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Photosynthetic gas exchange and the stable isotope composition of leaf water: comparison of a xylem-tapping mistletoe and its host

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ABSTRACT

Photosynthetic gas exchange and the stable isotopic composition of foliage water were measured for a xylem tapping mistletoe, *Phoradendron juniperinum*, and its host tree, *Juniperus osteosperma*, growing in southern Utah. The observed isotopic composition of water extracted from foliage was compared to predictions of the Craig–Gordon model of isotopic enrichment at evaporative sites within leaves. Assimilation rates of juniper were higher and stomatal conductance was lower than the values observed for the mistletoe. This resulted in lower intercellular/ambient CO_2 values in the juniper tree relative to its mistletoe parasite. For mistletoe, the observed foliage water hydrogen and oxygen isotopic enrichment was less than that predicted by the model. In juniper, foliage water hydrogen isotopic enrichment was also lower than that predicted by the evaporative enrichment model. In contrast, the oxygen isotopic enrichment in juniper foliage water was slightly greater than that predicted for the evaporative sites within leaves. Hydrogen isotopic enrichment in mistletoe foliage shows systematic variation with stem segment, being highest near the tips of the youngest stems and decreasing toward the base of the mistletoe, where isotopic composition is close to that of stem water in the host tree. In a correlated pattern, mid-day stomatal conductance declined abruptly in mistletoe foliage of increasing age.

Key-words: D/H ratios; $^{18}O/^{16}O$ ratios; transpiration; stomatal conductance; aridlands.

INTRODUCTION

Water in plant leaves is enriched in the heavy isotopes of oxygen and hydrogen relative to plant stem water (Dongmann *et al.* 1974; White 1988; Flanagan & Ehleringer 1991a). The enrichment occurs because of isotopic fractionation during leaf transpiration. There are two fractionation processes during transpiration, an equilibrium effect resulting from the phase change from liquid water to water vapour, and a kinetic effect caused by the different diffusion rates of the light and heavy water vapour in air. Isotopic exchange may also occur between water vapour in the atmosphere and leaf water. A model incorporating these fractionation and exchange processes, originally developed by Craig & Gordon (1965) for evaporation from the ocean, has been applied to leaf transpiration (Dongmann *et al.* 1974; White 1988; Flanagan *et al.* 1991b). Under controlled environmental conditions, the observed isotopic enrichment of bulk leaf water is less than that predicted by the model for the evaporative sites within leaves (Flanagan *et al.* 1991b). Much of the difference between the modelled and observed leaf water isotopic compositions may result from gradients within the leaf caused by a shifting balance between the bulk flow of unfractionated water into the leaf (convection) and the back diffusion of heavy isotope molecules away from the sites of evaporative enrichment (Farquhar & Lloyd 1993). The extent of the difference between modelled and observed leaf water isotopic compositions has been shown to be positively related to leaf transpiration rate (White 1988; Walker *et al.* 1989; Flanagan *et al.* 1991), a result consistent with the convection-diffusion model of isotope mixing within leaves.

The isotopic composition of plant leaf water is thought to influence the oxygen isotopic composition of atmospheric CO_2 (Francey & Tans 1987; Friedli *et al.* 1987). Francey & Tans (1987) have documented a latitudinal gradient in the $^{18}O/^{16}O$ values of atmospheric CO_2. They proposed that the gradient results from latitudinal variation in the isotopic composition of environmental waters (Dansgaard 1964) and isotopic exchange of oxygen in atmospheric CO_2 with oxygen in leaf (chloroplast) water catalysed by carbonic anhydrase. In support of the latter proposal, Farquhar & Lloyd (1993) and Farquhar *et al.* (1993) describe a mechanistic model of $C^{18}O/^{16}O$ fractionation during photosynthetic gas exchange and provide evidence for the role of oxygen isotopic exchange with chloroplast water as a primary factor influencing the $^{18}O/^{16}O$ vaues of atmospheric

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CO_2. Therefore, the model of leaf water isotopic enrichment may have important applications in studies of the oxygen isotopic composition of atmospheric CO_2 and global CO_2 exchange processes.

Before the evaporative enrichment model can be routinely applied in ecological and physiologial studies, it is necessary to know how well it predicts the isotopic composition of leaf water under field conditions. In this paper, we make comparisons between the predictions of the evaporative enrichment model and the observed isotopic composition of foliage water in a xylem-tapping mistletoe, *Phoradendron juniperinum*, and its host, *Juniperus osteosperma* (juniper), exposed to natural environmental conditions in the field. The mistletoe-juniper system is useful for studies of leaf water isotopic enrichment because both plants have the same source water isotopic composition, but they have different assimilation and transpiration rates (Ehleringer *et al.* 1985; Ehleringer, Cook & Tieszen 1986). Therefore, comparisons between the mistletoe and juniper can be used to study aspects of the influence of transpiration rate on foliage water isotopic enrichment under file conditions.

MATERIALS AND METHODS

Study site and plant species

The field component of the study was conducted at Coral Pink Sand Dunes State Park, Utah, USA (37° 3′ N, 112° 45′ W, elevation 1855 m). The study site was a Pinyon-Juniper woodland (West 1988), where *Juniperus osteosperma* was the dominant tree species and *Pinus edulis* was also abundant. *Artemisia tridentata* and *Chrysothamnus nauseosus* were the dominant shrub species. The vegetation occurred on deep, poorly developed, sandy soil. The two study species were a xylem-tapping mistletoe, *Phoradendron juniperinum*, and its host, *Juniperus osteosperma*.

Leaf photosynthetic gas exchange measurements

Measurements of CO_2 and water vapour flux were made on intact foliage samples on three branches in each of two trees with a portable photosynthesis system (Li-Cor 6200, LiCor Inc., Lincoln, NE, USA). Both mistletoe and juniper foliage were sampled at intervals throughout the day on 9 June and 23 July 1989. Measurements were made on young portions of foliage near branch tips for both mistletoe and juniper. Stomatal conductance measurements were also made during 23 June 1989 on different mistletoe branch segments with a portable porometer (Li-Cor 1600, LiCor Inc.), modified with a piece of clear teflon film under the gasket to obtain a seal around the mistletoe foliage.

Environmental measurements and sample collection

Measurements of leaf and air temperature were made with fine-wire copper-constantan thermocouples connected to a solid-state datalogger (CR21X, Campbell Scientific Inc., Logan, UT, USA). Sensors for leaf and air temperature were located at two positions within the canopy of a single tree. At each position, both mistletoe and juniper foliage temperatures were measured. Measurements of air relative humidity were made with a capacitance humidity proble (RH and Temperature Probe 207, Campbell Scientific Inc.) and measurement of photon flux density with a quantum sensor (LI 190SB, LiCor Inc.) both connected to the datalogger. The quantum sensor and humidity probe were located equidistant between the temperature sensors which were approximately one meter apart. Measurements of leaf temperature, air temperature and relative humidity were made so that the partial pressure of water vapour in the leaf intercellular air spaces, and ambient air could be calculated. Sensors were scanned every minute and readings were averaged for 30-min intervals throughout the day. Atmospheric water vapour was collected by pumping (TD-3LL Pump, Brailsford & Co. Inc., Rye, NY, USA) air through two glass traps cooled with an ethanol-dry ice slurry.

Measurement of the diurnal course of leaf and air temperatures, relative humidity and photon flux density were made during 23 June 1989. Foliage samples were collected every 2 h, from 500 to 2300 h, from both the mistletoe and juniper in locations immediately next to, and in the same orientation as the two positions for the leaf and air thermocouples. The samples collected included only the young portions of foliage near branch tips for both mistletoe and juniper. Juniper twig (stem) samples were collected at 500, 1300 and 1700 h. At midday different aged segments of mistletoe foliage were also collected (in association with the stomatal conductance measurements described above). Foliage and stem samples were removed from the plant and placed in a glass tube which was sealed with a rubber stopper and wrapped with parafilm. The plant samples were placed on dry ice and returned to the laboratory where they were stored in a freezer until water was extracted from the tissue using a cryogenic vacuum distillation apparatus [see Dalton (1989) for a schematic diagram of an apparatus similar to the one used in our studies]. Atmospheric water vapour was collected for a 4-h period starting at 1100 h.

Isotopic analysis

Water samples were prepared for measurements of the hydrogen isotopic composition by reacting approximately 5 mm^3 of water with zinc in an evacuated tube at 500°C (Coleman *et al.* 1982). The zinc was obtained from the laboratory of J.M. Hayes, Departments of

Chemistry and Geology, Indiana University. The resulting hydrogen gas was analysed for isotopic composition on either a Finnigan-MAT delta E or delta S gas isotope ratio mass spectrometer. Precision of the hydrogen isotopic analysis was determined by repeated sampling of a laboratory standard which had been calibrated in relation to SMOW obtained from the U.S. National Bureau of Standards and had also been analysed by two independent stable isotope laboratories. The precision of measurements of the laboratory standard was ± 1·4‰ for the delta E and ± 1·0‰ for the delta S mass spectrometers (± SD).

Water samples were prepared for measurements of the oxygen isotopic composition by converting the oxygen in water to CO_2 using the guanidine hydrochloride method (Wong, Lee & Klein 1987). The CO_2 gas was then analysed for oxygen isotopic composition on the mass spectrometers described above. Precision of the method was checked by making repeated measurements of SMOW. Precision was found to be ±0·31‰ for the delta E and ±0·12‰ for the delta S mass spectrometers during the course of this study.

Modelling leaf water isotopic composition

A model of isotopic fractionation, originally developed by Craig & Gordon (1965) for evaporation from the ocean, was used to model the isotopic composition of water at the evaporative sites within foliage. The model can be expressed in the following form (Flanagan & Ehleringer 1991a; Flanagan *et al.* 1991b):

$$R_e = \alpha^* \left[\alpha_k R_s \left(\frac{e_i - e_a}{e_i} \right) + R_a \left(\frac{e_a}{e_i} \right) \right] \quad (1)$$

where R is the molar ratio of the heavy to light isotope and the subscripts e, s and a refer to water at the evaporative sites within leaves, stem water and atmospheric water vapour, respectively; e is the partial pressure of water vapour and the subscripts i and a refer to the leaf intercellular air spaces and the ambient air, respectively; α^* is the equilibrium fractionation factor. The regression equations listed by Majoube (1971) were used to calculate alues for the parameter α^* at specific leaf temperatures. α_k Is the kinetic fractionation factor which is determined by the relative rates of molecular diffusion of the light and heavy isotope molecules in air. The values for α_k are: H/D = 1·025 and $^{16}O/^{18}O$ = 1·0285 (Merlivat 1978).

Stem water and atmospheric water vapour isotopic ratios were expressed relative to SMOW, using delta notation (‰) as shown below:

$$\delta = \left[\frac{R_{Sample}}{R_{Standard}} - 1 \right] \times 1000 \quad (2)$$

where R is the molar ratio of the heavy to light isotope (D/H or $^{18}O/^{16}O$). The modelled isotopic ratio of water at the evaporative sites and observed foliage water isotopic ratios were expressed relative to stem water, using discrimination notation (‰) as shown below:

$$\Delta = \left[\frac{R_{Sample}}{R_{Stem}} - 1 \right] \times 1000 \quad (3)$$

The model of Farquhar & Lloyd (1993) was used to relate the isotopic ratio of total foliage water (Δ_L) to that at the evaporative sites within leaves (Δ_E) as shown below:

$$\Delta_L = \frac{CD}{EL} \Delta_E \left(1 - e^{\frac{-EL}{CD}}\right) \quad (4)$$

where C is the molar concentration of water ($5{\cdot}5555 \times 10^4$ mol m^{-3}); D is the diffusivity of HDO ($2{\cdot}34 \times 10^{-9}$ m^2 s^{-1}), or $H_2{}^{18}O$ ($2{\cdot}66 \times 10^{-9}$ m^2 s^{-1}) in water (Wang 1954); E is the transpiration rate (mol m^{-2} s^{-1}); and L is the effective pathlength (m), averaged over the total leaf, for movement of water from leaf xylem veins (unfractionated) to the sites of evaporative, isotopic enrichment.

RESULTS

Photosynthetic gas exchange characteristics

Assimilation rates of juniper were higher and stomatal conductance was lower than the values observed for mistletoe (Fig. 1). This resulted in lower intercellular/ambient CO_2 values in the juniper tree relative to its mistletoe parasite (Fig. 1). There was a peak in assimilation and stomatal conductance at or before noon (local time) for both juniper and mistletoe. The gas exchange measurements were quite variable among different branches of a host tree, or among different mistletoe individuals. A portion of the variation was due to fluctuations in photon flux density during measurement periods.

Diurnal pattern of foliage water isotopic discrimination

The mistletoe and juniper plants were exposed to rather large, diurnal fluctuations in environmental conditions at the study site (Fig. 2). Leaf and air temperatures were closely coupled and varied approximately 18°C during the course of the day. Relative humidity was low, averaging approximately 12% during mid-day (Fig. 2).

Measurements of air and leaf temperature, and relative humidity were used along with the isotopic composition of both plant stem water and atmospheric water vapour to model isotopic discrimination at the evaporative sites within foliage, Δ_E, using Eqn 1. For oxygen isotopes, the model predicted relatively constant isotopic discrimination during the day. The model also predicted a relatively constant hydrogen isotopic discrimination with a slight decline during midday (Figs 3 & 4). The isotopic composition of the stem (source) water of the juniper tree remained constant during the day (mean value ± 1SD, $n = 3$; $\delta^{18}O = -13{\cdot}2‰ \pm 0{\cdot}3$; $\delta D = -100‰ \pm 0{\cdot}5$).

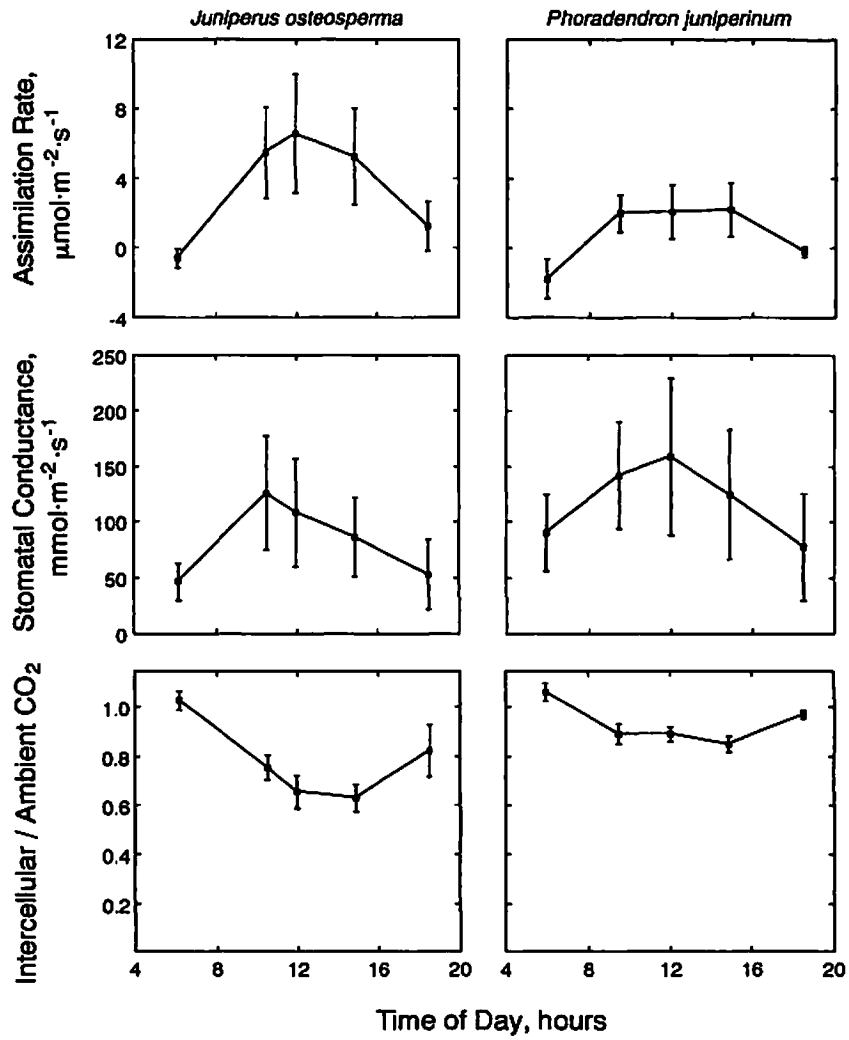

Figure 1. Diurnal changes in photosynthetic gas exchange characteristics in *Juniperus osteosperma* and *Phoradendron juniperinum* under field conditions at Coral Pink State Park, Utah, during 9 June 1989. The symbols represent the mean of six measurements ± 1SD.

For mistletoe, the observed hydrogen and oxygen isotopic discrimination remained relatively constant throughout the day in accordance with the evaporative enrichment model predictions, although the measured leaf water values were lower than values predicted by the model for evaporative sites within leaves (Fig. 3).

The observed foliage water hydrogen isotopic discrimination in juniper was also lower than that calculated by the evaporative enrichment model (Fig. 4). In contrast, the observed oxygen isotopic discrimination in juniper was slightly greater than that predicted for the evaporative sites within leaves (Fig. 4).

The mean difference between the modelled (Δ_E) and observed (Δ_L) hydrogen isotopic discrimination at midday, expressed as a fraction of Δ_E, was higher in mistletoe than in juniper (Fig. 5). This is consistent with the model of Farquhar & Lloyd (1993) and the higher transpiration rates observed for the mistletoe (Fig. 5). The mean effective pathlength, parameter L from Eqn 4, calculated from the observed field data shown in Fig. 5 was 21·6 ± 4·8mm (mean ± SD, $n = 6$) for mistletoe and 26·8 ± 9·5mm (mean ± SD, $n = 6$) for juniper.

Within-plant variation in foliage water isotopic discrimination

The observed hydrogen isotopic discrimination in mistletoe showed systematic variation with stem position. Isotopic discrimination was much higher near the tips of the younger stems than in older foliage segments closer to the base of the mistletoe, which had an isotopic composition close to that of stem water of the host tree (Fig. 6). Stomatal conductance, measured at midday, declined abruptly along sequential positions on a mistletoe stem, so that foliage approximately 10–15 nodes from the mistletoe tip had very little or no gas exchange (Fig. 6).

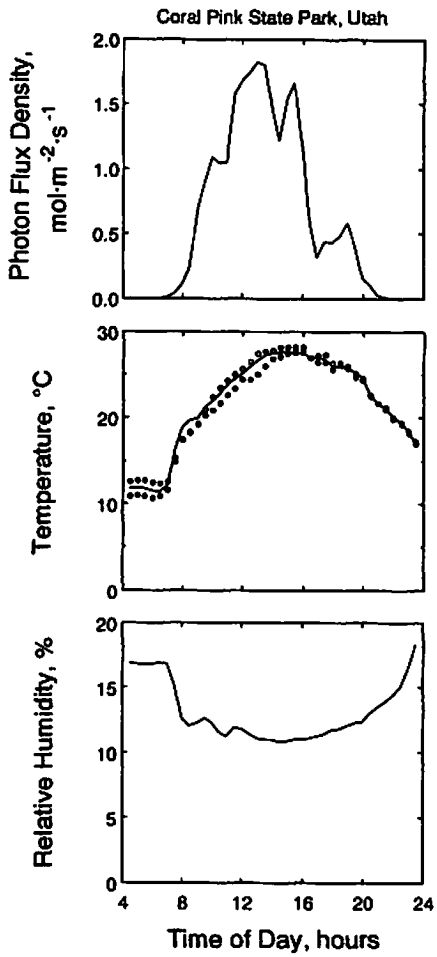

Figure 2. Diurnal changes in environmental parameters and leaf temperature of *Phoradendron juniperinum* and *Juniperus osteosperma* at Coral Pink State Park, Utah, on 23 June 1989. The solid line represents air temperature, the open circles represent juniper foliage temperature, and the solid circles represent mistletoe foliage temperature.

DISCUSSION

Diurnal pattern of leaf water isotopic discrimination

The evaporative enrichment model predicted relatively constant leaf water isotopic discrimination for both hydrogen and oxygen isotopes during the day (Fig. 3 & 4). However, there were small differences in the patterns for the two isotopes, with the model predicting a slight decline in the hydrogen isotopic composition of leaf water at midday. Variation in model predictions are due to the dissimilar relative magnitude of the kinetic and equilibrium fractionation factors for hydrogen and oxygen isotopes, as previously discussed (Flanagan & Ehleringer 1991b).

The observed foliage water isotopic discrimination in both the mistletoe and juniper followed qualitatively similar patterns throughout the day as that predicted by the evaporative enrichment model. Complete quantitative agreement between the calculations for the evaporative sites within leaves and the measured isotopic composition of leaf water was not obtained, however. In most cases, the model predicted a higher degree of heavy isotope enrichment than was actually observed in total foliage water (Figs 3 & 4). Several previous studies have also observed that the Craig & Gordon (1965) evaporative enrichment model overestimates the degree of isotopic enrichment observed in whole leaves (Allison, Gat & Leaney 1985; Bariac *et al.* 1989; Leaney *et al.* 1985; Walker & Brunel 1990; Walker *et al.* 1989; Yakir, DeNiro & Gat 1990), and the possible factors contributing to this difference have been previously discussed (Flanagan *et al.* 1991a, b). Of primary importance are gradients in the isotopic composition of water within the leaf. The gradients probably occur because of a shifting balance between the influx of source water (unfractionated) into the leaf and the back diffusion of heavy isotope molecules away from the sites of evaporative enrichment (Farquhar & Lloyd 1993). The Craig–Gordon (1965) model only predicts the isotopic composition of water at the evaporative, sites, and therefore, would be expected to overestimate the isotopic composition of whole leaf water.

For hydrogen isotopes, the extent of the difference between modelled and observed discrimination was larger for mistletoe than juniper. Such a result may occur because of the higher transpiration rates we observed for mistletoe (Fig. 5). Several other studies have also reported that xylem-tapping mistletoe species have higher transpiration rates than their host trees (Ullmann *et al.* 1985; Ehleringer *et al.* 1985, 1986). The Farquhar & Lloyd (1993) model of convection and diffusion of water within a leaf (Eqn 4), predicts a greater difference between modelled and observed leaf water isotopic compositions with a higher transpiration rate, assuming similar effective pathlengths for water movement in both the mistletoe and juniper (Fig. 5). Calculations of the effective pathlengths for water movement in foliage were similar for juniper (26·8 ± 9·5 mm) and mistletoes (21·6 ± 4·8 mm). These values were larger than calculations of the effective pathlength made for leaves of *Phaseolus vulgaris* (6–9 mm) under controlled environment conditions (Flanagan 1993). In both studies, the calculated effective pathlengths will be much larger than direct measurements of leaf anatomical properties (e.g. the distance from xylem veins to intercellular air spaces) because of the complex movement of water through leaves to the sites of evaporation (Farquhar & Lloyd 1993).

In this study, water extracted from juniper foliage had a higher degree of oxygen isotopic enrichment than that predicted by the evaporative enrichment model. A similar pattern was observed by Walker & Lance (1991) in barley grown in field plots. The recently emerged flag leaf of barley had water that was substantially more enriched in oxygen and hydrogen isotopes than the sequence of three leaves lower down the stem, which had more similar leaf water isotopic compositions. The flag leaf had heavy isotope compositions much higher than that predicted by the evaporative enrichment model, a result that Walker &

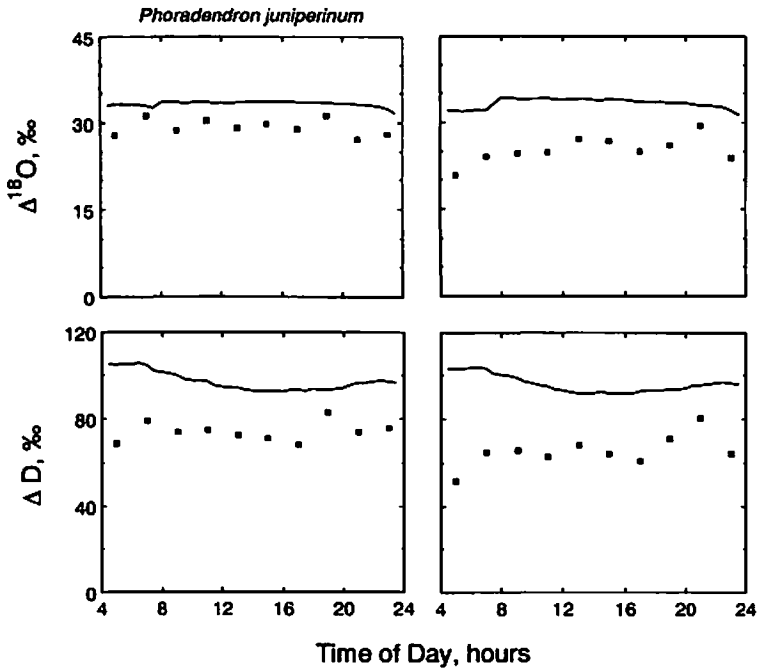

Figure 3. Diurnal pattern of change in the foliage water stable isotopic discrimination in *Phoradendron juniperinum* under field conditions at Coral Pink State Park, Utah, on 23 June 1989. The solid line represents calculated discrimination (using Eqns 1 & 3) at the sites of evaporation within leaves. The solid symbols represent measurements of isotopic discrimination on total water extracted from *P. juniperinum* foliage. Stem water isotopic composition (mean ± SD, $n = 3$): $\delta^{18}O = -13{\cdot}2‰ \pm 0{\cdot}3$, $\delta D = -100‰ \pm 0{\cdot}5$. Isotopic composition of atmospheric water vapour: $\delta^{18}O = -23{\cdot}7‰$, $\delta D = -165‰$.

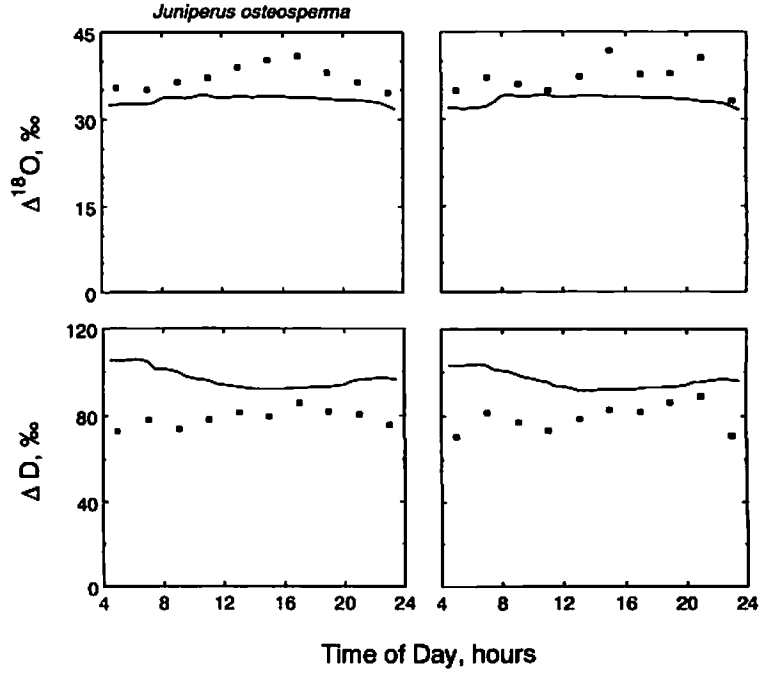

Figure 4. Diurnal pattern of change in the foliage water stable isotopic discrimination in *Juniperus osteosperma* under field conditions at Coral Pink State Park, Utah, on 23 June 1989. The solid line represents calculated discrimination (using Eqns 1 & 3) at the sites of evaporation within leaves. The solid symbols represent measurements of isotopic discrimination on total water extracted from *J. osteosperma* foliage. Stem water isotopic composition (mean ± SD, $n = 3$): $\delta^{18}O = -13{\cdot}2‰ \pm 0{\cdot}3$, $\delta D = -100‰ \pm 0{\cdot}5$. Isotopic composition of atmospheric water vapour: $\delta^{18}O = -23{\cdot}7‰$, $\delta D = -165‰$.

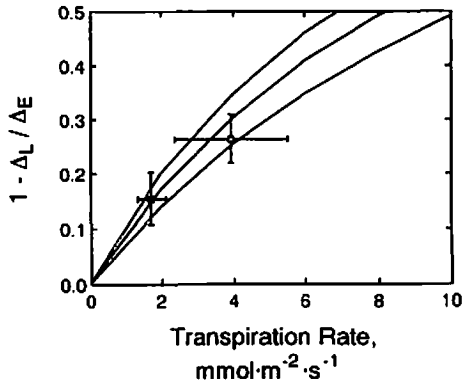

Figure 5. The influence of transpiration rate on the fractional difference between the modelled (Δ_E) and observed (Δ_L) leaf water hydrogen isotopic discrimination in *Juniperus osteosperma* (solid symbol) and *Phoradendron juniperinum* (open symbol). The discrimination values used in the calculation were taken from midday values shown in Figs 3 and 4 (mean ± 1SD, $n = 6$). The transpiration data were measured at midday on 23 July 1989 in the same tree used for the discrimination measurements (mean ± 1SD, $n = 3$). The lines represent calculations done with Eqn 4, using different values for the effective pathlength for water movement (from bottom to top the L values were: 20, 25, 30 mm).

Lance (1991) suggested was caused by the flag leaf containing enriched water imported from the other tissues in addition to that received from soil water. However, it is important to note that our results differ from that observed by Walker & Lance (1991) with barley because only the oxygen isotopic composition of juniper foliage water was enriched above that predicted by the model (Fig. 4). The observed hydrogen isotopic discrimination was below that predicted by the model. The reason for the different patterns observed for hydrogen and oxygen isotopes in juniper is not known.

Within-plant variation in foliage water isotopic discrimination

The isotopic composition of foliage water in the mistletoe is much more enriched in deuterium near the tips of the younger stems than in the older foliage segments closer to the base of the mistletoe (Fig. 6). The correlated sequential decline in stomatal conductance in different positions along a mistletoe stem may have been associated with complete stomatal closure in some sections of the foliage. If no water vapour loss occurs in some sections of the foliage, then there is no opportunity for foliage water to become enriched in heavy isotope. The reason for stomatal closure on the older mistletoe stem sections is presently unclear, since the tissue was green and healthy looking. Calvin (1970) described anatomical evidence of stomatal occlusion due to compression and necrosis of the guard cells on older stems of *Phoradendron flavescens*. An alternative explanation for changes in leaf water isotopic enrichment associated with leaf position is that isotopically enriched water is imported by young leaves from adjacent tissue, as discussed above. However, the maximal isotopic enrichment observed in young mistletoe foliage is similar to that predicted by the evaporative enrichment model (compare Figs 3 & 6). We suggest that the progressive decline in isotopic enrichment from young to older foliage along a mistletoe stem is probably due to complete stomatal closure in portions of older stem segments. The lack of gas exchange observed in older sections of mistletoe foliage may have important consequences for models of mistletoe growth that assume all green tissue is largely self-sufficient for carbon acquisition (Marshall & Ehleringer 1990).

Other studies have also observed systematic leaf-age, canopy-position or within-leaf variation in the isotopic composition of leaf water. Walker *et al.* (1989) have observed that younger leaves of wheat (*Triticum aestivum*) have higher leaf water δD values than older leaf tissue. During the summer months, there was approximately a 5‰ difference between 1- and 3-year-old needles of *Pinus radiata* in the top and middle of the tree canopy for both leaf water δD and the $\delta^{18}O$ values (Allison *et al.* 1985). The younger leaf tissue had the more enriched isotopic composition. This pattern was only observed in the summer months; during the spring, no differences were apparent among different needle age classes. Allison *et al.* (1985) also showed that needles collected from the top of the *Pinus radiata* trees had higher δD and $\delta^{18}O$ values that the same

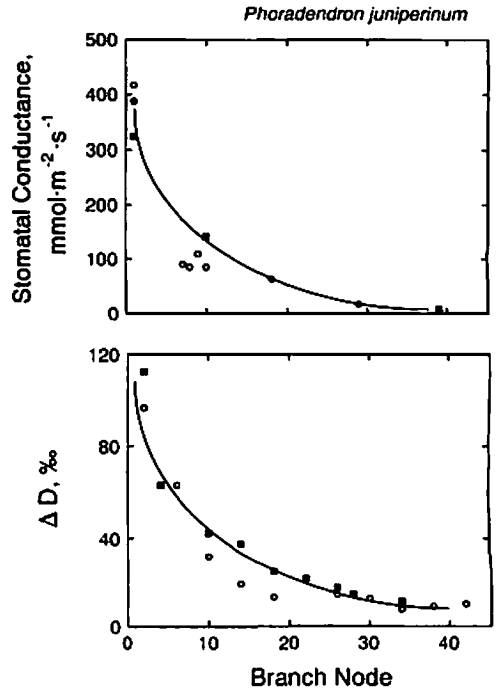

Figure 6. Variation in midday stomatal conductance and foliage water hydrogen isotopic discrimination in stem segments of increasing age in *Phoradendron juniperinum* at Coral State Park, Utah, on 24 June 1989. The different symbols represent measurements made on different mistletoe plants.

age class of needles located in the middle of the tree crown. The canopy-position variation in leaf water isotopic composition was suggested to be associated with possible gradients in relative humidity within the canopy. Luo & Sternberg (1992) have shown large spatial variability in δD values within the mesophyll of a single, large leaf from *Pterocarpus indicus*. They attributed the spatial variation in δD values to non-uniform stomatal closure across the leaf surface.

Application of the evaporative enrichment model in ecological studies

In general, there was good agreement between the predictions of the modelled isotopic composition of water at the evaporative sites within leaves and total water extracted from foliage. Much of the difference between the modelled and observed foliage water values should result from gradients in the isotopic composition of water within leaves (Farquhar & Lloyd 1993; Flanagan 1993), and patchy stomatal closure across the surface of leaves. Young, newly expanding foliage may also import isotopically enriched water from adjacent foliage, thereby causing some difference between modelled and observed isotopic compositions, although this would occur in only a small portion of the total foliage of a plant. These factors should have little effect on the application of the evaporative enrichment model in studies of the isotopic composition of atmospheric CO_2. The isotopic ratio of water in chloroplasts, which are located near sites of evaporation within leaves, is likely very similar to that predicted by the evaporative enrichment model. Exchange of oxygen contained in atmospheric CO_2 with leaf water would occur in chloroplasts near open stomata and should be little affected by gradients in the isotopic ratio of water in the rest of the leaf (Farquhar & Lloyd 1993; Flanagan 1993).

A final consideration in applying the evaporative enrichment model is that it is necessary to measure the isotopic composition of stem water in plants being compared under field conditions. Co-occurring plant species can have diverse rooting patterns and can take up water from different regions within the soil profile, resulting in differences in stem water isotopic composition (White *et al.* 1985; Dawson & Ehleringer 1991; Ehleringer *et al.* 1991; Sternberg *et al.* 1991; Flanagan, Ehleringer & Marshall 1992). Gradients in the isotopic composition of water within soils can occur because of seasonal variation in the isotopic ratio of precipitation input to the soil (Dansgaard 1964; Gat 1980), differences in the evaporation in the uppermost soil layers (Allison, Barnes & Hughes 1983), or because of differences between bulk soil moisture and groundwater (Fontes 1980). Proper interpretation of leaf water isotopic composition depends on knowledge of the source, stem water isotopic composition.

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