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The relationship between stable oxygen and hydrogen isotope ratios of water in astomatal plants

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Abstract—Isotopic fractionation of leaf water during transpiration is influenced by both equilibrium and kinetic factors. Previous workers have predicted that the influence of each factor varies depending upon the path of water loss, whether centralized through stomata, or diffuse through the cuticle. We studied the relationship between the δD and $\delta^{18}O$ values of leaf and stem waters of laurel sumac, *Rhus laurina* (Nutt.) T. & G., and its parasite, dodder, *Cuscuta subinclusa* D. & H., growing in the field. Stomatal transpiration, associated with more stagnant boundary layers, predominates in *R. laurina;* cuticular transpiration, associated with more turbulent boundary layers, is most important in the largely astomatal *C. subinclusa.* We also studied the diurnal variation in the δD and $\delta^{18}O$ values of leaf waters of two astomatal plants, *Chiloschista lunifera* (Rchb. F.) J.J.S and *Stylites andicola* Amstutz, and two stomatal plants, *Tillandsia balbisiana* Schult. and *Lilaeopsis schaffneriana* (Schlecht.) C. & R., growing with them under the same conditions in the laboratory. Slopes, m, for the relation $\delta D = m\delta^{18}O + b$ were significantly higher for stem waters in *C. subinclusa* than for leaf waters in *R. laurina* (1.77), consistent with the difference in the boundary layers through which water was lost in the two species. The magnitude of diurnal heavy isotope enrichment of tissue water was smaller in *C. subinclusa* than in *R. laurina,* which is also consistent with predictions concerning evapotranspiration through different types of boundary layers. The slopes, m, in plant waters in the laboratory experiments, conducted at high humidity, were not different than those observed during evaporation of water from pans, regardless of plant anatomy. The observation suggests that cuticular transpiration is important in influencing isotopic fractionation of water only at low humidity. Our results indicate that the isotopic composition of water vapor released by plants in arid regions may be influenced by the relative proportions of stomatal versus cuticular transpiration.

INTRODUCTION

LEAF WATER BECOMES enriched in the heavy isotopes of oxygen and hydrogen (^{18}O and D) during evapotranspiration (GONFIANTINI *et al.,* 1965; WERSHAW *et al.,* 1970; DONGMANN *et al.,* 1974; ZUNDEL *et al.,* 1978; FARRIS and STRAIN, 1978; LEANEY *et al.,* 1985; STERNBERG *et al.,* 1986). This biologically mediated process influences the isotopic composition of oxygen and hydrogen that is incorporated into organic matter (STERNBERG, 1988). The isotopic composition of molecular oxygen, the carbon and oxygen isotope composition of atmospheric carbon dioxide, and the atmospheric concentrations of other trace and greenhouse gases are all affected by transpiration of water by plants (DOLE *et al.,* 1954; FRANCEY and TANS, 1987; MOONEY *et al.,* 1987; YAKIR *et al.,* 1989).

Two of the more important factors controlling enrichment of heavy isotopes in leaf water are equilibrium fractionations between liquid and vapor phases of water and kinetic fractionations that influence the rate at which different isotopic forms of water escape from the leaf surface (DONGMANN *et al.,* 1974). The relative importance of equilibrium versus kinetic isotope effects during leaf water evaporation can be studied by examining the relationship between the enrichment of D relative to ^{18}O in leaf water (ALLISON *et al.,* 1985; COOPER and DENIRO, 1989). Characteristically, this involves comparisons of the slope, *m,* for the following relation, obtained from analyses of leaf waters collected during diurnal cycles of evapotranspiration:

$$\delta D = m\delta^{18}O + b.$$

δD and $\delta^{18}O$ are the isotopic compositions of leaf water, given in per mil in the conventional δ notation, where

$$\delta^{18}O \text{ or } \delta D = [(R_{sample}/R_{SMOW}) - 1] \times 1000.$$

R is $^{18}O/^{16}O$ or D/H; SMOW is Standard Mean Ocean Water.

Kinetic fractionation effects are larger for $H_2{}^{18}O$ relative to $H_2{}^{16}O$ than for $DH^{16}O$ relative to $H_2{}^{16}O$ (DANSGAARD, 1964; MERLIVAT, 1978; GAT, 1980). As a result, *m,* the slope in the leaf water equation, $\delta D = m\delta^{18}O + b$, will be lower in plants for which kinetic fractionation plays a larger role during

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evapotranspiration than in plants for which equilibrium processes dominate. Kinetic fractionation effects are influenced by the nature of the boundary layer through which water evaporates (DONGMANN *et al.*, 1974; FARRIS and STRAIN, 1978; LEANEY *et al.*, 1985; ALLISON *et al.*, 1985). When stagnant boundary layer conditions exist, kinetic processes become more important. With more turbulent boundary layers, water molecules encounter less resistance to irreversible loss to the atmosphere, so that equilibrium-dominated processes involved in the evaporation of water become more significant. If the boundary layer adjacent to the leaf is more turbulent, evaporation will proceed faster, kinetic effects will be less important, and the slope, m, in the equation $\delta D = m\delta^{18}O + b$ will be higher. Hence, comparative studies of the slope, m, potentially can be used to characterize boundary layers associated with different plants. Unfortunately, several complications have precluded widespread application of this idea.

First, the possibility that different proportions of leaf water in various species are subject to evapotranspiration (LEANEY *et al.*, 1985; YAKIR *et al.*, 1989, 1990) could affect the slopes of the leaf water equations. As an example, consider two plants, both using the same source water, but with different proportions of leaf water subject to heavy isotope enrichments. Slopes for the leaf water lines in the two plants would be influenced by the relative abundances in the leaves of unfractionated source water, which lies on the meteoric water line, $\delta D = 8\delta^{18}O + 10$, and fractionated leaf water, for which slopes much lower than eight have been observed (BRICOUT *et al.*, 1972; LESAINT *et al.*, 1974; ALLISON *et al.*, 1985; STERNBERG *et al.*, 1986; COOPER and DENIRO, 1989).

Nevertheless, a means for studying the influence of pools of fractionated versus unfractionated plant water on the slope, m, in the leaf water equation $\delta D = m\delta^{18}O + b$ can be found in the proposal that the fraction of leaf water subject to heavy isotope enrichment is proportional to the magnitude of daily heavy isotope enrichment observed in total leaf water (LEANEY *et al.*, 1985). Two predictions follow from this proposal. First, plants with low daily ranges in leaf water isotopic composition should have higher slopes, m, because they contain a higher proportion of unfractionated (meteoric) source water. Second, the lines, $\delta D = m\delta^{18}O + b$, describing leaf water isotopic content in such plants during the daily cycle of heavy isotope enrichment should have a minimum point closer to meteoric source water. In a separate study of nine species (COOPER and DENIRO, 1989), we observed that plants with the smallest daily range of leaf water isotopic variability actually had the lowest, not the highest, slopes, although minima for the lines $\delta D = m\delta^{18}O + b$ were in fact closer to the meteoric source water for these plants. Thus only one of two predictions of LEANEY *et al.* (1985) was validated by our study.

A second complication in applying the relation $\delta D = m\delta^{18}O + b$ to studies of boundary-layer changes within stomata and adjacent to leaves is species-specific variability in the patterns of water utilization (COOPER and DENIRO, 1989). Low humidity should increase the stagnant nature of leaf boundary layers (FARRIS and STRAIN, 1978; ALLISON *et al.*, 1985) because of partial or complete stomatal closure, resulting in a longer path length before water molecules are irreversibly lost to the atmosphere. As an example, ALLISON *et al.* (1985) attributed a lower slope, m, observed in needle waters of Monterey pine, *Pinus radiata*, to more arid conditions during the summer relative to the winter. COOPER and DENIRO (1989), however, observed that plants growing in more arid locations did not necessarily exhibit lower slopes in the equation $\delta D = m\delta^{18}O + b$. Rather, we observed that different species growing under the same environmental conditions showed different responses to the physical factors that are presumed to influence boundary layer characteristics associated with transpiration. COOPER and DENIRO (1989) concluded that biological differences in water utilization among different species had an important influence on the relation $\delta D = m\delta^{18}O + b$. An association was found between the residence time of water within a plant and the slope, m: We proposed that this residence time may affect apparent or actual kinetic fractionation factors for D and ^{18}O (COOPER and DENIRO, 1989).

A third complication that is pivotal to understanding biological effects upon enrichment of heavy isotopes in leaf waters is the role of stomatal versus cuticular transpiration. FARRIS and STRAIN (1978) proposed that loss of water through the cuticle, the wax-covered surface of leaves, is associated with more turbulent boundary conditions than loss of water through stomata, which are pores through which plants exchange gases with the atmosphere. This proposal was based on differences in topography between stomata, which are recessed into the leaf, and the cuticle, for which there is no physical barrier impeding air flow close to the surface of the leaf. Yet the analysis of the needle water line for Monterey pine (ALLISON *et al.*, 1985) indicates that the cuticular-stomatal transpiration ratio, which would be greater under summer water-stressed

conditions, is not as important as lowered humidity in determining the slope, m, in the relation $\delta D = m\delta^{18}O + b$. The interplay of species-specific effects with physical factors such as humidity has prevented an unambiguous test of the proposal of FARRIS and STRAIN (1978) regarding stomatal versus cuticular transpiration as it relates to changes in slope, m.

It was our intent in the present work to test the influence of diffusional boundary-layer conditions as they relate to stomatal versus cuticular transpiration. We studied the relationships between δD and $\delta^{18}O$ values of waters in *Cuscuta subinclusa* D. & H., a largely non-photosynthetic parasite, and its host, *Rhus laurina* (Nutt.) T. & G., a common southern California chaparral shrub. The host-parasite relationship permits control of physical factors influencing leaf and tissue water enrichment because both plants are subject to the same humidity and temperature. To a large extent, this study also allowed control of stomatal distribution as it influences evapotranspiration, because the genus *Cuscuta* is characterized by an almost complete lack of stomata (MACLEOD, 1962). The mature parasite has no roots and is dependent upon the host plant for water and nutrients obtained through haustoria. Although it is not known what proportions of host water are derived from isotopically fractionated leaf water versus unfractionated source water, xylem to xylem water conduction exists between *Cuscuta* and its hosts (ASHTON, 1976). Xylem are the non-living water conduction vessels in vascular plants. Thus vegetative cells in *C. subinclusa* are likely to have similar sources of water as do vegetative cells in *R. laurina*. The major difference between the two plants is that water evaporating from *C. subinclusa* will be lost predominantly through the cuticle, while water lost from *R. laurina* will be evaporated primarily through stomata, with a much smaller loss through the cuticle.

We followed this work up with a laboratory study of the relationship between the δD and $\delta^{18}O$ values of tissue waters of two additional astomatal plants, a largely leafless orchid, *Chiloschista lunifera* (Rchb. F.) J.J.S, and a specialized isoetid, or fern ally, *Stylites andicola* Amstutz. The latter has minimal gas exchange with the atmosphere and obtains inorganic carbon from the sediments through lacunae, which are air spaces within the stalk (KEELEY *et al.*, 1984; STERNBERG *et al.*, 1985). We also studied heavy isotope enrichment patterns during transpiration with two control, stoma-bearing plants growing under the same conditions, *Tillandsia balbisiana* Schult. and *Lilaeopsis schaffneriana* (Schlecht.) C. & R.

We expected the slope, m, in the equation $\delta D = m\delta^{18}O + b$ to be higher for the astomatal plants than for the stoma-bearing species. This hypothesized increase would be due to evaporation occurring through the plant surface in association with a more turbulent boundary layer in the astomatal plants than the less turbulent boundary layer associated with recessed stomata in the stoma-bearing plants. We tested for variability in slopes due to differing proportions of tissue water subject to enrichment of heavy isotopes by evaluating the total range of water isotopic variability over diurnal periods.

MATERIALS AND METHODS

For the field experiment, samples consisted of 3 to 4 g of leaves from a single laurel sumac bush, *R. laurina*, and of leafless stems of dodder, *C. subinclusa*, growing on the same bush. Samples were collected and stored immediately in sealed tubes every 3 hours for 48 hours from 1400 local time, 24 September 1987 to 1400, 26 September 1987. The plants sampled were growing along the access road to the Pasadena, California, water supply in Arroyo Seco Canyon, adjacent to the Jet Propulsion Laboratory. Two samples of *C. subinclusa* were collected at each sampling time, one a sample of stems that were attached to the host and the second, stems that had been removed from the *Rhus laurina* bush at the beginning of the experiment. These detached stems were left in the general vicinity dangled from a dead branch of the sumac bush. The intention of detaching the parasitic stems was to gain some insight into the scope of oxygen and hydrogen isotope variability of water within the parasite that was distinct from variation in the host that was then transmuted to the attached parasitic stems. Air temperature and humidity were measured at the time of each collection.

For the laboratory experiments, *C. lunifera* was obtained from a retail orchid importer (Spencer Howard, Orchid Imports, North Hollywood, California), who obtained the plants from sources in Thailand. *S. andicola* and *L. schaffneriana* were taken from a collection at Occidental College, Los Angeles, that had been cultivated for over a year from plants collected in Peru and Columbia, respectively. The *T. balbisiana* used in the experiment was purchased from a local source.

Samples consisting of 1 g of roots (*C. lunifera*) and a 3 to 4 g whole leaf of *T. balbisiana* were collected every 4 hours for 24 hours during the first laboratory experiment, on April 13 and 14, 1988. Both species were growing in a covered glass case adjacent to a lighted window; artificial room lighting was turned off at night. (All of the plants except *L. schaffneriana* exhibit Crassulacean acid metabolism, in which CO_2 uptake and water loss can occur at night.) Since both *C. lunifera* and *T. balbisiana* obtain water from atmospheric vapor, two collections of the atmospheric vapor present in the glass case during the experiment were made for isotopic analyses of source water. Water vapor collections were made in the following manner. A glass trap, filled with molecular sieve pellets, size 5A (Linde, Tarrytown, New Jersey) was placed in Dewar flask containing liquid nitrogen. The inlet to the trap was connected through a flow meter to another trap containing Pyrex glass beads, which was also immersed in liquid ni-

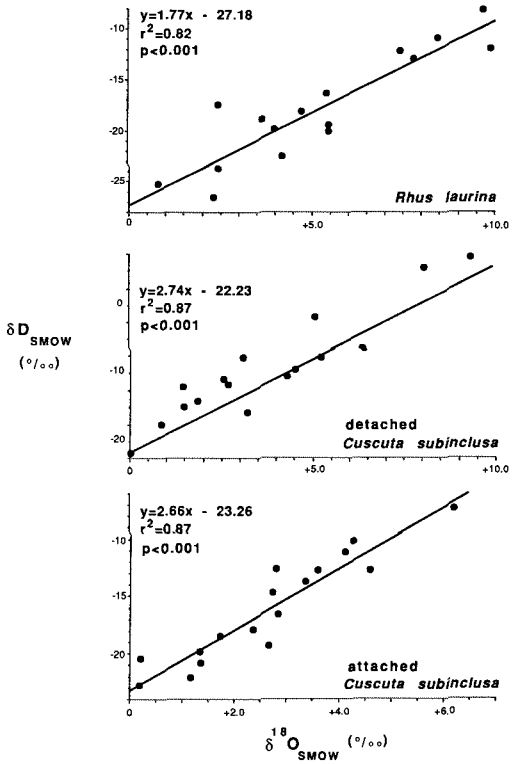

FIG. 1. Stable hydrogen and oxygen isotopic compositions plotted against one another and regression lines (of the form $\delta D = m\delta^{18}O + b$) for leaf waters from *R. laurina* (top), stem waters from *C. subinclusa* (detached from host at beginning of experiment) (middle), and for stem waters from *C. subinclusa* (attached to host) (bottom).

trogen. The inlet to the initial trap was connected through rubber tubing to a disposable Pasteur pipette, the tip of which was placed within 1 cm of plant surfaces within the terrarium. Air flow into the molecular sieve trap during two 40 minute periods, one during the day and one at night, resulted in water vapor being trapped on the Pyrex beads in the initial trap. The rate of air flow was variable, depending upon the amounts of oxygen and nitrogen condensed on the molecular sieve. After sufficient collection of atmospheric water vapor, the initial trap was then isolated, its water distilled and analyzed isotopically.

Conditions in the second laboratory experiment using *S. andicola* and *L. schaffneriana*, on July 26 and 27, 1988, were similar except for the following differences. Six collections in the 24 h period were made, rather than seven. All samples collected consisted of approximately 1 g of leaf material. The plants were growing together in a plastic container filled with water-logged sand. The container was placed in an ice-filled cooler, which was partially closed at night. Source water was sampled directly from the water-logged sand for isotopic analysis.

All plant samples were kept frozen in sealed tubes until water was extracted by quantitative freeze drying (STERNBERG *et al.*, 1986). For the field experiments, oxygen isotope ratios were determined by equilibrating 0.5 to 1.0 ml water samples with approximately 300 μmoles of carbon dioxide for 48 hours, purifying the equilibrated carbon dioxide cryogenically, analyzing the CO_2 mass spectrometrically, and using mass balance considerations to calculate the original oxygen isotope composition of the water (EPSTEIN and MAYEDA, 1953). For the laboratory experiments (for all plants except *T. balbisiana*, where 0.5 ml of water was analyzed as described above), because of the small size of the water samples, oxygen isotope ratios were determined by equilibrating 75 to 100 μL of water with approximately 500 μmoles of carbon dioxide for 48 hours and then proceeding in the same manner as above. Hydrogen gas was extracted by passing approximately 10 μL of water over uranium metal heated to 700°C, which releases hydrogen gas that was collected using a uranium hydride pump prior to mass spectrometric analyses (FRIEDMAN and HARDCASTLE, 1970). Precision, based on repeated mass spectrometric determinations of secondary water standards analyzed concurrently with the samples, was found to be ±0.2 per mil for $\delta^{18}O$ values and ±2 per mil for δD values. Accuracy of D/H ratios was evaluated by running the samples and SMOW directly against SMOW in the mass spectrometer. For small water volume (75 to 100 μL) determinations of $\delta^{18}O$ values, machine standards of CO_2 were prepared and used within the mass spectrometer by equilibration of CO_2 with a well-calibrated secondary water standard, matching the volumes used for each water sample. This served to correct small systematic errors resulting from high CO_2/H_2O ratios used in small water volume equilibrations.

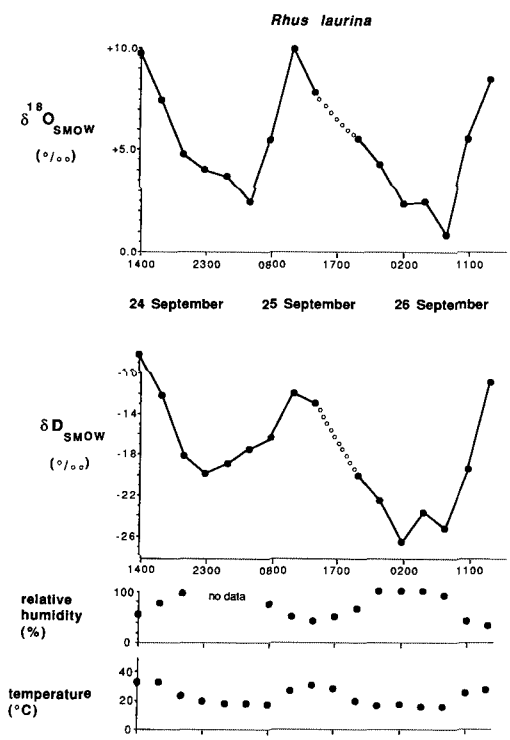

FIG. 2. Stable oxygen and hydrogen isotope ratios of leaf water of *R. laurina* over the course of the sampling period. Anamolous δD and $\delta^{18}O$ values of water collected at 1700, 25 September, were excluded due to probable errors during collection or distillation. These data were also excluded from Fig. 1.

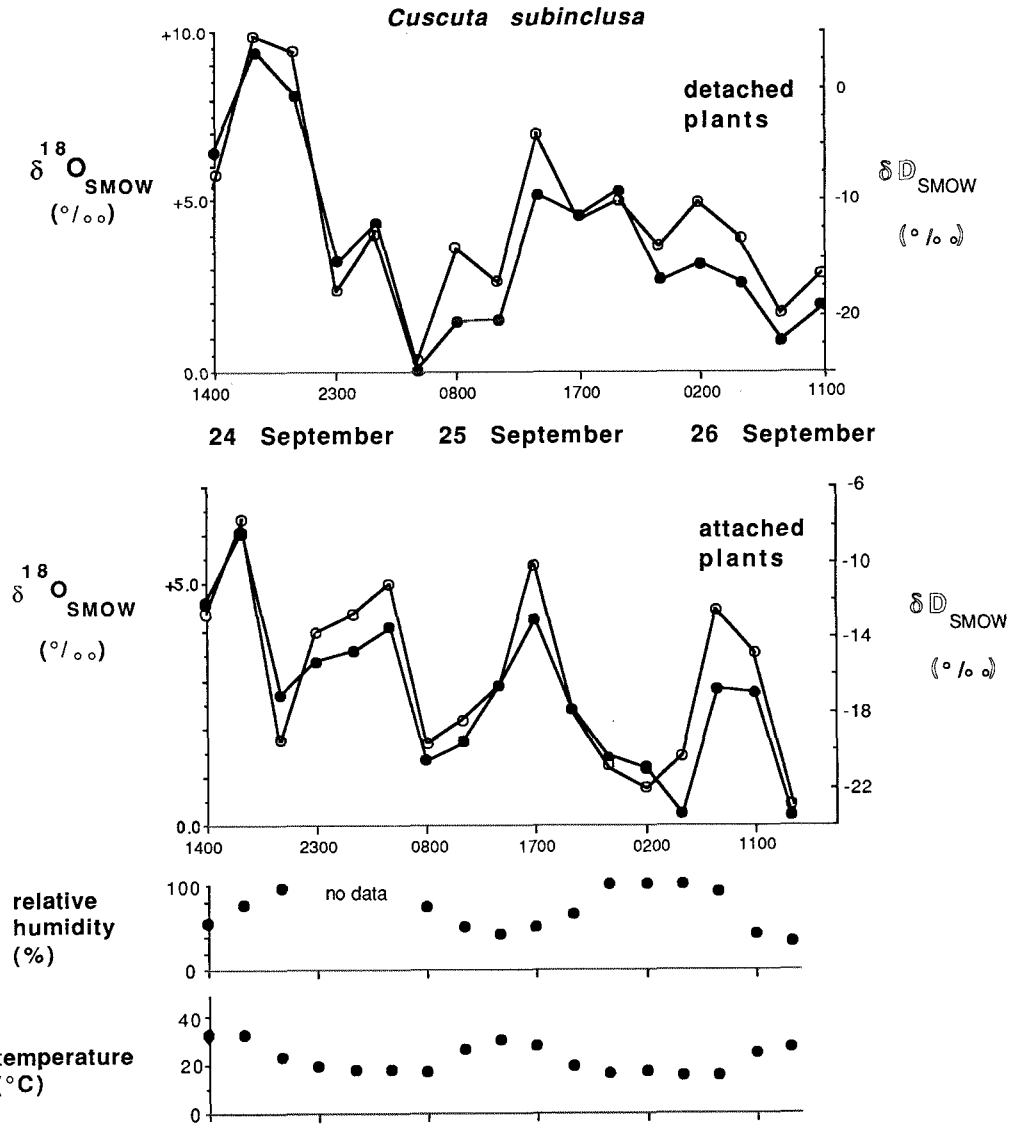

FIG. 3. Stable oxygen (darkened circles) and hydrogen isotope ratios (clear circles) of stem water of *C. subinclusa* (detached from host at beginning of experiment) (top) and stem water of *C. subinclusa* (attached to host) (bottom), over the course of the sampling period. Relative humidity and temperature at the time sampling are also indicated.

RESULTS

Field experiment

The slopes, m, in the relationship $\delta D = m\delta^{18}O + b$ for water of *R. laurina* leaves are 1.77 and 2.74 for *C. subinclusa* stems that had been detached from the host at the start of the experiment, and 2.66 for *C. subinclusa* stems that remained attached to *R. laurina*. These slopes were derived from regression analyses of δD and $\delta^{18}O$ values for each of the three sets of plant samples (Fig. 1). Analysis of covariance (SACHS, 1984) indicated that slopes for the two types of *C. subinclusa* samples did not differ, but both were significantly higher than the slope for *R. laurina*. During the course of the experiment, both the detached *C. subinclusa* and *R. laurina* showed a regular diurnal fluctuation with heavy isotopic enrichment at a minimum at dawn and at a maximum in the late morning or the afternoon (Figs. 2 and 3). Heavy isotope enrichment in attached *C. sub-*

Table 1. Daily range of tissue water isotopic enrichment

Sample	$\Delta\delta D$ (‰)	$\Delta\delta^{18}O$ (‰)
C. subinclusa (detached from host)	29	9.3
C. subinclusa (attached to host)	15	6.0
R. laurina	18	9.8

inclusa did not follow a smooth diurnal pattern (Fig. 3), and the overall magnitudes of enrichment for D and ^{18}O were somewhat smaller than for either the detached *C. subinclusa* or *R. laurina* (Table 1).

The magnitudes of D- and ^{18}O-enrichment for detached *C. subinclusa* and *R. laurina* were correlated with air temperature at a significant level ($p < 0.05$), but only *R. laurina* showed a significant negative correlation with relative air humidity. Heavy isotope enrichment in attached *C. subinclusa* was not correlated with either temperature or humidity (Table 2).

Laboratory experiments

Overall variability in δD and $\delta^{18}O$ values of tissue water in the four species grown in the laboratory are shown in Figs. 4 and 5. A plot of tissue water δD versus $\delta^{18}O$ values collected at each sampling time yields a regression line for each species, but the least-squares fit equation corresponding to *S. andicola* is not statistically significant (Table 3). The δD and $\delta^{18}O$ values of water vapor in the first experiment were −98 and −12.6 (day) and −99 and −13.5 (night), respectively. The δD and $\delta^{18}O$ values for source water in the second experiment were −90 and −11.3, respectively.

DISCUSSION

The results of the field study are consistent with the proposal that the bulk of water transpiring from a largely astomatal plant, *C. subinclusa,* passes through a more turbulent boundary layer than the bulk of water transpiring from *R. laurina.* Slopes of leaf water, m, in the equation $\delta D = m\delta^{18}O + b$, for stem or leaf water were significantly higher for the parasite than for the host.

By contrast, in the laboratory experiment slopes, m, in the equation $\delta D = m\delta^{18}O + b$, observed for tissue water distilled from three of four species studied showed no clear pattern with respect to the

Table 2. Relationships between leaf and stem water isotopic ratios and air temperature (T) or relative humidity (H)

Sample	m	b	r^2	p	n
		T (°C) = $m\delta^{18}O + b$			
C. subinclusa (detached from host)	1.49	16.24	0.40	$0.005 < p < 0.01$	16
C. subinclusa (attached to host)	1.76	17.54	0.21	$0.05 < p < 0.10$	17
R. laurina	1.94	11.65	0.74	$p < 0.0001$	16
		T (°C) = $m\delta D + b$			
C. subinclusa (detached from host)	0.45	27.18	0.32	$0.01 < p < 0.025$	16
C. subinclusa (attached to host)	0.44	29.32	0.11	$0.10 < p < 0.25$	17
R. laurina	0.99	39.59	0.74	$p < 0.0001$	16
		H (%) = $m\delta^{18}O + b$			
C. subinclusa (detached from host)	−0.30	73.68	0.001	$p > 0.25$	14
C. subinclusa (attached to host)	−2.54	75.83	0.03	$p > 0.25$	14
R. laurina	−6.74	109.68	0.59	$0.001 < p < 0.005$	13
		H (%) = $m\delta D + b$			
C. subinclusa (detached from host)	0.25	75.03	0.01	$p > 0.25$	14
C. subinclusa (attached to host)	−1.13	50.51	0.05	$p > 0.25$	14
R. laurina	−3.07	15.94	0.54	$0.001 < p < 0.005$	13

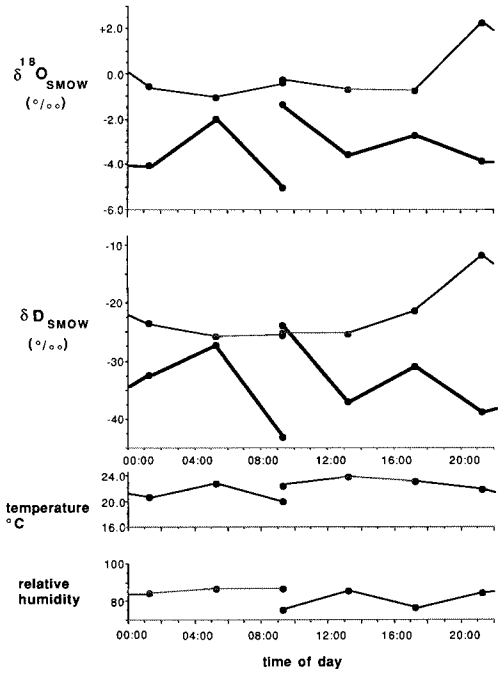

FIG. 4. $\delta^{18}O$ and δD values of tissue water, air temperature and relative humidity (%) at the indicated time of day for *C. lunifera* (thickened lines) and *T. balbisiana* (narrow lines).

presence or absence of stomata (Table 1). Evaporation from surface bodies of water in mid-continental humid regions results in slopes, *m*, ranging from 4 to 6 (ALLISON *et al.*, 1985), within the range we observed for these plants. Heavy isotope enrichment, as it affects the slope, *m*, is apparently little different in at least three of the four species studied from that which occurs during water evaporation from surface bodies of water. No conclusions can be drawn concerning the fourth species, *S. andicola*, because the least-squares fit regression line generated by plotting leaf water δD and $\delta^{18}O$ values (Fig. 2; Table 2) is not statistically significant. It should also be noted that the relation $\delta D = m\delta^{18}O + b$ for the stoma-bearing bromeliad *T. balbisiana* is somewhat tenuous because it is highly dependent on ^{18}O- and D-enriched water collected from a leaf sampled at 20:00 (Fig. 4). All other individual *T. balbisiana* leaves sampled during the experiment showed small variations in ^{18}O and D content.

A major difference between the field and laboratory experiments was the high relative humidity in the laboratory growth chambers. The role that low relative humidity plays in driving the leaf water isotope enrichment process (FARRIS and STRAIN, 1978) was apparent in the magnitude of isotopic enrichment for the field and laboratory experiments. Unlike the field experiment, the ranges of δD and $\delta^{18}O$ values observed for tissue water of plants studied in the laboratory were small. Over the 24 hour lab experiments, $\delta^{18}O$ values changed by little more than one per mil in *S. andicola*, with changes of only about two per mil in the remaining three species. These changes are comparatively small. We observed diurnal variability in leaf water $\delta^{18}O$ values up to ten per mil in the field; ranges as large as 30 per mil have been observed in desert plants (COOPER and DENIRO, 1989).

The high slopes, *m*, in the equation $\delta D = m\delta^{18}O + b$ determined for an astomatal plant, *C. lunifera*, and a stoma-bearing plant, *L. schaffneriana* grown with it, indicate that our original hypothesis, that astomatal plants will exhibit higher slopes than stoma-bearing plants, must be rejected at higher relative humidities. The laboratory study could not have been conducted under conditions of lower humidity, because such conditions would have interfered with basic metabolism in each of the plants. *T. balbisiana* and *C. lunifera* are epiphytes in moist tropical forests, and are specifically dependent on water vapor for photosynthesis. *L. schaffneriana* and *S. andicola* are wetland species that grow in

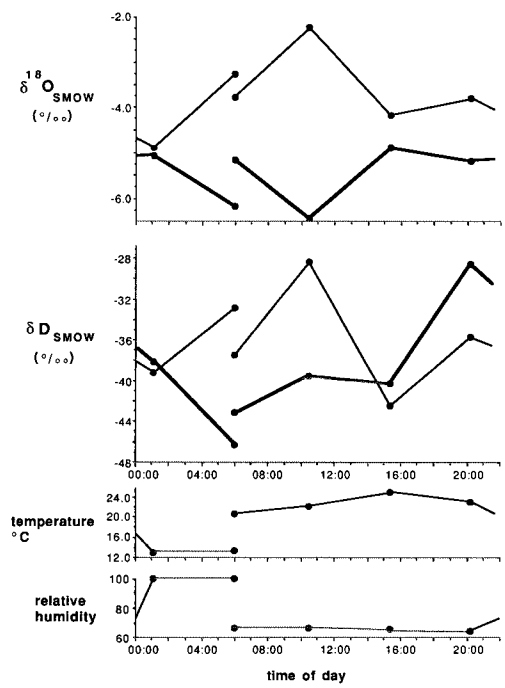

FIG. 5. $\delta^{18}O$ and δD values of tissue water, air temperature and relative humidity (%) at the indicated time of day for *S. andicola* (thickened lines) and *L. schaffneriana* (narrow lines).

Table 3. Regression equations of the form $\delta D = m\delta^{18}O + b$ for tissue water: laboratory experiments

Species (Anatomy)	m (±SE)	b	r^2	n	p
C. lunifera (no stomata)	4.89 (±0.86)	−17.56	0.867	7	$0.001 < p < 0.005$
T. balbisiana (stomata)	4.15 (±0.80)	−21.75	0.843	7	$0.001 < p < 0.005$
S. andicola (no stomata)	3.27 (±4.31)	−21.46	0.126	6	$p > 0.25$
L. schaffneriana (stomata)	4.94 (±1.29)	−17.77	0.787	6	$0.01 < p < 0.025$

bog ecosystems. This illustrates a fundamental problem with studies of the tissue water isotope composition of astomatal plants. Most astomatal vascular plants, and most stoma-bearing plants that appear suitable as control species, grow under humid conditions. Leaf water enrichment of ^{18}O and D is low under these conditions, and the enrichment that does occur appears to proceed much as it does in open-water evaporation processes; i.e., the slope, m, in the equation $\delta D = m\delta^{18}O + b$ is greater than four. Thus, under conditions of high humidity, the presence of stomata and the boundary layer characteristics of stoma-bearing leaves do not affect the heavy isotope enrichments that occur during evaporation.

Our results are also consistent with a separate study (COOPER and DENIRO, 1989) that indicates that variable inputs of unfractionated meteoric water do not significantly influence the slope, m. Using the reasoning of LEANEY *et al.* (1985) discussed in the introduction, we might have expected plants with larger diurnal ranges of leaf water isotope composition to have lower slopes, m, signifying a smaller contribution of unfractionated meteoric water. Compared with the attached parasites used in the field experiment, the detached parasites removed from the host plant at the beginning of the experiment no doubt had a higher proportion of tissue water subject to heavy isotope evaporation enrichments, and this is reflected in both the larger $\delta^{18}O$ and δD ranges for the detached parasites (Table 1). Nevertheless, the slope, m, does not differ significantly between the detached and attached parasites. The different evaporative ranges in detached and attached *C. subinclusa* are no doubt in part due to unavoidable injuries in detaching the stems at the beginning of the experiment. Nevertheless, it is remarkable what little difference there is between the tissue water relationships for the detached ($\delta D = 2.74\delta^{18}O - 22.2$) and attached parasites ($\delta D = 2.66\delta^{18}O - 23.3$). These relationships held for at least 48 hours despite the obvious differences in water stress and the certainty that water loss occurred through broken stems in the detached plants.

Despite predominant cuticular transpiration that is presumably associated with a well-mixed boundary layer, *C. subinclusa* probably does not lose much water over the course of a day compared to *R. laurina,* and hence the isotopic composition of its stem water does not respond to humidity and temperature changes as does leaf water in *R. laurina.* The same explanation accounts for the smaller heavy isotope enrichment ranges in attached *C. subinclusa* compared to *R. laurina.* The occurrence of significant correlations between temperature and stem water heavy isotope enrichments in the detached *C. subinclusa* suggests that this insulating physiology and anatomy may have been disturbed when the parasite was removed at the start of the experiment. Our experimental results support the proposal that water evaporation through the cuticle proceeds through a more turbulent boundary layer than that associated with stomatal transpiration (FARRIS and STRAIN, 1978), but that this evapotranspiration is only important under the conditions of lower humidity that increase the magnitude of leaf water heavy isotope enrichment. The relative importance of cuticular versus stomatal transpiration, particularly under arid conditions, could thus affect the relationship between the D and ^{18}O content of atmospheric water vapor. Recently, efforts have been undertaken to use the isotopic variability of atmospheric water vapor as an element in global circulation models (JOUZEL *et al.,* 1989). Isotopic fractionation during the hydrological cycle in the soil-plant-atmosphere continuum could be altered by the ratios of cuticular to stomatal transpiration in different vegetation types. Changes in vegetation type have been shown to alter regional rates of evapotranspiration (MOONEY *et al.,* 1987). Increasing desertification, together with vegetation and climate changes associated with human activity, can no doubt cause changes in the global hydrological cycle. Unfortunately, the isotopic fractionation processes in plants that could help provide information on the magnitude of elements of the global hydrological cycle and the fluxes of other gases exchanged by vegetation have been studied only in a preliminary way.

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