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RESPONSE

Response to the comment of V. J. Terwilliger on "A mechanistic model for interpretation of hydrogen and oxygen isotope ratios in tree-ring cellulose," by J. S. Roden, G. Lin, and J. R. Ehleringer (2000) *Geochim. Cosmochim. Acta* 64:21–35.

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1. INTRODUCTION

Roden et al. (2000) developed and tested a model describing the factors influencing the δD and $\delta^{18}O$ values of α-cellulose in stems. Our goal was to develop a model to aid in the interpretation of δD and $\delta^{18}O$ values of tree-ring cellulose. Roden et al. (2000) concluded that the δD and $\delta^{18}O$ values of stem cellulose recorded information about both source water and humidity. Supporting evidence for the Roden et al. (2000) model from both experimental greenhouse and field conditions were presented in Roden and Ehleringer (1999a, 1999b, 2000). Terwilliger (2001) discussed some possible complications with this model, especially as they relate to inputs from different carbon sources during cellulose synthesis. The issue regarding the inputs of carbon reserves during tree-ring production certainly needs investigation.

First, some terminology requires clarification. In the plant literature, the terms *autotrophy* and *heterotrophy* refer to photosynthetic assimilation and nonphotosynthetic metabolism, respectively. However, in Terwilliger (2001), the terms *autotrophic* and *heterotrophic* input seem to indicate the fraction of carbon that comes from current leaf photosynthate and stored reserves, respectively. We would suggest that when discussing inputs from different carbon sources for cellulose synthesis the terms *current photosynthate* and *stored reserves* should be adopted to reduce confusion (which we do in the rest of this article).

Terwilliger (2001) suggests that "it is premature to assign constants to the model to ubiquitously describe heterotrophic and autotrophic inputs to wood cellulose δ values." However, in the models of Roden et al. (2000), autotrophic and heterotrophic parameters describe the biochemical isotopic fractionation associated with photosynthetic carbon fixation and carbohydrate metabolism during cellulose synthesis, respectively, and not the sources of carbon. These fractionation factors (ε_{HH}, ε_{HA}, ε_{O}) are variables in the equations of Roden et al. (2000), not constants, and the variation in the literature estimates of each are discussed. In each case, the mechanisms of isotopic fractionation are associated with fundamental biochemical reactions that are likely conserved among different species. Thus, although estimates of these parameters could certainly be improved, it is likely that the range of values for each may end up being quite narrow. The biochemical isotopic fractionation that occurs when sucrose is converted to cellulose is unlikely to differ regardless of whether the source of sucrose came from the leaf or from stored reserves (although the nonexchangeable fractions may differ).

2. SPECIES CHOICE AND EXPERIMENTAL DESIGN

The heart of this section in Terwilliger (2001) is that the two studies (Terwilliger and DeNiro, 1995; Roden and Ehleringer, 1999b) represent "end members of what trees can do" with regard to stored reserves. There was no assumption in Roden and Ehleringer (1999b) that all the organic H incorporated in a tree ring will come from current photosynthate. In their experiment, the outer two thirds of the tree ring was sampled because the earliest cellulose produced could possibly have had isotopic signatures unrelated to the experimental conditions (i.e., from stored reserves). Roden et al. (2000) also highlighted the need for subdividing tree rings because the H and O isotopes of cellulose in early-season xylem may not represent either current meteoric source water or current humidity conditions.

Although avocado seedlings may represent the maximum capacity of trees to utilize stored reserves, two areas of caution need to be addressed before any conclusions regarding "end members" can be made. First, do mature trees have comparable amounts of carbon storage as germinating seedlings, which are dependent on the carbon sources in the seed? Terwilliger (2001) asserts that trees have substantial stored reserves but admits that little is known how those reserves are tapped for growth. Second, does the form of carbon reserves in an avocado seed (lipids) add a complicating factor to the interpretation of the isotopic signals in the cellulose? Carbohydrate and lipid carbon reserves will exhibit differences in isotopic exchange capacities during subsequent cellulose synthesis (Luo and Sternberg, 1992).

3. SIMILARITIES AND DIFFERENCES IN RESULTS

In this section, Terwilliger (2001) restates many of the observations of Terwilliger and DeNiro (1995) and additionally refers to potential reinterpretations of the author's original work. Roden et al. (2000) did not conclude that "humidity information could be consistently obtained," only that humidity information was not eliminated during cellulose synthesis. Teasing out humidity information from tree-ring records may

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still be difficult. Our comment on Terwilliger and DeNiro (1995) was that they concluded the δD values of stem cellulose do not contain a humidity-derived signal, which is in disagreement with our observations.

4. VARIABILITY IN THE BALANCE BETWEEN HETEROTROPHIC AND AUTOTROPHIC EFFECTS

This section of Terwilliger (2001) is not a criticism of Roden et al. (2000) but instead a speculative discussion on the role of different carbon sources for plant growth: autotrophic (i.e., derived from current photosynthate) versus heterotrophic (i.e., derived from storage, such as from reserves in roots and seeds). Any carbon source that proceeds through metabolic pathways leading to storage products could be subject to different fractionation events than those of autotrophic carbon fixation. This is especially true if the storage product is not a carbohydrate (e.g., lipids, Luo and Sternberg, 1992).

However, a simple modification of the general models for isotopic exchange during cellulose synthesis (Sternberg et al., 1986; Yakir and DeNiro, 1990; Luo and Sternberg, 1992; Roden et al., 2000) could potentially account for these fractionation events.

The δD or $\delta^{18}O$ of cellulose can be described by the general model,

$$\delta_c = f \cdot (\delta_w + \epsilon_H) + (1 - f) \cdot \delta_{non\text{-}exchangeable} \quad (1)$$

where the subscripts *c*, *w*, and *nonexchangeable* indicate the isotopic composition of cellulose, medium water, and the nonexchangeable stable isotopes of the substrate, respectively, ε_H is the heterotrophic fraction factor for cellulose synthesis, and f is the proportion of isotopic exchange with medium water. For the fraction that exchanges with medium water, it matters not whether the hydrogen or oxygen were derived from current photosynthate or stored reserves because they will be exchanged. However, if a portion of the nonexchangeable fraction comes from stored reserves, then differences in cellulose δD and $\delta^{18}O$ may be observed. Another proportionality factor is required to account for different carbon sources,

$$\delta_c = f \cdot (\delta_w + \epsilon_H) + (1 - f) \cdot [(f_s \cdot \delta_{NES}) + (1 - f_s) \cdot \delta_{NES}] \quad (2)$$

where the subscripts *NES* and *NEC* indicate the nonexchangeable stable isotopes of stored reserves and the nonexchangeable stable isotopes of current photosynthate, respectively, and f_s is the proportion of the total substrate used in cellulose synthesis that is derived from stored reserves. The contribution of current photosynthate is estimated as

$$\delta_{NEC} = \delta_{wi} + \epsilon_A \quad (3)$$

where δ_{wl} is the δD or $\delta^{18}O$ value of leaf water and ε_A is the autotrophic fractionation factor for hydrogen or oxygen during photosynthetic carbon fixation. If there is no contribution of stored reserves ($f_s = 0$) then Eqn. 2 reverts to the model described in Roden et al. (2000).

Although Eqn. 2 may satisfy the objections of Terwilliger (2001)), it has three major obstacles to becoming a useful model. First, what is the value of $\acute{E}_s$, how do we estimate it for mature trees and how do we handle seasonal variation in f_s? Second, what is the value of δ_{NES}? Do we know all of the discrimination events involved in postphotosynthetic fractionation? Third, how do we handle multiple sources of stored carbon reserves? Eqn. 2 could potentially be expanded to include multiple sources, each with its own proportionality factor and value for δ_{NES}, but clearly the unknowns become overwhelming at some point. Until these questions can be answered, the equations of Roden et al. (2000) remain the most applicable model for the interpretation of hydrogen and oxygen isotope ratios in cellulose. However, we would also reiterate the caution that tree-rings need to be carefully sampled and even subdivided to ensure that the signals of interest are measured rather than those from stored reserves or previous year's climatic conditions.

In summary, we see no compelling reason to alter the model of Roden et al. (2000). That study developed and tested a model to explain the factors influencing the δD and $\delta^{18}O$ values of stem cellulose. It is a model that should be further tested but which accounts for why humidity signals are sometimes observed in tree-ring cellulose studies and at other times apparently are not. Certainly this is an important point to understand if we are going to use tree rings for paleoclimatic reconstructions.

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