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**Communication**

# Spatial D/H Heterogeneity of Leaf Water[1]

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## ABSTRACT

**The mean $\delta D$ value of petiole water of *Pterocarpus indicus* Willd ($\delta D$ = −9.0 ± 2.5‰, $n$ = 3) was not significantly different from the mean value of stem water (−8.3 ± 2.8‰, $n$ = 3). $\delta D$ values of main vein water ranged from −11.1 to +12.0‰ ($n$ = 14) and increased along the main vein from petiole to the tip of leaves. Mesophyll water was highly enriched in deuterium (mean $\delta D$ = +32.0 ± 2.0‰, $n$ = 19) when compared with stem, petiole, and vein water. $\delta D$ values of mesophyll water for different areas of the lamina, however, were not homogenous and could differ by as much as 20‰.**

Leaf water becomes isotopically enriched relative to stem water during transpiration (12). Although several models have been proposed to explain $\delta D$[2] and $\delta^{18}O$ values of leaf water (2, 5, 6, 8, 10–13), a perplexing question still has not been solved: $\delta D$ and $\delta^{18}O$ values of leaf water are always lower than those predicted by previous models (3, 4, 8, 11, 13, 15). Several hypotheses have been proposed to explain this discrepancy. A number of investigators have suggested that leaf water is composed of two fractions: vein water, which is not isotopically enriched, and mesophyll water, which undergoes evaporation and thus becomes isotopically enriched (8, 11). Others have suggested that there are three distinct water compartments: vein water; apoplastic water, which becomes isotopically enriched; and symplastic water, which lags in isotopic enrichment because of slow mixing with the apoplastic pool (14, 15). Recently, it has also been suggested that there are patches of leaf water that do not become enriched because of areas of stomatal closure with the subsequent cessation of transpiration (6).

Here, we tested two hypotheses: first, whether leaf vein and mesophyll water have different isotopic composition, and second, whether there are spatial differences in isotopic enrichment for different patches of the leaf mesophyll.

## MATERIALS AND METHODS

Leaves of a *Pterocarpus indicus* Willd. shrub, grown in the University of Miami Biome, were used for these studies. The shrub was growing in an open, sunny location, and the leaves intercepted sunlight evenly. The leaves were an average of 0.12 ± 0.002 m long and 0.066 ± 0.003 m wide ($n$ = 4) with a well-defined central vein. Mean specific leaf weight was 105 g/m$^2$, and mean leaf water content was 52.5 ± 0.5% ($n$ = 4).

Leaf discs were taken at 13:30 h on bright, sunny days (September 24 and October 21) by using a glass tube (length 0.15 m with an inner diameter 0.3 × $10^{-2}$ m) that was previously made into a break-seal at one end. The open end of the glass tube was used to punch out a leaf disc at the various locations of the lamina between the veins (main and branch veins). The leaf disc was pushed to the bottom of the ampule with a glass rod, and the ampule was immediately flame sealed about 2.5 × $10^{-2}$ m from the break-seal end. The main vein for each leaf sample was cut into five to six segments (about 0.025 m apart between segments), and the mesophyll tissue was completely removed from the vein segments. Each of the segments was similarly sealed into a glass ampule. Samples of the petiole and the stem were sealed into different ampules. The sampling procedure took no longer than 4 min for 10 discs to minimize changes in leaf water content during sampling. Samples in the sealed glass tubes were broken in vacuum, and the water was distilled from the tissue with a boiling water bath; hydrogen gas preparation from distilled water was by the method of Bigeleisen *et al.* (1).

## RESULTS AND DISCUSSION

The mean $\delta D$ value of petiole water (−9.0 ± 2.5‰, $n$ = 3) was not significantly different from that of stem water (−8.3 ± 2.8‰, $n$ = 3). $\delta D$ values of the leaf main vein water (ranging from −11.1‰ to +12.0‰, $n$ = 14), although lower than mesophyll water (+32.0 ± 2.0‰, $n$ = 19), were not homogeneously lower than those of mesophyll. Values ranged from those typical of stem and petiole water and subsequently increased toward the tip to values intermediate between mesophyll and stem water (Fig. 1). Thus, the first hypothesis with regard to depletion of vein water relative to the mesophyll water holds true; $\delta D$ values of leaf vein water are lower than leaf mesophyll water. However, leaf vein water cannot be

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[2] Stable isotope ratios of hydrogen and oxygen are expressed here as a $\delta$ value, where

$$\delta X\ (‰) = \left(\left[\frac{R_{sample}}{R_{SMOW}}\right] - 1\right) \times 10^3$$

$X$ represents D or $^{18}O$, $R_{sample}$ is the molar ratio of heavy to light isotope of hydrogen or oxygen, and $R_{SMOW}$ is the mole ratio of the respective isotope for standard mean ocean water.

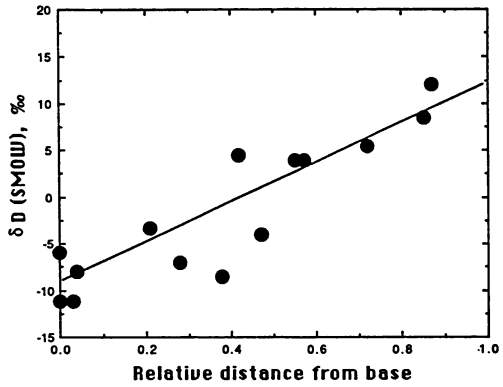


**Figure 1.** $\delta D$ values of main vein water ($y$) plotted against relative distances ($x$) from the base ($y = -9.51 + 21.46x$, $r^2 = 0.800$, $P \leq 0.01$).

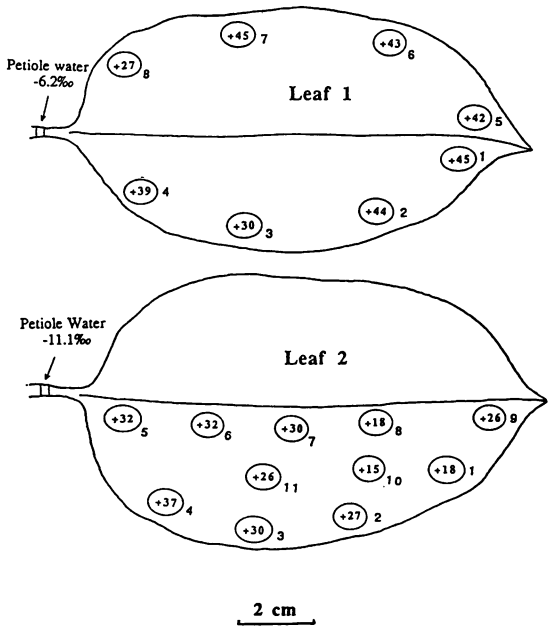


**Figure 2.** Heterogeneity of hydrogen isotopic composition for different areas of leaves. The value in each circle indicates the $\delta D$ value (‰) of water extracted from leaf disc. The number near each circle indicates the order of disc sampling.

treated as a distinct isotopically depleted water pool; rather, there is a gradual mixing between isotopically depleted water from the stem with isotopically enriched mesophyll water, leading to a gradient in isotopic enrichment from the base of the leaf to the tip.

Differences in $\delta D$ values of water from different leaf discs could be large (Fig. 2). For example, in leaf 2 the fourth disc was 22‰ higher than the 10th disc. Thus, leaf water shows a spatial heterogeneity, which is not necessarily associated with an intracellular and extracellular compartment as has been previously proposed (15). We speculate that this heterogeneity may be associated with patches of isotopically enriched and depleted water in the mesophyll as suggested by Flanagan *et al.* (6). These results are consistent with the observation that patches of stomatal closure occur in leaves exposed to low humidity (4). Patches of stomatal closure have also been previously demonstrated in sunflower leaves (7). An alternative to the patch hypothesis is the possibility that leaf discs with water showing low isotopic enrichment occur where a higher proportion of microveins containing depleted water exists. This possibility is unlikely because by the mass balance principle vein water would have to make up in some cases >50% by volume of leaf discs to account for the 20‰ isotopic variability observed in leaf mesophyll. This proportion for leaf vein is far above that previously calculated by stereological methods (9). Patches showing water with different isotopic enrichment could not be an artifact of sampling time, because there were no trends in $\delta D$ values of mesophyll water with the time of sampling as shown in Figure 2.

Previous models predicting the isotopic ratios of leaf water assumed that there is a pool of isotopically depleted water in the vein, which made up 30% of the leaf water (8). These estimates of the proportion of vein water are too high when compared to anatomical studies (9). Our observation of isotopically depleted water patches in the leaf indicates that deuterium-depleted water is not only limited to the vein but can occur in mesophyll patches as well, which allows for the possibility of a larger proportion of isotopically depleted leaf water. Flanagan *et al.* (6) observed that the discrepancy between modeled and measured values of leaf water isotopic composition was greater when transpiration was the highest. They suggested that during low transpiration there was a greater back flow of isotopically enriched water in the vein, thus increasing overall $\delta D$ and $\delta^{18}O$ values of leaf water. Our results demonstrate this back flow, because the $\delta D$ value of the main vein water increased progressively from the base to the tip of the leaves.

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