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Analysis of plant water stable isotopes using the water-vapor equilibrium method

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Plant water stable isotopes ($\delta^{18}O$, $\delta^{2}H$) have been used in eco-hydrological, biogeochemical and hydrological studies to e.g., quantify terrestrial water fluxes or to determine plant water sources. Current plant water extraction methods for isotope measurements are either expensive, labor-intensive or can lead to isotopic fractionation. Recent studies employed a new, extraction-free measurement method that was originally developed for the analysis of isotopes in sediment pore water: the water-vapor equilibrium method. It still needs to be tested if this method can be reliably used for isotope analysis of plant samples and how to best prepare the samples. Therefore, we investigated the effects of various preparation steps when measuring the plant water stable isotopes using this new method. We chose tomato and strawberry plants and prepared roots, shoots, leaves and fruits by either grinding or cutting them into pieces. Further, the necessary sample amount and the effect of equilibration time was evaluated. We investigated the effect of the preparation steps on mean values, standard deviations and a measurement device-specific value (LWV) that indicates a negative impact of volatile organic compounds (VOC) on reported isotope values. Results showed that an equilibration time longer than 24 hours is not advisable as the relationship between $\delta^{18}O$ and $\delta^{2}H$ of all plant samples worsened with R^2 declining from 0.97 to a minimum of 0.16. Additionally, the LVW indicated the influence of VOC with progressing equilibration time. Optimum amounts of plant material for roots were 3 g while for all other plant parts 5 g was necessary. In contrast to cut samples, kinetic fractionation effects were observed for grinded samples which could also be apparent fractionation effects because of the observed changes in LWV indicative of VOC interferences. For both plants the successive enrichment of the irrigation water from roots to leaves was observed. Fruits showed differences in their isotopic composition of the water stored inside the fruit compared to the water in the skin, with the inside water closer to the applied irrigation water. The intersection of the dual-isotope plot of all measured plant samples with the local meteoric water line was close to the applied irrigation water, making it theoretically possible to acquire information about the plant source water and enrichment factors in future studies when using the water-vapor equilibration method. From the findings of this study protocols can be established for sample preparation and plant water stable isotope analysis using the water-vapor equilibrium method.