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AN030

Induction Module CRDS analysis of water isotope fractionation along a *Pinus spp.* branch and leaf
Single-step method for analysis of evapotranspiration

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Keywords:

Material: Plants, evapotranspiration, water cycle
Process: Stable isotopes, δD, $\delta^{18}O$, IM-CRDS

Summary and Relevance:

Stable isotope ratios of hydrogen ($^{2}H/^{1}H$; δD) and oxygen ($^{18}O/^{16}O$; $\delta^{18}O$) in water have long been used to probe physiological and ecological processes. Studies at natural abundance levels have been used to understand leaf evapotranspiration [1], metabolic processes [2], and water source competition among plant communities [3]. However, the analytical methodology required for these measurements is expensive, long and tedious. The approach requires vacuum extractive equipment to isolate the matrix-bound water using potentially hazardous materials (e.g., uranium oxide, liquid nitrogen) and extreme conditions (e.g., vacuum at 250 ^{o}C with simultaneous trapping at -196 ^{o}C) [1-3]. It often takes days to prepare only several samples while the entire set-up, from vacuum line to isotope ratio mass spectrometer (IRMS), is expensive and requires highly specialized technical training. In addition, large sample sizes are often needed to successfully isolate enough water, so fine-scale studies (e.g., millimeter-to-centimeter scale gradients of δD and $\delta^{18}O$ across a single leaf) are often not accessible.

The recent development of the Induction Module coupled to a Cavity Ring-Down Spectrometer (IM-CRDS) is a much simplified, more rapid approach for the analysis of water isotopes in biological matrices. In this technique, a localized electric field rapidly heats a metal sample holder facilitating the complete extraction of matrix-bound water from the sample. During extraction, the water is simultaneously swept to the CRDS analyzer for measurement of δD and $\delta^{18}O$ allowing a single-step protocol. The whole process (extraction, detection and analysis) can be accomplished within 5 – 15 minutes and requires low water volumes (1 – 3 µL), accommodating small sample sizes. The technology is a significant advance, both in terms of sample throughput efficiency and materials and training costs, over vacuum line extraction coupled to IRMS.

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Process:

In this application note, IM-CRDS is used to probe the variations in δD and δ^{18}O of water in a *Pinus spp.* from branch through leaf tip. A branch of ~40 cm in total length (20-cm with leaves) was collected on the evening of July 7, 2011 from Santa Clara, California, and placed in a ziplock bag until analysis the next morning. The first 10 cm of the branch (from base) was cut and discarded. Thin slices (~0.05 mm thick, 7 mm diameter) were taken at 1 cm intervals along the length of the branch. The thin slices were placed in stainless steel strips that were crimped with pliers and immediately placed in the sample vial for analysis (Figure 1). The first leaf along the length of the branch was then removed, and the base of the leaf placed inside a 3.5 cm long stainless steel sample coil. The coil was prepared by wrapping a stainless steel strip around a thin metal cylinder (1 mm o.d.). Once the base of the leaf was lined up with the base of the coil, the leaf was cut, the coil tightened by pulling on both ends and immediately placed in a sample vial for analysis (Figure 1). Data for the branch are single points of extremely thin slices, while the leaf is the water contained in a 3.5 cm long segment (1 x 0.2 mm thick). A total of 11 samples were collected in a clock time of 99 minutes (9 minutes per sample). Isotopes are reported in the delta notation.[1] Calibration was done once using three in-house water standards spanning δD of -106.10 to 4.56 ‰ and δ^{18}O = -14.11 to 0.54 ‰. A 6 mm diameter filter paper hole-punch was wet with 3 µL of the standard, placed in a stainless steel strip and analyzed immediately. Five replicates of each standard were analyzed, with a mean precision and accuracy of ±0.12/1.28 ‰ and 0.74/5.49 ‰ for δ^{18}O/δD, respectively.

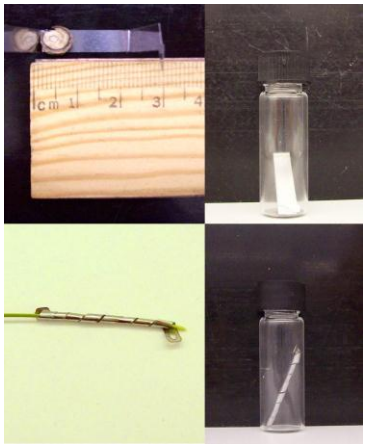

Figure 1 Sample holders for branch slices (top) and leaf (bottom).

Results:

Very little variation along the 7.5 cm length of branch is observed (δD = -55.02 ± 0.98 ‰ and δ^{18}O = -5.06 ± 0.26 ‰, Figure 2, top). The near proximity of the branch samples, coupled to limited evapotranspiration from the inner xylem through the bark, easily explain this result. The leaf, however, displays starkly different behavior that is in agreement with previous studies (Figure 2, bottom) [1]. From branch to leaf tip,

[1] The delta notation is defined as: $\delta^{n}X=1000\times[(R_S - R_{Ref})/R_{Ref}]$; where X is some element, n is the number of its heavier stable isotope (e.g., 18 for oxygen), R_S is the ratio of the heavy to the light isotope of the sample (e.g., $^{18}O/^{16}O$) , and R_{ref} is the same ratio, but of a reference material. In this notation, rather than $\delta^{2}H$, the convention is δD (for deuterium).

δD and $\delta^{18}O$ are enriched by 75.11 and 27.39 ‰, respectively. The enrichment is linear with distance away from the branch with δD increasing by 2.75 ‰ cm^{-1} (r^2 = 0.99) and $\delta^{18}O$ by 1.06 ‰ cm^{-1} (r^2 = 0.98). Evapotranspiration of water through stomatal openings of the leaf explain this enrichment profile.

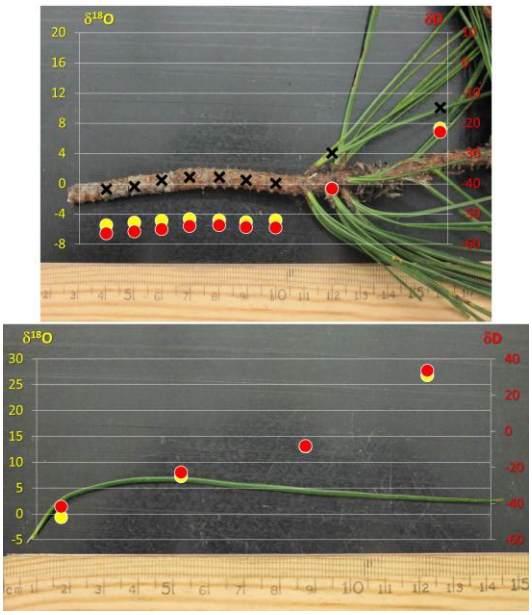

Figure 2 Top: δD and $\delta^{18}O$ profiles along the length of stem and one leaf (black × denotes sample slice location on the branch, each data point of the branch is a single 0.05 mm slice). Bottom: δD and $\delta^{18}O$ profiles along the length of leaf (each data point is the integrated signal of 3.5 cm lengths of leaf).

The correlation between δD and $\delta^{18}O$ is a long-standing tool to evaluate evaporative processes, including evapotranspiration in plants [1-3]. A relationship defined by $\delta D = 8 \times \delta^{18}O + 10‰$ gives the global meteoric water line (GMWL)
and describes evaporative processes that are in isotopic equilibrium [4]. Evaporation that is not in equilibrium will fall off the GMWL. In this work, the slope of the *Pinus spp.* water line (δD vs. $\delta^{18}O$, Figure 3) is 2.7, which is in agreement with a previous study of pine leaves showing increased evaporation compared to the GMWL [1].

This data describes a transpiration mechanism in which isotopically light water ($H_2{}^{16}O$) preferentially evaporates from the highly regulated stomatal openings, concentrating the isotopically heavy water ($HD^{16}O/H_2{}^{18}O$) along the length of the leaf.

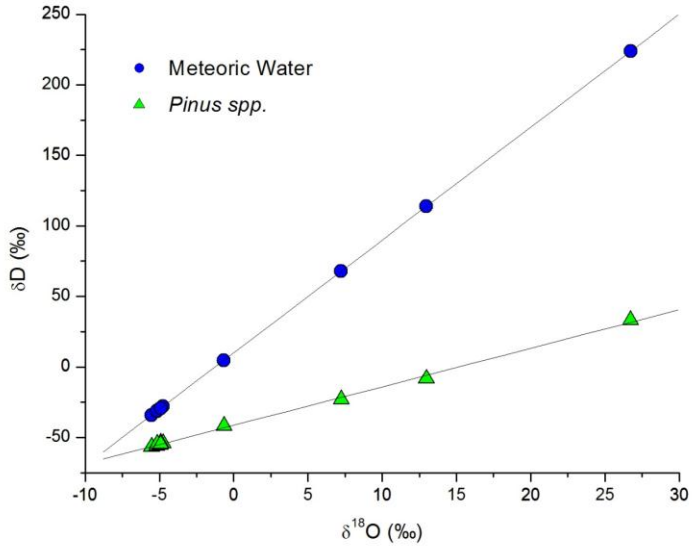

Figure 3 Relationship between stable isotopes of water in pine branch/leaf compared to ideal meteoric water line. The decreased slope of the *Pinus spp.* is indicative of excessive evaporation of water due to evapotranspiration.

Comments:
The greatly simplified approach offered by IM-CRDS allowed this data to be collected in about 1.5 hours, and sample prep required only a ruler, razor blade and stainless steel strips. In addition,

the robustness of the CRDS analyzer with the low maintenance requirements of the IM allows this technique to be used in the field for *in situ* analysis. The IM-CRDS opens the possibility of using natural abundance stable isotopes to rapidly probe physiological processes such as stomatal conductance, shifting water source, or label tracing, like never before.

References:

[1] Allison *et al.* (1985) Chem. Geology 58:145-156
[2] Sternberg *et al.* (1986) Plant Physiol. 82:428-431
[3] Dawson and Ehleringer (1991) 350:335-337
[4] Craig (1961) Science 133:1702-1703

Induction Module product details can be found at:
http://www.picarro.com/isotope_analyzers/im_crds