H_2O pass, open only when sufficient light and water are available. It is reasonable to assume, therefore, for paleoclimate investigations, that the $\delta^{18}O$ in total tissue or in cellulose from tree-ring specimens will have recorded both a temperature and a humidity component. Recent evidence on cellulose from Pinus radiata (Wilson and Grinsted, 1977), and on whole wood tissue from King Billy pine (Arthrotaxis selaginoides) (Pearman et al., 1976) indicate that $\delta^{13}C$ in wood is temperature dependent. Gray and Thompson (1976) have shown that the $\delta^{18}O$ of cellulose from white fir correlates well with annual average temperatures. Thus, the combination of $\delta^{18}O$ and $\delta^{13}O$ measurements on tree-ring sequences should be a useful probe into past climates. Moreover, when isotope data from trees in a continental wide network of sample points are coupled with dendroclimatological results from the same or nearby moisture-sensitive trees, a very powerful and comprehensive paleoclimatic tool may result.

HOW DO THESE RESULTS RELATE TO PLANT SCIENCE RESEARCH?

The modelized relationship between the $\delta^{18}O$ observed in plant tissue and the ratio between transpiration and photosynthesis signals to us one of the obviously possible applications to plant science. This isotope method would offer the possibility of a fast and cheap method for screening a large population of different plant species or different varieties or different individuals for water use efficiency. Thus, the same technique would be useful to retrieve/predict climate and to screen for better crops for the climates to come.

SUMMARY

We have discussed two models to attempt an explanation of the stable oxygen isotope ratios observed in plant tissue and the implications for two types of research: paleoclimate reconstruction, and plant science research. A computer model now seems feasible which would generate possible temperature and humidity conditions from $\delta^{18}O$

data stored in plant cellulose, perhaps ultimately from dendrochronologically dated tree rings, and such a model might provide us with a simple and cheap tool to gain insight into the physiology of modern plants.

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