

Establishing the environmental controls and mechanisms shaping the $^2\text{H}/^1\text{H}$ ratios of leaf wax *n*-alkanes

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The extent to which water source, atmospheric conditions, and leaf water ^2H -enrichment affect $\delta^2\text{H}$ values of terrestrial plant leaf waxes impacts interpretations of $\delta^2\text{H}$ variation of leaf waxes as a proxy for hydrologic conditions. To elucidate the effects of these parameters, we analyzed $\delta^2\text{H}$ values of *n*-alkanes from two tree species grown hydroponically under monitored atmospheric conditions. *Populus fremontii* and *Betula occidentalis* saplings were each grown under one of six isotopically distinct waters [-120, -60, 0, 60, 120 and 180‰] and under either 40% or 75% relative humidity. We observed *n*-alkane $\delta^2\text{H}$ values of both species were linearly related to source water $\delta^2\text{H}$ values, but with slope differences associated with differing humidities. A Craig-Gordon model was used to predict the $\delta^2\text{H}$ values of leaf water and modeled leaf water values were linearly related to observed *n*-alkane $\delta^2\text{H}$ values. These observations support a constant biosynthetic fractionation factor between leaf water and *n*-alkanes for each species under these growth conditions. However, we calculated small differences in the biosynthetic fractionation factor between the two species. Recent studies have suggested that the biosynthetic fractionation may be similar but slightly different among species, and the results from this study ostensibly support these findings. At present, it remains unclear if these apparent interspecies differences in biosynthetic fractionation reflect specific-species biochemistry or inapt model parameterization. Nonetheless, our conclusions suggest the need for measurements of or accurately modeled $\delta^2\text{H}$ values of source water, atmospheric water, and humidity conditions at the time of lipid synthesis to assess biosynthetic fractionations between leaf water and lipids.