

Hydrogen isotope fractionation during water uptake by woody xerophytes

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Received: 19 September 2006 / Accepted: 30 November 2006 / Published online: 17 January 2007
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Abstract Stable isotope measurements are employed extensively in plant–water relations research to investigate physiological and hydrological processes from whole plant to ecosystem scales. Stable isotopes of hydrogen and oxygen are routinely measured to identify plant source water. This application relies on the assumption that no fractionation of oxygen and hydrogen isotopes in water occurs during uptake by roots. However, a large fraction of the water taken up through roots in halophytic and xerophytic plants transverses cell membranes in the endodermis before entering the root xylem. Passage of water through this symplastic pathway has been hypothesized to cause fractionation leading to a decrease in ^2H of root xylem water relative to that in the surrounding soil medium. We examined 16 woody halophytic and xerophytic plant species in controlled conditions for evidence of hydrogen isotope fractionation during

uptake at the root–soil interface. Isotopic separation ($\Delta^2\text{H} = \delta^2\text{H}_{\text{soil water}} - \delta^2\text{H}_{\text{xylem water}}$) ranging from 3‰ to 9‰ was observed in 12 species. A significant positive correlation between salinity tolerance and the magnitude of $\Delta^2\text{H}$ was observed. Water in whole stem segments, sapwood, and roots had significantly lower $\delta^2\text{H}$ values relative to soil water in *Prosopis velutina* Woot., the species expressing the greatest $\Delta^2\text{H}$ values among the 16 species examined. Pressurized water flow through intact root systems of *Artemisia tridentata* Nutt. and *Atriplex canescens* (Pursh) Nutt. caused the $\delta^2\text{H}$ values to decrease as flow rate increased. This relationship was not observed in *P. velutina*. Destroying the plasma membranes of root cells by excessive heat from boiling did not significantly alter the relationship between $\delta^2\text{H}$ of expressed water and flow rate. In light of these results, care should be taken when using the stable isotope method to examine source-water use in halophytic and xerophytic species.

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Keywords Hydrogen isotope fractionation · Roots · Xerophyte · Halophyte · Transpiration

Introduction

Differences in the physical properties of the stable isotopes of hydrogen and oxygen in water result in isotopic separation or fractionation during

physicochemical reactions (Chacko et al. 2001; Dansgaard 1964; McMillan 1985). These fractionation events provide important information about numerous physical and biological processes. Precipitation formed by the fractionating processes of evaporation and condensation produces isotopically distinguishable meteoric waters across elevational gradients and between cold and warm seasons (Craig 1961, Dansgaard 1964; Kendall and Caldwell 1998). Plant-available pools of water in the unsaturated soil and deeper groundwater therefore are often isotopically distinguishable, establishing the means to use isotope ratio measurements to trace sources of water used by plants (Dawson 1996; Flanagan and Ehleringer 1991; Williams and Ehleringer 2000). The isotopic composition of xylem water in the plant represents a mixture of different plant water sources. Quantification of the proportion of water taken up from these sources is less complex provided that there is no fractionation of water during uptake at the root.

Several greenhouse and field-based studies have verified that water is not altered isotopically during uptake by roots (Dawson and Ehleringer 1991; Dawson and Ehleringer 1993; Thorburn et al. 1993; Walker and Richardson 1991; Washburn and Smith 1934; Wershaw et al. 1966; White et al. 1985; Zimmermann et al. 1967; Ziegler et al. 1976). However, mangrove species (*Laguncularia racemosa* Gaert., *Rhizophora mangle* L., and *Avicennia germinans* L.) and the salt-tolerant (halophytic) plant, *Conocarpus erecta* L., do fractionate hydrogen isotopes during water uptake (Lin and Sternberg 1993). Fractionation by roots in such species may lead to error in determination of the proportional use of isotopically distinct water sources (Lin and Sternberg 1992; Sternberg and Swart 1987).

The magnitude of hydrogen isotope fractionation during water uptake by roots in mangrove was positively correlated with growth and transpiration rates (Lin and Sternberg 1993). Mangroves have a highly developed Casparian strip on the radial cell walls of the root endodermis that impedes apoplastic movement of water, forcing water to move across cell membranes (Atkinson et al. 1966; Waisel et al. 1986). Lin and Sternberg (1993) hypothesized that the fractionation of

hydrogen isotopes in water occurred as water moved symplastically through the endodermis. Several studies have observed fractionation of isotopes of water across biological membranes and membranes composed of clay (Coplens and Hanshaw 1973; Karan and Macey 1980; Phillips and Bentley 1987). The energy required to dissociate individual water molecules from water aggregates before passing through the plasma membrane in the root endodermis is less for $^1\text{H}^1\text{H}^{16}\text{O}$ than for $^1\text{H}^2\text{H}^{16}\text{O}$ (Chacko et al. 2001). Substitution of ^{18}O for ^{16}O in water causes only a slight relative difference in vibrational energy between the isotopic species compared to the substitution of ^2H for ^1H , such that fractionation of isotopes in water during uptake by roots is expected to be more apparent for hydrogen than oxygen. If a large proportion of water enters the root symplastically, root xylem water is likely to be more depleted in ^2H compared to the soil solution. As the quantity of water entering the roots through the symplastic pathway increases, the magnitude of isotopic separation ($\Delta^2\text{H} = \delta^2\text{H}_{\text{soil water}} - \delta^2\text{H}_{\text{xylem water}}$) should also increase. Under conditions where apoplastic movement of water predominates, no fractionation is likely to occur because water aggregates can enter the plant without the dissociation into single water molecules from water aggregates.

The hypothesis proposed by Lin and Sternberg (1993) for fractionation in mangroves predicts that other species relying predominately on a symplastic pathway for water movement from soil to root xylem should fractionate hydrogen isotopes of water. The degree of suberization and lignification of the root cell walls forming the Casparian strip determines the extent of symplastic water movement from the root cortex into the root stele and xylem. Casparian strip development on the radial walls of the endodermis varies widely among plant species, and is most extensive in halophytes (Poljakoff-Mayber 1975; Waisel 1972). Glycophytic and mesophytic species have much less Casparian strip development, so symplastic movement of water is proportionally less important in these species. The Casparian strip and suberization of epidermal and cortical cell walls are also highly developed in woody xerophytes (Nobel and Sanderson 1984; Passioura

1981). Thus woody species that exhibit xerophytic and halophytic properties are likely to fractionate hydrogen isotopes during water uptake by roots.

The objectives of this study were to investigate the extent of hydrogen isotope fractionation during water uptake in woody plants of arid and semi-arid regions and identify the mechanism responsible for this fractionation. We hypothesized that symplastic water movement fractionates hydrogen isotopes in water, while apoplastic water movement does not. We predicted that salinity tolerant desert xerophytes with a well-developed Casparian strip in the root endodermis should fractionate hydrogen isotopes during water uptake to a greater extent than those species with lower salinity tolerance. We further predicted that if isotopic fractionation during uptake from soil occurred, the isotopic composition of root xylem water should be similar to that of shoots reflecting the location of fractionation at the root–soil interface. Finally, the isotopic separation ($\delta^2\text{H}_{\text{soil}} - \delta^2\text{H}_{\text{xylem water}}$) should increase as flow rate through the root system increases. Destroying the plasma membranes with excessive heat by boiling should remove the relationship between $\delta^2\text{H}$ and flow rate. Sixteen woody species from arid and semi-arid zones of the American Southwest varying in salinity tolerance were examined for hydrogen isotope fractionation. *Prosopis velutina* Woot., a widespread warm-desert shrub, was used in most of the experiments described here because prior studies (Ellsworth, unpublished results) suggested that it fractionated hydrogen isotopes in water during uptake by roots. *P. velutina* was solely used in two greenhouse experiments to locate the tissue where hydrogen isotope fractionation originated and to determine if oxygen isotope fractionation also occurred. A root pressurization experiment was conducted to identify the degree that flow rate through the symplastic and apoplastic pathways affected isotopic separation.

Methods

Several pot experiments were conducted to evaluate the magnitude, pattern, and extent of fractionation in desert shrubs and trees.

Extent of hydrogen isotope fractionation among woody xerophytes

Sixteen shrub and tree species native to semi-arid or arid areas of southwestern North America were evaluated for their capacity to fractionate hydrogen isotopes in water during uptake (Table 1). All but *Ephedra viridis* Coville, a gymnosperm, were woody angiosperms. In addition, one mesophytic species, *Lycopersicon esculentum* L. (tomato), was included for comparison as this species was shown not to fractionate either hydrogen or oxygen isotopes in an earlier study (Lin and Sternberg 1993). Salt-intolerant and tolerant taxa were included (Table 1). Every individual of each species except *Tamarix chinensis* Luor and *Prosopis* spp. was 2 years old and nursery grown in 4-L pots. The three *Prosopis* species were 3-year-old and nursery grown in 20-L pots. Cloned *Tamarix* stems from parent plants of unknown age were propagated and grown in 20-L pots. The three *Prosopis* species were 1.5–2 m tall and had basal stem diameters of 1.5–3 cm. *Larrea tridentata* (Sessé & Moc. ex DC.) Coville and *Artemisia ludoviciana* Nutt. were 50 cm tall and had multiple basal stems with diameters ranging from 0.4 to 0.7 cm. The stems were herbaceous except at the base of the main stems. All other species were 50–70 cm tall and single-stemmed with basal stem diameters of 1–1.5 cm. The tomato plants were 40 cm tall and had basal stem diameters of 1 cm. The plants were grown in a greenhouse in full sun with a 16 h photoperiod. The mean daytime and nighttime temperatures were 25 and 21°C, respectively, and daytime relative humidity was 19%.

The original potting soil used in the nursery where the plants were purchased was carefully removed from the root system and the plants were repotted in with sandy loam soil with 0.8% organic matter. The pH of the soil was 7.8, and the electrical conductivity was 0.69 dS m⁻¹. A 20–20–20 NPK fertilizer was applied weekly in the irrigation water to ensure adequate nutrients were available for plant growth. Three weeks prior to the collection of stems and soils, the pots were completely covered with plastic to prevent evaporation from the soil surface. At this time the pots were thoroughly flushed with isotopically

Table 1 Species examined for fractionation of hydrogen isotopes in water during uptake by roots, the number of individuals (n) examined for each species, and the salinity tolerance of each species reported as electrical conductivity (EC: dS m^{-1})

Scientific name	n	EC (dS m^{-1})
<i>Acacia constricta</i> Benth.	4	N/A
<i>Acacia greggii</i> Gray	3	N/A
<i>Artemisia ludoviciana</i> Nutt.	6	N/A
<i>Artemisia tridentata</i> Nutt.	4	6 ^a
<i>Atriplex canescens</i> (Pursh) Nutt.	6	20 ^b
<i>Chrysothamnus nauseosus</i> (Pallas ex Pursh) Britt.	6	6 ^a
<i>Ephedra viridis</i> Coville	4	10 ^a
<i>Flourensia cernua</i> DC.	6	N/A
<i>Krascheninnikovia lanata</i> (Pursh) A.D.J. Meeuse & Smit	3	10 ^a
<i>Larrea tridentata</i> (Sessé & Moc. ex DC.) Coville	5	1.84 ^c
<i>Lycopersicon esculentum</i> L.	4	2.5 ^d
<i>Olneya tesota</i> Gray	6	N/A
<i>Prosopis glandulosa</i> Torr.	3	8 ^e
<i>Prosopis pubescens</i> Benth.	6	9.4 ^e
<i>Prosopis velutina</i> Woot.	5	15 ^b
<i>Simmondsia chinensis</i> (Link) C. Schneider	4	10 ^b
<i>Tamarix chinensis</i> Luor	4	25 ^f
Total	79	

The EC values represent the threshold level after which plant growth is adversely affected. EC values were obtained from published studies.

^a Feucht (2001)

^b Forti (1986)

^c El-Ghonemy et al. (1980)

^d Maas (1985)

^e Jackson et al. (1990). The values used are threshold values when the plant is significantly damaged by the salinity concentration

^f Houerou (1986)

uniform water ($\delta^2\text{H} = -131\text{‰}$) and watered regularly with the same water.

To minimize contamination of transpiration-derived, ^2H and ^{18}O -enriched water in the stems, all stems were collected distal from leaves and herbaceous material. Unlike all other species, stems collected from *L. tridentata* and *A. ludoviciana* were from the base of the main stems and were close to transpiring tissue. Numerous green leaves were removed from the collected twig segments of *Artemisia tridentata* Nutt. The root crown of the tomato was collected to avoid tissues affected by evaporative enrichment of ^2H and ^{18}O

(Thorburn and Mensforth 1993). A single soil sample was collected from each pot. Each species was represented by 3–6 individuals (Table 1). The collection order of the individuals of each species and the species was randomized.

Magnitude of isotopic separation ($\Delta^2\text{H} = \delta^2\text{H}_{\text{soil water}} - \delta^2\text{H}_{\text{xylem water}}$) was calculated for all the species and correlated with salinity tolerance. Salinity tolerance (dS m^{-1}) was defined quantitatively as the electrical conductivity that begins to adversely affect plant growth. Values of salinity tolerance were obtained from the literature for 12 of the 16 species. Values of salinity tolerance for five species (*Artemisia ludoviciana*, *Acacia greggii* Gray, *A. constricta* Benth., *Olneya tesota* Gray, and *Flourensia cernua* DC.) were not available.

Variation in $\delta^2\text{H}$ and $\delta^{18}\text{O}$ among tissues and organs in *Prosopis velutina*

Prosopis velutina was identified as a species that fractionated hydrogen isotopes in water and was used to investigate the isotopic variation among different plant tissues and organs. Five *P. velutina* plants were nursery grown in 20-L pots of organic soil, consisting of wood chips, decomposed leaf litter, and a small amount of sand. Plants were 3 years old and had a basal stem diameter of 1–2 cm and a height of 1 m. The plants were grown outdoors with daily maximum temperatures of 38°C, minimum nighttime temperatures of 19°C, and 15% minimum daytime relative humidity. The pots were completely covered and sealed with black plastic and watered with isotopically uniform water ($\delta^2\text{H} = -65\text{‰}$, $\delta^{18}\text{O} = -9.4\text{‰}$) daily for 10 days prior to sample collection. Covering the pots 10 days prior to collection prevented evaporation, and any isotopically heavy water was flushed from the pot with subsequent irrigations. Whole stem segments (including bark) and soils were collected from 1100 to 1300 h when the trees were likely transpiring at maximum rates. Leaves, young stems, old stems, and soil from the top and bottom of each pot were collected in vials and kept frozen until the water was extracted from the samples by cryogenic vacuum distillation. The young, green stems were probably not completely

suberized and likely subject to evaporative enrichment of ^2H and ^{18}O .

An additional experiment was conducted to verify that fractionation occurred in roots rather than down stream in the stem and to determine the contribution of evaporatively enriched water in the bark to xylem water of sapwood. Five 2-year-old *P. velutina* plants were grown in 4-L pots consisting of a mixture of fine organic matter, wood chips, and sand. The plants had a basal diameter of approximately 1 cm and height of 50–75 cm. Plants were soaked in a bucket of water for 1 h to flush existing water from the soil 5 days before the tissues were collected for isotopic analysis. Plants were then removed from the water bath, and the soil medium was flushed with an additional 8 L of water. Water used to irrigate and flush the root system of the plants was isotopically uniform ($\delta^2\text{H} = -134\text{‰}$, $\delta^{18}\text{O} = -17.0\text{‰}$). The pots were wrapped and sealed with plastic to prevent evaporation from the soil surface. After the pots were allowed to drain, the holes in the bottom of the pots were sealed. The plants were flushed again with water of the same isotopic composition three more times in 5 days prior to harvesting tissue. The plants were kept in the sun throughout the day in a greenhouse at 31°C maximum daytime and 19°C minimum nighttime temperatures. Minimum daytime relative humidity was 17%.

Whole stem segments containing both sapwood and bark, sapwood only, bark only, and roots were collected separately from the five plants. Tissue samples were collected from four plants between 0930 and 1730 h. Tissues from the fifth plant were collected at 400 h when the plant was expected to be transpiring minimally. Bark was quickly removed from the sapwood, and each tissue component was placed in separate vials for water extraction and isotopic analysis. Another stem was collected intact with bark and sapwood together. The taproot was quickly cleaned, blotted dry of any external soil and moisture and placed in a vial. A single soil sample was collected from each pot. The plants were small, so all the stems were in close proximity to leaves and young herbaceous stems. None of the collected tissues was from large stems with extensive bark development. The isotopic composition of the leaf

water was calculated using the Craig and Gordon model, which was modified to calculate the isotopic composition of leaf water (Yakir and Sternberg 2000). The magnitude of isotopic separation ($\Delta^2\text{H} = \delta^2\text{H}_{\text{soil water}} - \delta^2\text{H}_{\text{xylem water}}$ and $\Delta^{18}\text{O} = \delta^{18}\text{O}_{\text{soil water}} - \delta^{18}\text{O}_{\text{xylem water}}$) was calculated for all plant parts.

Hydrogen isotope fractionation in live and heat-killed intact root systems

Three individuals of three species (*P. velutina*, *A. tridentata*, and *Atriplex canescens*) were used in a root pressurization experiment to examine the relationship between water flow rate and $\delta^2\text{H}$ of the expressed water for live and heat-killed root systems. The role of symplastic water movement in hydrogen isotope fractionation was evaluated by immersing the root systems in boiling water for 35 min to destroy the root cell membranes and comparing the $\delta^2\text{H}$ of expressed water to that in live root systems. Isotopic fractionation should not be apparent in the heat-killed root systems, which allows only for apoplastic water movement. Whole root systems were placed in a Scholander-type pressure chamber filled with water. Flow rate of water exuding from the stem was maintained by regulation of pressure applied to the chamber.

Three weeks prior to the pressurization experiment, each pot was covered with plastic to prevent evaporation as was done in the previous experiments. The root system was flushed several times then soaked for 2 h with water isotopically identical with the water used in the pressurization chamber ($\delta^2\text{H} = -132\text{‰}$), ensuring isotopic uniformity within the pressurization chamber. After flushing, the root system was completely submerged in water in the pressure chamber with only the cut stem protruding out of the top of the lid. Water was collected for at least 1 h at each pressure, so that at least 3 ml of water exuded from root system to ensure that the water collected from the root system for isotopic analysis was water that entered the root system under that pressure. Water exuding from the stem was collected in pre-weighed micro-centrifuge tubes filled with oven-dried glass wool. The tubes were weighed again after water collection to calculate the volume of water collected. Flow rate

measurements (ml h^{-1}) were made by collecting the water exuding from the stem in 1 min intervals. Only water collected in the last 5 min at each pressure was analyzed for isotopic composition. All treatments were placed under a series of pressures in the following order: 0.3, 0.5, 0.7, 0.9, 0.7, 0.3 MPa. Each root system was placed in boiling water for 35 min after the first pressurization on the live root system. The same pressurization method was followed on the heat-killed root system.

A composite sample consisting of five consecutive 1-min water samples was used for isotopic analysis. The water from the micro-centrifuge tubes with glass wool was distilled using a cryogenic vacuum line. Water from the pressurization chamber also was collected to measure the initial isotopic composition of the water in the pressurization chamber.

To test the extent that experimental error had on the isotopic composition of the samples during collection and distillation, a mock pressurization experiment was conducted on samples of known isotopic composition. The mock pressurization experiment was conducted and these water samples were distilled and analyzed for isotopic composition in the same manner as above. The isotopic composition of the water used in the mock pressurization experiments was compared to the original $\delta^2\text{H}$ value of the water used in this experiment to calculate the effect of evaporation and experimental error.

$\delta^2\text{H}$ and $\delta^{18}\text{O}$ analyses

Stem, root, and soil samples were stored frozen until the water was quantitatively extracted by cryogenic vacuum distillation (Ehleringer and Osmond 1989). Extraction efficiency for all samples, defined as the proportion of water extracted from the sample, was greater than 97%. All water samples collected were prepared for hydrogen isotope analysis by reacting 2 μl of extracted water with 100 mg of zinc at 500°C for 1 h in sealed Pyrex tubes (method modified from Coleman et al. 1982). The $\delta^2\text{H}$ value of the resulting H_2 gas was analyzed on a dual-inlet stable isotope ratio mass spectrometer (Optima, Micromass UK Ltd., Manchester, UK) at the University of Wyoming Stable Isotope Facility (UWSIF), Department of Renewable

Resources, University of Wyoming, Laramie, WY, USA, or at the University of Arizona Laboratory of Isotope Geochemistry, using a dual-inlet stable isotope ratio mass spectrometer (Delta S, Finnigan MAT, San Jose, CA, USA) and an on-line chromium reduction furnace (HDevice, Finnigan MAT, San Jose, CA, USA; Nelson and Dettman 2001). Precision of the analysis using chromium reduction calculated from lab internal working standards also was 0.8‰. Oxygen isotopic composition of all samples was measured at UWSIF by CO_2 equilibration using a Gas Bench II connected to a Delta^{Plus} XP mass spectrometer (ThermoFinnigan, San Jose, CA, USA) or at the University of Arizona Laboratory of Isotope Geochemistry by CO_2 equilibration (Epstein and Mayeda 1953). The precision of the analysis for $\delta^{18}\text{O}$ was 0.08‰ at UWSIF and 0.15‰ at the University of Arizona Laboratory of Isotope Geochemistry. Standard lab waters were calibrated against the international standard V-SMOW and SLAP (International Atomic Energy Agency (IAEA) 1995), which were used in linear corrections of values obtained from the mass spectrometer. Reported values are in per mil (‰) relative to V-SMOW.

Paired student's *t*-tests were used to determine if the δ values of stem xylem water and soil were significantly different ($P < 0.05$). Paired student's *t*-tests in the pot experiments and linear regressions in the pressurization experiment were calculated using SigmaPlot for Windows (Version 8.02, SPSS Inc. 2002). The slopes and *y*-intercepts of all linear regressions were compared by covariance analysis of the two groups where *y* was the dependent variable and *x* the co-variable (Armitage 1980).

Results

Extent of hydrogen isotope fractionation among woody xerophytes

Isotopic separation ($\Delta^2\text{H} = \delta^2\text{H}_{\text{soil}} - \delta^2\text{H}_{\text{plant}}$) was highest in *P. velutina* ($9 \pm 1\text{‰}$) and lowest in *Chrysothamnus nauseosus* (Pallas ex Pursh) Britt. ($3 \pm 1\text{‰}$; Fig. 1). Isotopic separation for five species (*L. esculentum*, *A. tridentata*, *Prosopis pubescens* Benth., *E. viridis*, and *L. tridentata*) was not significantly different than zero.

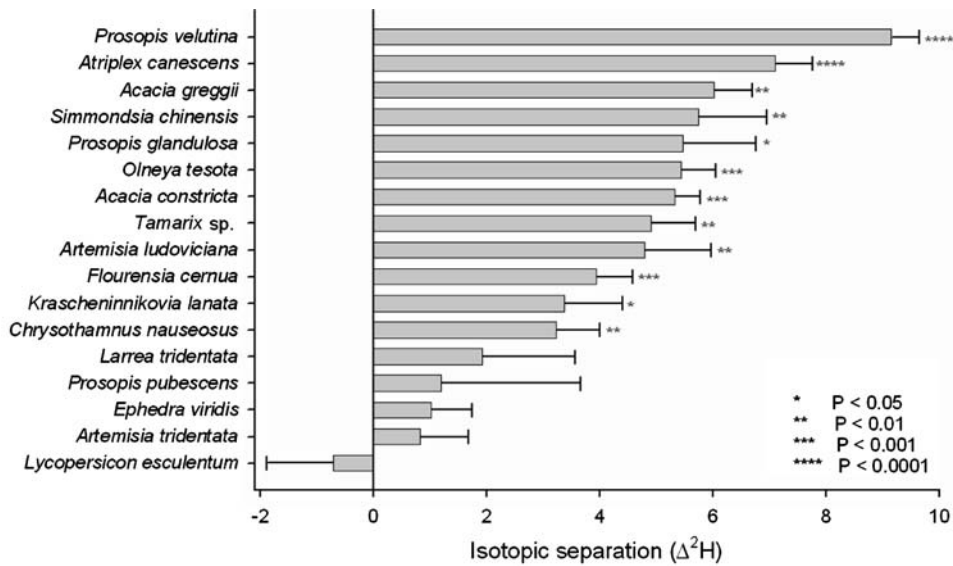
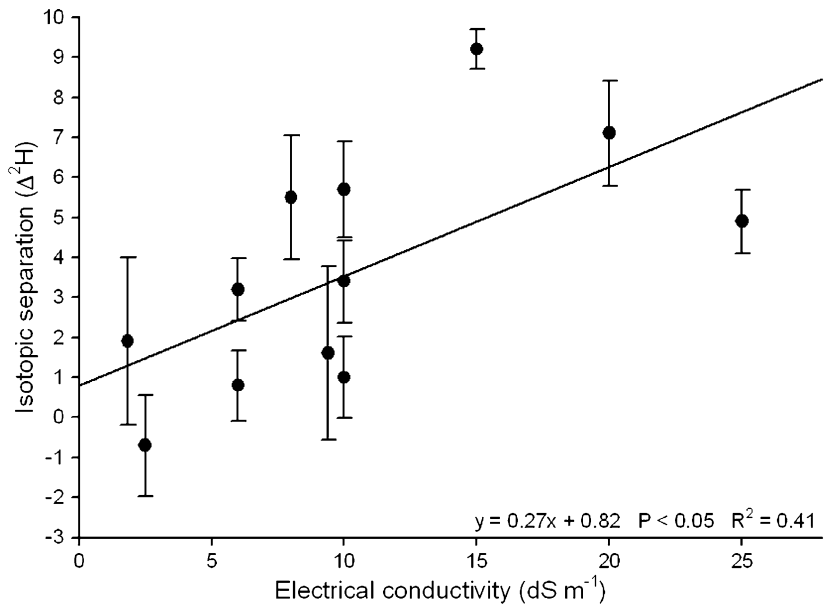


Fig. 1 Magnitude of isotopic separation ($\Delta^2\text{H} = \delta^2\text{H}_{\text{soil water}} - \delta^2\text{H}_{\text{xylem water}}$) for 16 species of semi-arid and arid shrubs and trees and the herbaceous mesophyte *Lycopersicon esculentum*

Fig. 2 The relationship between the magnitude of isotopic separation ($\Delta^2\text{H} = \delta^2\text{H}_{\text{soil water}} - \delta^2\text{H}_{\text{xylem water}}$) and salinity tolerance, defined as the minimum soil electrical conductivity negatively affecting plant growth. Values of salinity tolerance were obtained from published studies. Information on salinity tolerance for five species (*Acacia constricta*, *Acacia greggii*, *Artemisia ludoviciana*, *Flourensia cernua*, and *Olneya tesota*) were not available



The magnitude of fractionation increased with increasing salt tolerance among 12 species for which salinity tolerance information was available (Fig. 2, $\delta^2\text{H} = 0.29[\text{EC}] + 0.63$, $P < 0.05$, $R^2 = 0.39$). Species that did not fractionate hydrogen isotopes in water had the lowest reported salinity tolerance of these 12 species, ranging in tolerable electrical conductivity (EC) from 1.84 to 10 dS m⁻¹ (Table 1). The range of salinity tolerance for species that significantly fractionated the

hydrogen isotopes was from 6 to 20 dS m⁻¹ (Table 1).

Variation in $\delta^2\text{H}$ and $\delta^{18}\text{O}$ among tissues and organs in *Prosopis velutina*

Leaf water of *P. velutina*, as expected, had higher $\delta^2\text{H}$ ($\Delta^2\text{H} = -59 \pm 1\%$, $P < 0.0001$) and $\delta^{18}\text{O}$ ($\Delta^{18}\text{O} = -25.1 \pm 0.6\%$, $P < 0.001$) values compared to the soil water (Fig. 3). Mean $\Delta^{18}\text{O}$ value

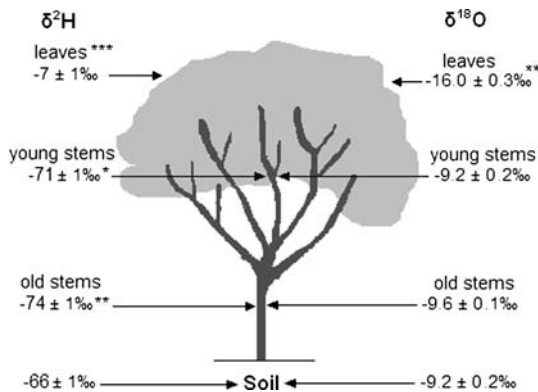


Fig. 3 Mean isotopic composition of water extracted from woody, green stems (young), woody stems with well-developed bark (old), leaves and soil from five potted individuals of *P. velutina*. Two-tailed paired *t*-tests were used to determine the statistical significance of mean differences between the isotopic composition of water from stems and soil samples. $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values of plant organs that were significantly different from that of the soil are marked with an asterisk (* $P < 0.01$, ** $P < 0.001$, *** $P < 0.0001$). Mean $\delta^{18}\text{O}$ values of water extracted from old stems were slightly more positive than that of soil water ($P = 0.07$)

was -0.3 ± 0.3 ‰ for woody, green (young) stems ($P = 0.36$) and 0.4 ± 0.2 ‰ for older stems ($P = 0.07$). Mean $\Delta^2\text{H}$ value of water in young and old stems was 5 ± 1 ‰ ($P < 0.01$) and 8 ± 1 ‰ ($P < 0.001$), respectively. The $\Delta^2\text{H}$ and $\Delta^{18}\text{O}$ values of xylem water of the young stems were intermediate between that of the xylem water of the old stems and that of the leaves (Fig. 3). The two soil samples collected from each pot were similar isotopically.

The pattern of variation among plant tissues and organs of *P. velutina* was similar for hydrogen and oxygen isotopes, with the water in bark having the highest $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values and water in roots having the lowest $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values relative to soil (Fig. 4). The $\delta^2\text{H}$ value of water in bark was higher by 6 ± 1 ‰ ($P < 0.01$) than that of the sapwood. However, the $\delta^2\text{H}$ value of water in bark was not significantly higher than that of source water ($P = 0.82$), while the $\delta^{18}\text{O}$ value of water in bark was higher by 0.7 ± 0.1 ‰ ($P < 0.01$). The $\delta^2\text{H}$ value of water in whole stem segments was only slightly higher than that of sapwood (2 ± 1 ‰, $P = 0.06$). Although the bark was thin, it was 33 to 43% of the water in the entire stem, contributing substantially to the isotopic composition of the stem. The plant organ exhibiting the largest

positive isotopic separation in hydrogen isotope values compared to soil water was the root ($\Delta^2\text{H} = 7 \pm 1$ ‰, $P < 0.05$), but the mean $\delta^{18}\text{O}$ value of water in roots was identical to that of soil (i.e., $\Delta^{18}\text{O} = 0$). Water in whole stem segments and sapwood had significantly lower $\delta^2\text{H}$ values than the soil source water ($P < 0.01$ and $P < 0.001$, respectively). The $\delta^{18}\text{O}$ values of water in whole stem segments and sapwood collected separately were higher than that of the soil water, having $\Delta^{18}\text{O}$ values of -0.4 ± 0.1 ‰ ($P < 0.05$) and -0.4 ± 0.1 ‰ ($P < 0.01$), respectively.

Isotopic separation in whole stem segments, sapwood, and root of the plant collected at predawn was less than that measured in the same organs and tissues of plants collected at midday ($\Delta^2\text{H}$ at predawn = 3, 2, and 5, respectively). Bark, collected at predawn, had a higher $\delta^2\text{H}$ value than bark collected from plants at midday ($\Delta^2\text{H} = -6$). For plants collected at predawn and midday, bark had higher $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values than all other plant parts except leaves.

Isotopic variation in the whole stem segments, sapwood, and bark was a mixture of xylem water and phloem enriched in ^2H and ^{18}O by cuticular evaporation in the stems and backflow from the leaves. The $\delta^2\text{H}$ values of water in whole stem segments, sapwood, and bark were intermediate between the $\delta^2\text{H}$ values of the roots and the calculated $\delta^2\text{H}$ value of the leaf (Fig. 4). The $\delta^{18}\text{O}$ values of water in roots, whole stem segments, sapwood, and bark were intermediate between the $\delta^{18}\text{O}$ values of the soil water and the calculated $\delta^{18}\text{O}$ value of the leaf (Fig. 4).

Hydrogen isotope fractionation in live and heat-killed intact root systems

Evaporation during sample collection and fractionation during sample distillation and isotopic analysis did not have any significant effect on the isotopic composition of the water samples. The mean $\delta^2\text{H}$ value of five 20 μl samples of water used to test the effect of sample collection and processing was only 2 ± 1 ‰ different than control water not subjected to the collection and extraction processes, and that of samples greater than 20 μl in volume was 1 ± 1 ‰ different than control samples. Water samples collected during the

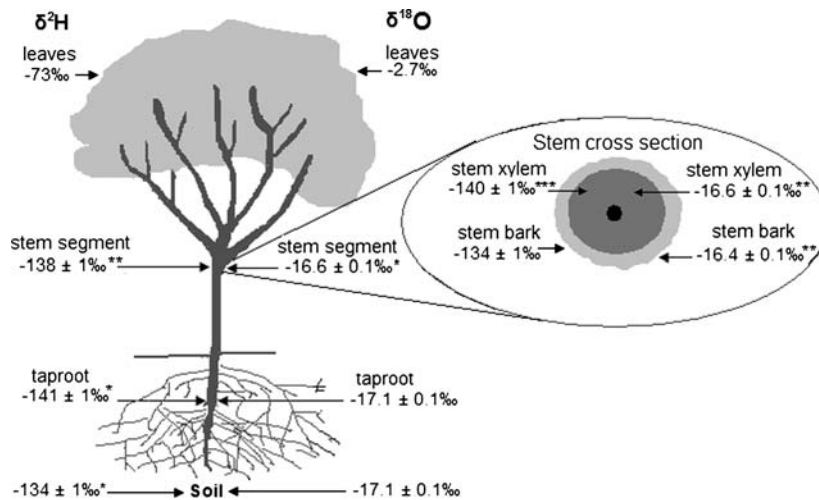


Fig. 4 Mean isotopic composition of leaves, whole stem segments, sapwood, bark, and roots of four potted *Prosopis velutina* plants. The isotopic composition of the leaf was calculated a modified form of the Craig and Gordon model (Yakir and Sternberg 2000). Two-tailed paired *t*-tests were used to determine the statistical significance of mean differences between the isotopic composition of water

from plant tissue and soil samples. $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values of plant organs that were significantly different from that of the soil are marked with an asterisk (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). The inset represents sapwood (inner region) and bark (outer region) of the stem and their respective isotopic compositions

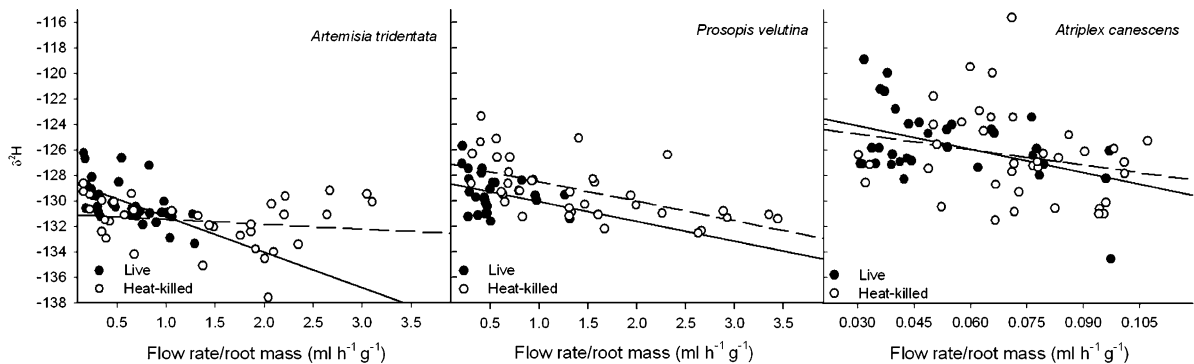


Fig. 5 The relationship between the $\delta^2\text{H}$ of water expressed from whole, severed root systems and flow rate per unit root mass of three xerophytic woody species. Data are composites from three individuals of each species. Statistically significant linear regressions (solid lines) were observed for data from live root systems of *Artemisia*

tridentata ($y = -2.8x - 128.5$, $R^2 = 0.34$, $P < 0.001$) and *Atriplex canescens* ($y = -61.3x - 122.2$, $R^2 = 0.2$, $P < 0.01$). Disruption of the plasma membranes from excessive heat by boiling did not significantly alter the relationship between flow rate and the $\delta^2\text{H}$ of water expressed from the root systems in these species (dashed lines)

pressurization experiment that were 20 μl or less in volume were discarded from the analyses.

$\delta^2\text{H}$ values of the expressed water from the live root systems decreased significantly with increasing flow rate per unit root mass in *A. tridentata* ($y = -2.8x - 128.5$, $R^2 = 0.34$, $P < 0.001$) and *A. canescens* ($y = -61.3x - 122.2$, $R^2 = 0.2$, $P < 0.01$). The relationship between flow rate and $\delta^2\text{H}$

values of water expressed from the root was not significant in the live root system of *P. velutina* ($P = 0.12$; Fig. 5).

Disruption of the plasma membranes of the root by excessive heat from boiling did not significantly alter the relationship between flow rate per unit root mass and $\delta^2\text{H}$ in any of the three species when the relationship was compared over the

same range of flow rates in both live and heat-killed root systems (Fig. 5). The relationship between flow rate and $\delta^2\text{H}$ was significantly negative in the heat-killed root system of *A. tridentata* ($y = -2.1x - 129.9$, $R^2 = 0.22$, $P < 0.05$) and in *P. velutina* ($y = -2.9x - 125.8$, $R^2 = 0.24$, $P < 0.05$), but neither relationship was significantly different than the relationship produced in the corresponding live root systems. The relationship between flow rate per unit root mass and $\delta^2\text{H}$ was not significant in heat-treated root systems of *A. canescens*, nor was the relationship significantly different from the relationship produced from the corresponding live root system.

Discussion

Extent of hydrogen isotope fractionation among woody xerophytes

All 12 species that fractionated hydrogen isotopes in water during uptake in this study are adapted to saline or xeric environments. Such species often develop highly suberized and lignified cell walls in their roots, especially the Casparian strip on the endodermis cell walls, to prevent water loss to dry or highly saline soils and to exclude salts from entering the root (Craig et al. 1990; Nobel and Sanderson 1984; Poljakoff-Mayber 1975; Reinoso et al. 2004; Serrato Valenti et al. 1991; Serrato Valenti et al. 1992; Sharma 1982; Vartanian 1981). Both salinity and drought tolerance result in part from suberization of the endodermis and exodermis, which proportionally increases symplastic movement of water in the root (Feder and Taube 1952; Passioura 1981; Stewart and Friedman 1975). Though some plants such as *A. canescens* and *Tamarix chinensis* use salt secretion from salt glands and storage in tissues to remove excess salt, the adaptive features of salt exclusion such as suberization of the root endodermis and exodermis are still present in most halophytic species to reduce salt concentrations entering the root (Scholander et al. 1966; Shannon 1997). The water movement through the symplastic pathway potentially fractionates hydrogen isotopes in water (Karan and Macey 1980).

With 12 of the 16 desert species examined in this experiment exhibiting hydrogen isotope fractionation, halophytic and xerophytic species must be examined for fractionation of hydrogen isotopes in water during uptake by roots before plant water source studies that use isotopic composition of xylem water are conducted. Only a small quantity of desert species were included in this study, yet numerous species were found to fractionate hydrogen isotopes in water, having $\Delta^2\text{H}$ values from $3 \pm 1\text{‰}$ in *Chrysothamnus nauseosus* to $9 \pm 1\text{‰}$ in *P. velutina* (Fig. 1). Other halophytic and xerophytic species, yet to be examined, are likely also to fractionate hydrogen isotopes in water during uptake from soil, potentially leading to errors in water source calculations using linear mixing models. For example, if the differences in the $\delta^2\text{H}$ values of the plant water sources are small and hydrogen isotope fractionation in water during uptake by roots results in a large $\Delta^2\text{H}$ values, such as 9‰ in *P. velutina*, miscalculations in water source use would be quite large and would lead to misinterpretations of plant water-source use and the role the species plays in hydrologic processes at the ecosystem or larger scales.

Since anatomical features of roots conferring salinity tolerance cause symplastic movement of water in the root, salinity tolerance was used as an indicator of the predominance of the symplastic pathway in water movement (Fig. 2). Species with high salinity tolerance tended to fractionate hydrogen isotopes more so than those with limited salinity tolerance. The relationship between salt tolerance and apparent fractionation reported here provides more evidence that the halophytic properties of salt tolerant plants, such as the reliance on the symplastic pathway for water movement into the root, contributes to the fractionation of hydrogen isotopes in water.

Another mechanism potentially accounting for the lower observed $\delta^2\text{H}$ values of xylem water than the source water is hydrogen ion exchange between water entering the root and apoplastic H^+ pumped into the apoplast and from organic acids secreted from the root. Fractionation of the apoplastic H^+ is high (670‰; Luo et al. 1991). Assuming the apoplastic pH is 5.5 and incoming water had an extremely high

pH of 9.0, the quantity of H^+ needed to change the pH from 5.5 to 9.0 would cause a decrease of the hydrogen isotope ratio of apoplastic water by only 0.002‰. The decrease in 2H of water entering the root by exchange with apoplastic H^+ apparently is not sufficient to produce the low δ^2H values of stem xylem water measured in this experiment.

Variation in δ^2H and $\delta^{18}O$ among tissues and organs in *Prosopis velutina*

Low δ^2H values in xylem water in young and old stem classes relative to source water likely was the result of an ultrafiltration process occurring as water entered the root through a symplastic pathway. Low δ^2H values in xylem water in young and old stems was not a result of cuticular evaporation or isotopic contamination from leaf water because these processes would lead to higher rather than lower δ^2H values in xylem water relative to source water. Isotopic variation in the soil did not account for the isotopic variation expressed in the stems as the two soil samples collected from each pot showed no significant difference in either 2H or ^{18}O . The old stems had marginally lower δ^2H values ($P = 0.07$) than source water. One would expect some depletion of ^{18}O , albeit small, as water enters the plant according to our hypothesis of isotopic fractionation occurring as water passes through the plasma membranes in the roots. However, because of the relative mass differences, it is expected that the depletion of the heavy isotope in stem xylem water compared to that of the soil was principally found for hydrogen (Fig. 3).

Isotopic composition of young stems, whole stem segments, bark, and sapwood in both experiments represented a mixture of 2H -enriched phloem water from the leaves and 2H -depleted root xylem water. Leaf water, enriched in 2H and ^{18}O by evaporation during transpiration is the likely source for the enrichment in ^{18}O and 2H of water in young stems with respect that in the old, suberized stems (Fig. 3). Isotopically heavy leaf water is also the cause of ^{18}O and 2H enrichment in water of bark, whole stem segments, and sapwood relative to root xylem water (Fig. 4, Dawson and Ehleringer 1993). On the other

hand, root xylem water depleted in 2H , caused δ^2H values in young and old stems, whole stem segments, sapwood, and roots to be lower than the source water for both experiments. The older, woody stems of these *P. velutina* plants had fully developed protective bark and were likely suberized, so that evaporative enrichment in 2H and ^{18}O was minimal. Mature stems with developed bark generally have a lower proportion of their water in the phloem, so the effects of contamination from isotopically heavy phloem water are smaller than in young stems (Dawson and Ehleringer 1993; Thorburn et al. 1993). As a result, evaporation from the young stems and backflow in the phloem from the leaves caused greater enrichment in 2H than in the older stems (Fig. 3). Also sufficient exchange occurred between isotopically heavy phloem water and xylem water to cause the xylem water in the sapwood to be enriched in ^{18}O with respect to the soil water and to be enriched in 2H with respect to the root xylem water (Fig. 4).

Fractionation of hydrogen isotopes during uptake in *P. velutina* occurred in the root. The most negative δ^2H values of water observed in potted *P. velutina* plants was from root xylem water samples, indicating that the root–soil interface was the likely location of fractionation (Fig. 4). However, the $\delta^{18}O$ of xylem water in roots was not significantly lower than that of source water, indicating minimal fractionation of oxygen isotopes in water during uptake. The genus *Prosopis* is reported to exclude salt in roots to tolerate high salinity (Reinoso et al. 2004). *Prosopis* species develop lignified and suberized root endodermis and exodermis cell walls and a Casparian strip that develops faster and closer to the root tip than in salt-intolerant species, forcing water to move *via* the symplastic pathway (Reinoso et al. 2004; Serrato Valenti et al. 1991; Serrato Valenti et al. 1992).

The magnitude of isotopic fractionation of 2H in water during uptake by roots in *P. velutina* varied between midday and predawn periods. At predawn, during a period with minimal transpiration, a low Δ^2H value was observed, but did not reach isotopic equilibrium with the soil. Bark collected at predawn was enriched in 2H with respect to the soil water. The first possibility suggests that

when transpiration dropped to low levels at pre-dawn, isotopically light xylem water moving up from the root contributed less to the $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values of the water in the plant tissues, making the overall contribution of isotopically heavy water in the phloem proportionally larger, resulting in higher $\delta^{18}\text{O}$ and $\delta^2\text{H}$ values. The second possibility, as hypothesized Lin and Sternberg (1993), is that the fractionating process of ultrafiltration can occur as water enters the root from the rhizosphere and as water leaves the root. At low transpiration rates, the fractionation event of water entering the root would be only slightly higher than the fractionation event of water leaking from the root, therefore the magnitude of isotopic separation would be negligible.

Hydrogen isotope fractionation in live and heat-killed intact root systems

The hypothesis that water movement through the symplastic pathway fractionates hydrogen isotopes in water during uptake by roots when symplastic water movement predominates is consistent with observations that the $\delta^2\text{H}$ values of the water expressed from the root system decreased with increasing flow rate in *A. canescens* and *A. tridentata*. *A. tridentata* apparently did not fractionate hydrogen isotopes in water in the survey study using 16 woody xerophyte and halophyte taxa reported above. Stems collected from *A. tridentata* in the survey study were attached to several green leaves. It is likely that the xylem water was enriched in ^2H and ^{18}O by evaporation and back flow through the xylem as whole stem segments were sampled.

The relationship between flow rate and $\delta^2\text{H}$ of expressed xylem water was not significant in live root systems of *P. velutina*, although hydrogen isotope fractionation was observed in the experiments described above. The range of pressures used in this experiment to produce a gradient in flow rate resulted in only a small range of flow rates of water expressed from the live root system of *P. velutina*. A larger range of flow rates may have resulted in greater variation in $\delta^2\text{H}$ values of the expressed water from the live root system and a significant relationship between flow rate and $\delta^2\text{H}$ of the expressed water from the root.

Destroying the plasma membranes by excessive heat did not alter the relationship between flow rate and $\delta^2\text{H}$ of the water expressed from the root. Flow rates of water moving through the heat-killed root systems of *A. tridentata* and *P. velutina* increased with respect to the live root systems, showing that resistance to flow decreased after the root system was immersed in boiling water. However, this increase in flow rate did not alter the relationship between flow rate and $\delta^2\text{H}$, contrary to our prediction. Immersing the root systems of *A. canescens* in boiling water to disrupt the root plasma membranes did not increase flow rates or alter the relationship between $\delta^2\text{H}$ of water expressed from the root system and flow rate. Possibly the plasma membranes were not fully destroyed by excessive heat.

Evaporation during collection of water samples and fractionation during distillation and isotopic analysis of water samples did not have any significant effect on the isotopic composition of the samples. Therefore these results were not the result of fractionation due to experimental design. Water collected from the pressurization chamber before the pressurization experiment was conducted did not vary isotopically from water samples collected from the pressurization experiment after the experiment was concluded; therefore, isotopic heterogeneity was not responsible for the isotopic variation of water samples collected from the root system during the pressurization experiment.

Differences between positive pressure and tension could explain the results that $\delta^2\text{H}$ values of the water expressed from root systems were higher at lower flow rates with respect to the source water in the pressurization chamber. As the flow rate increased, $\delta^2\text{H}$ values became more similar to the $\delta^2\text{H}$ value of the source water ($\delta^2\text{H} = -132\text{‰}$). The positive pressure needed to push water through the xylem conduits is of the same magnitude but opposite sign as the tension required to pull water through the xylem (Tyree and Sperry 1989). Though the response of pressure to flow is the same if the pressure is positive or negative, the effect that positive pressure has on the ultrafiltration process of water passing through a membrane is unknown. This higher $\delta^2\text{H}$ values with respect to the source water at lower

flow rates may be due to the positive pressure instead of tension that was exerted on the root system. Positive pressure might have eliminated the fractionating process of ultrafiltration. If water leaking from the root still underwent the fractionating process of ultrafiltration, the effect of water entering and leaving the root would cause water inside the root to be enriched in ^2H with respect to soil water. As the net quantity of water entering the plant increases, the proportional importance of water leaking from the root would decrease. As a result, the $\delta^2\text{H}$ values of the water entering the root would approach that of the source water. In this case the expected results would be similar to the results obtained in this experiment where the water expressed from the severed root system would have lower $\delta^2\text{H}$ values than the source water at low flow rates and similar to the source water at higher flow rates.

Conclusions

Although this study did not fully resolve the mechanism responsible for fractionation, it does provide more evidence to support the hypothesis that symplastic movement of water during uptake fractionates hydrogen isotopes. Halophytic and xerophytic species rely more heavily on the symplastic water transport pathway in the roots than the apoplastic pathway due to the high degree of endodermal and exodermal cell wall development associated with salt and drought tolerance, and consequently fractionate hydrogen isotopes in water. Twelve of the 16 xerophytic and halophytic species examined in this study exhibited fractionation of hydrogen isotopes in water during uptake by roots. The magnitude of isotopic separation was positively correlated with salinity tolerance, showing that adaptations to salinity tolerance result in hydrogen isotope fractionation. Roots, having the largest $\Delta^2\text{H}$ values, were the apparent location of fractionation, and the isotopic composition of whole stem segments was a mixture of ^2H -enriched leaf water transported through the phloem and ^2H -depleted xylem water from the root. The $\delta^2\text{H}$ values of xylem water did not accurately reflect the $\delta^2\text{H}$ values of soil water. However, $\delta^{18}\text{O}$ values of

xylem water of the same species that fractionated hydrogen isotopes in water did accurately reflect the $\delta^{18}\text{O}$ values of the soil water. Also, as predicted, the $\delta^2\text{H}$ values of water expressed from live root systems decreased as flow rate through the roots increased. However, disrupting root plasma membranes by excessive heat did not have a consistent effect on the relationship between flow rate and $\delta^2\text{H}$ among three species examined.

Acknowledgements We thank Enrico Yepez, Victor Resco, Rico Gazal, and Ayme Ahrens for their help with the greenhouse experiments. We also thank Ann Hild and Brent Ewers for their help during the manuscript preparation.

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