On the Controls of Leaf-Water Oxygen Isotope Ratios in the Atmospheric Crassulacean Acid Metabolism Epiphyte

*Tillandsia usneoides*¹[W][OA]

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Previous theoretical work showed that leaf-water isotope ratio (δ¹⁸O) of Crassulacean acid metabolism epiphytes was controlled by the δ¹⁸O of atmospheric water vapor (δ¹⁸Ov) and observed δ¹⁸Ow could be explained by both a non-steady-state model and a “maximum enrichment” steady-state model (δ¹⁸O-M), the latter requiring only δ¹⁸Ov and relative humidity (h) as inputs. δ¹⁸Ov, therefore, should contain an extractable record of δ¹⁸Ov. Previous empirical work supported this hypothesis but raised many questions. How does changing δ¹⁸Ov and h affect δ¹⁸Ow? Do hygroscopic trichomes affect observed δ¹⁸Ow? Are observations of changes in water content required for the prediction of δ¹⁸Ow? Does the leaf need to be at full isotopic steady state for observed δ¹⁸Ow to equal δ¹⁸O-M? These questions were examined with a climate-controlled experimental system capable of holding δ¹⁸Ov constant for several weeks. Water adsorbed to trichomes required a correction ranging from 0.5% to 1%. δ¹⁸Ov could be predicted using constant values of water content and even total conductance. Tissue rehydration caused a transitory change in δ¹⁸Ov, but the consequent increase in total conductance led to a tighter coupling with δ¹⁸Ov. The non-steady-state leaf water models explained observed δ¹⁸Ov (y = 0.93x - 0.07; r² = 0.98) over a wide range of δ¹⁸Ov and h. Predictions of δ¹⁸O-M agreed with observations of δ¹⁸Ov (y = 0.97x - 0.99; r² = 0.92), and when h > 0.9, the leaf did not need to be at isotopic steady state for the δ¹⁸O-M model to predict δ¹⁸Ov in the Crassulacean acid metabolism epiphyte *Tillandsia usneoides*.

Tropical and subtropical epiphytic higher plants have long been a curiosity to plant physiologists because of the multiple and unique constraints on physiology that the epiphytic lifeform represents (Mez, 1904; Benzing, 1970; Medina and Troughton, 1974). While the competition for light has no doubt led the plants to the tree tops, the concomitant loss of roots effectively removed the plants from an environment of abundant rainfall to one of arid conditions (Griffiths et al., 1986; Smith et al., 1986a; Winter and Smith, 1996). In a sense, the plants shifted biomes by immigrating vertically instead of horizontally. This shift led to morphological adaptations like the development of modified leaf hairs to absorb water and the formation of tanks via the overlapping of leaf bases as well as physiological adaptations such as maintaining high leaf osmotic potential and, perhaps most notably, the evolution of the CO₂-concentrating mechanism known as Crassulacean acid metabolism (CAM) photosynthesis (Medina and Troughton, 1974; Benzing et al., 1976; Griffiths and Smith, 1983; Smith et al., 1986b; Martin and Schmitt, 1989; Martin et al., 2004; Ohrui et al., 2007). This unique physiology of tropical and subtropical CAM epiphytes also presents an intriguing contrast to the general way we view oxygen isotope ratios (δ¹⁸O) in plant water and organic material (Helliker and Griffiths, 2007; Helliker and Noone, 2009).

The enrichment of ¹⁸O in leaf water during plant transpiration leads to a suite of physiology-based tracers that inform us of current and past plant-environment interactions. Leaf water δ¹⁸O (δ¹⁸Ow) labels CO₂ and allows for partitioning of the gross components of net CO₂ flux from ecosystem to regional scales (Yakir and Wang, 1996; Ciais et al., 1997; Styles et al., 2002; Cuntz et al., 2003; Ogée et al., 2004; Hollinger et al., 2005). The production of isotopically distinct O₂ by photosynthesis allows for global-scale estimates of productivity over millennia (Guy et al., 1993; Luz et al., 1999; Hoffmann et al., 2004). The isotopic record of δ¹⁸O in leaf and tree ring cellulose allows for the reconstruction of growth environment and/or physiological responses to that growth environment (Epstein et al., 1977; Anderson et al., 1998; Switsur and Waterhouse, 1998; Barbour and Farquhar, 2000; Barbour et al., 2000; Roden and Ehleringer, 2000; Ferrio and Voltas, 2005; Poussart and Schrag, 2005; Helliker and Richter, 2008). Much of the error associated with the above δ¹⁸O applications could be decreased with better estimates of the isotope ratio of atmospheric water vapor (δ¹⁸Ov), a primary control on leaf-water ¹⁸O enrichment (Farquhar and

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underpinnings for CAM epiphytes. \( \delta^{18}O \) is a control on CAM epiphyte primary controls of CAM epiphyte growth habit (Reyes-Garcia et al., 2008) and the reconstruction of \( \delta^{18}O \) (Helliker and Griffiths, 2007). It was recently shown through empirical and theoretical work that lichen thalli obtain equilibrium with \( \delta^{18}O \) even under nonsaturating conditions (Hartard et al., 2009). The lichen system of equilibrium with \( \delta^{18}O \) represents an important contrast to that of CAM epiphytes, and this is discussed later. The theoretical work presented by Hartard et al. (2009), however, is extremely helpful for the interpretation of isotopic signals in CAM epiphytes.

Helliker and Griffiths (2007) developed the theoretical underpinning for \( \delta^{18}O \) as a control on CAM epiphyte \( \delta^{18}O \). They extended the thoughts of Farquhar and Cernusak (2005) to show that at the high nocturnal \( h \) experienced by CAM epiphytes, gross exchange fluxes of water vapor into the leaf from the atmosphere can be several times the transpirational flux out of the leaf. Model simulations showed that constant environmental conditions and continuous water loss led to an isotopic steady state where both \( \delta^{18}O \) and transpired water \( \delta^{18}O \) converged to a single isotopic value controlled by the exchange of \( \delta^{18}O \) and described by the following “maximum enrichment” equation (Farquhar and Gan, 2003; Helliker and Griffiths, 2007; Hartard et al., 2009):

\[
\begin{align*}
R_{L-M} &\approx R_{L} \frac{R_{h} \delta}{\delta^{18}O_{a}} - \alpha^{K} + \alpha^{K} \delta h
\end{align*}
\]

where the steady-state \( \delta^{18}O \) \( (R_{L}) \) is determined solely by \( \delta^{18}O \) \( (R_{h}) \), \( h \), the temperature-dependent equilibrium fractionation factor \( \alpha^{*} \), and the balance of the ratio of diffusivities of light to heavy water molecules through the stomata and through the leaf boundary layer, \( \alpha^{K} \) (Flanagan et al., 1991; Farquhar and Lloyd, 1993; Farquhar and Cernusak, 2005). The non-steady-state analytical solution for \( \delta^{18}O \) developed by Hartard et al. (2009; Eq. 6 in “Materials and Methods,” hereafter referred to as the Hartard-Cuntz solution) clearly demonstrates that, through time in the nonsteady state, \( \delta^{18}O \) is continuously moving toward the value of \( R_{L-M} \) (\( \delta^{18}O_{L-M} \)). In a similar manner, the simulations of Helliker and Griffiths (2007) suggested that even at values of nocturnal \( h \) of 0.8, which is low for a CAM epiphyte (Garth, 1964; Smith et al., 1986b), \( \delta^{18}O \) was controlled almost entirely by the isotope ratio of atmospheric water vapor and the maximum enrichment equation above would ultimately predict \( \delta^{18}O_{L} \).

The simulations also showed that the approach to steady state was faster and occurred through less water loss as \( h \) increased. In general, their simulations showed that \( \delta^{18}O \) approached the steady-state value, or \( \delta^{18}O_{L-M} \) much faster than \( \delta^{18}O_{E} \), and this yields an important prediction that is tested by our study: the leaf does not need to be at full isotopic steady state for \( \delta^{18}O \) to be at or near the steady-state, maximum enrichment value (\( \delta^{18}O_{L-M} \)).

There are many unresolved questions as to the agreement between models and observations that underpin the efficacy of using \( \delta^{18}O \) to reconstruct \( \delta^{18}O \). The empirical work of Helliker and Griffiths (2007) did show support for the modeling exercises, but only over a narrow range of conditions. Their experimental setup was limited, as it was developed to demonstrate a preliminary proof of concept. Also, the invariably high \( h \) and noisy \( \delta^{18}O \) (±1.2‰) of Helliker and Griffiths (2007) could lead one to erroneously conclude that \( \delta^{18}O \) was in direct equilibrium with \( \delta^{18}O \) even when \( h \) was less than unity, which should not be the case. The primary controls of CAM epiphyte \( \delta^{18}O \) are \( h \) and \( \delta^{18}O \) and the manner in which changes in \( \delta^{18}O \) and \( h \) affect \( \delta^{18}O \) through time must be assessed. Like many CAM epiphytes, the study species Tillandsia usneoides has a heavy covering of hygroscopic trichomes. While the water adsorbed to these trichomes does not lead to water uptake by living cells at subsaturating conditions (Martin and Schmitt, 1989), the water is inevitably sampled and extracted for \( \delta^{18}O \) determination. Hence, the isotopic offset caused by these trichomes must be
determined. Total plant conductance to water loss ($g_{pv}$; stomatal and boundary layer conductance) controls the rate of exchange of tissue water with water vapor and the relative water content (RWC) of the plant. Therefore, the effect of changes in RWC on $\delta^{18}O_L$, through both water loss and rehydration, must be determined. Previous work has shown that changes in water volume are not important to predictions of $\delta^{18}O_L$ (Cernusak et al., 2002; Cuntz et al., 2007), and a similar finding in CAM epiphytes could greatly simplify our interpretation of observed $\delta^{18}O_L$. In summary, the goal of this study was to construct controlled-environment experiments to assess how well observed $\delta^{18}O_L$ could be predicted over a range of conditions through both steady-state and non-steady-state approaches of Helliker and Griffiths (2007) and Hartard et al. (2009).

RESULTS

Representative chamber measurements of nocturnal $h_i$, air temperature, and $\delta^{18}O_L$ over a 16-d experimental period are presented in Table I. Mean values of $\delta^{18}O_L$, $h_i$, and the number of days that $T. usneoides$ strands were in the chamber for each experimental run are presented in Table II. An image of the experimental setup and representative diurnal cycles are presented in Supplemental Figure S1. Data from the four initial experimental runs that were used to test the chamber setup were not included. Throughout this paper, $h = e_i/e_s$, and it is assumed that $h$ equaled relative humidity in all chamber studies because the chamber fans ensured little boundary layer development around the $T. usneoides$ ramets. Support for this assumption is provided from several early runs of the chamber setup, where $T. usneoides$ leaf temperatures were measured and were not significantly different from measures of air temperature (data not shown), so $e_i$ was equal to $e_{sat}$ at air temperature. The chamber held temperature and $h$ relatively constant (typical SD for temperature = 0.08°C and for $h = 0.015$; Table I). Maintaining constant $h$ above 0.9 for long periods of time is difficult and, to my knowledge, has not been attempted in many plant gas-exchange studies. Condensation in tubing and within the chamber, and directly on any chamber temperature-control device like the Peltier unit, can systematically and dynamically alter $h$ within the chamber. There were occasional, unexplained changes in $h$, as seen on days 12 and 13 (Table I). These changes were manifested as a systematic drop in $h$ that was caused by a slight drop in $e_i$ and not by changes in temperature (thus, not a change in $e_s$). Fortunately, such variability in $h$ did not appear to compromise the long-term stability of $\delta^{18}O_L$. The source for $\delta^{18}O_L$ was the 23-L capacity temperature-controlled water tower, the volume and specific heat of which led to a low multiday SD for $\delta^{18}O_L$ of $\pm 0.15\%$.

To quantify the amount of water adsorbed to trichomes (Fig. 1) and to determine how the $\delta^{18}O$ of this trichome water should affect observed $\delta^{18}O_L$ (Fig. 2), the gain in mass from water adsorption to $T. usneoides$ trichomes was determined at 23 separate settings of $h$ from 0.34 to unity. The data in Figure 1 represent the water adsorbed to trichomes expressed as RWC; therefore, this value is comparable to RWC values elsewhere in this study. Below an $h$ of about 0.8, the water adsorbed to trichomes was less than 8% RWC, how-

| Experimental Day | Chamber Air Temperature | $h$ | $\delta^{18}O_L$ |
|------------------|-------------------------|----|-----------------|
| 1                | 27.5 ± 0.03             | 0.96 ± 0.01 | -16.8 |
| 2                | 27.5 ± 0.06             | 0.97 ± 0.01 | -16.8 |
| 3                | 27.6 ± 0.20             | 0.96 ± 0.02 | -17.0 |
| 4                | 27.5 ± 0.04             | 0.98 ± 0.01 | -17.0 |
| 5                | 27.5 ± 0.09             | 0.96 ± 0.01 | -17.0 |
| 6                | 27.5 ± 0.12             | 0.96 ± 0.01 | -17.0 |
| 7                | 27.4 ± 0.19             | 0.97 ± 0.01 | -17.0 |
| 8                | 27.5 ± 0.14             | 0.98 ± 0.01 | -17.0 |
| 9                | 27.5 ± 0.13             | 0.98 ± 0.01 | -17.0 |
| 10               | 27.5 ± 0.07             | 0.98 ± 0.01 | -17.0 |
| 11               | 27.4 ± 0.17             | 0.97 ± 0.01 | -17.0 |
| 12               | 27.3 ± 0.19             | 0.93 ± 0.01 | -17.0 |
| 13               | 27.3 ± 0.20             | 0.94 ± 0.01 | -17.0 |
| 14               | 27.5 ± 0.14             | 0.96 ± 0.02 | -17.0 |
| 15               | 27.5 ± 0.04             | 0.98 ± 0.01 | -17.0 |
| 16               | 27.5 ± 0.00             | 0.98 ± 0.01 | -17.0 |
| Multiday mean    | 27.5 ± 0.967            | 0.967 ± 0.15 | -17.1 |
| SD               | 0.08 ± 0.015            | 0.015 ± 0.15 | 0.15 |

Table II. Mean values of $h$, $\delta^{18}O_L$, and the number of days that T. usneoides strands were in the chamber for each experimental run
ever, above 0.8, the trichome water increased to more than 40% at $h = 1$. While the latter value may seem high, these results are consistent with water hydration studies of nonlipid plant cuticle components (Dominguez and Heredia, 1999). These data were used to parameterize the model of Hallwood and Horrobin (1946), which was then used to predict the “trichome offset” for a variety of $T. \text{usneoides}$ RWC and $h$ values (Fig. 2). For the model values of Figure 2, RWC includes both tissue water and water adsorbed to trichomes. The expected isotopic offset can be as high as 2% if RWC is low and the $h$ during sampling is high; however, for most of the sampling scenarios encountered in this study, the trichome offset was between 0.5‰ and 1‰, which is still well above the measurement precision of 0.2‰. These results show that the trichome offset must be considered for this study, because sampling occurred during nocturnal periods for the plants, when $h$ was highest. All steady-state and non-steady-state predicted values of $\delta^{18}O_L$ were corrected for trichome water adsorption based on these observations (see “Materials and Methods”). In field sampling conditions, trichome offsets should be less of a worry because of typically low $h$ during the day. For example, at an $h$ of 0.65 and RWC of 100%, the trichome offset is near the sampling precision for $\delta^{18}O$ (approximately 0.2‰). If the RWC is decreased to 50% (thereby increasing the proportional representation of trichome water), the offset increases to 0.36‰.

To examine how $\delta^{18}O_L$ changed as a consequence of rehydration, $T. \text{usneoides}$ strands that were not watered for 1 week were placed in enriched water and removed at set times from 10 min to 24 h (Fig. 3). A mass balance using the $\delta^{18}O$ of wetting water (11.5‰), the initial $\delta^{18}O_L$ ($-3.3$‰ ± 0.31‰), and the final $\delta^{18}O_L$ (3.7‰ ± 0.57‰) showed that approximately 47% of the final water volume was the wetting water or that plant water volume nearly doubled due to the rewetting. After 5 h of submersion in water, the $\delta^{18}O_L$ was statistically indistinguishable from $\delta^{18}O_L$ after 24 h submersed (Fig. 3). These results are similar to previous work showing that $T. \text{usneoides}$ attains saturated water content in approximately 4 h. After only 20 min submersed, $\delta^{18}O_L$ became significantly enriched by 2.5‰, and approximately 70% of the “new” water was absorbed. Therefore, substantial changes in $\delta^{18}O_L$ can occur over relatively short time spans due to water absorption.

Figure 4 shows $g_{\text{tot}}$ (mmol m$^{-2}$ s$^{-1}$; stomatal and boundary layer conductance) for water vapor from $T. \text{usneoides}$ versus RWC for average chamber $h$ ranging from 0.556 to 0.997. There was a highly significant ($r^2 = 0.78$, $P < 0.001$) correlation between RWC and $g_{\text{tot}}$. Across treatments, there was also a significant effect of vapor pressure deficit (VPD) on $g_{\text{tot}}$ but VPD explained only about 17% of the variation in $g_{\text{tot}}$ (data not shown).

A general empirical sense of how the control of $\delta^{18}O_L$ by $\delta^{18}O_w$ is regulated by $h$, and that as $h$ approaches unity $\delta^{18}O_L$ approaches equilibrium with $\delta^{18}O_w$, can be seen in Figure 5. Helliker and Griffiths (2007) showed that, under reasonably constant conditions, $\delta^{18}O_L$ tended toward maximum enrichment (Eq. 4 in “Materials and Methods”) after 4 d. Figure 1 shows the difference between $\delta^{18}O_L$ and $\delta^{18}O_w$ ($\delta^{18}O_L - \delta^{18}O_w$) plotted against $h$ for every $\delta^{18}O_L$ sample that had been in the chamber for at least 4 d ($n = 169$). The data showed a tight relationship ($y = -44.9x + 52.8$; $P < 0.0001$, $r^2 = 0.87$) where $h$ explained 87% of observed $\delta^{18}O_L$. However, when the range of $h$ was restricted to 0.8 and above, conditions more in line with natural field conditions, $h$ explained the observed data scatter less well ($y = -59x + 660.00$; $P < 0.0001$, $r^2 = 0.67$).

Figures 6 and 7 show day-to-day observations and predictions of $\delta^{18}O_L$ for representative chamber studies at three high values (0.96 and 0.92 in Fig. 6; 0.97 in Fig. 7A), one middle value (0.84; Fig. 7B), and one low value (0.56; Fig. 7C) of mean $h$. The leaf water models (Eqs. 1, 4, and 6) were parameterized with observed nocturnal $h$, temperature, $\delta^{18}O_w$, and $g_{\text{tot}}$ data for each day, and the solutions were corrected for water adsorbed to trichomes ($\delta^{18}O_{\text{trichome}}$) using observed RWC and the em-
Rehydration of plants during experimental runs did cause a significant change in $\delta^{18}O_b$, but the change was short term. In four out of 12 of the high-$h$ experimental runs ($h > 0.9$), two populations of T. usneoides were maintained within the chamber, one wetted and one nonwetted. The frequency of the wetting varied from 2 to 7 d, and the rehydration water was enriched to artificially enhance the difference between the wetted and nonwetted $\delta^{18}O_b$. Representative data from these comparisons are presented in Table III. As in all experiments, all plants were fully hydrated on day 0. From day 1 to day 6, all plants received no rehydration water. On day 7, $\delta^{18}O_b$ samples were taken and half of the plants were submerged in the wetting water for 1 h. When all plants were sampled for $\delta^{18}O_b$ on day 10, there was no significant difference between the wetted and nonwetted plants. The wetted plants were again rehydrated on day 10 (and again on day 14), and in each subsequent sampling period, there were no significant differences between the wetted and nonwetted plants. $\delta^{18}O_b$ for the wetted plants was sampled shortly after rehydration on day 10 to show that the wetting water did in fact cause a significant enrichment of $\delta^{18}O_b$, yet this difference was not apparent on day 14. With one exception, the results in Table III are comparable to all the wetted versus nonwetted comparisons. In only one part of one trial was there a significant difference between wetted and nonwetted strands. In this trial, the wetted plants were rehydrated every 2 d and the nonwetted plants were very dry (RWC < 80%) and exchanging (losing) very little water with chamber water vapor ($g_{tot}$ was very low).

**DISCUSSION**

It is a long-term goal to use the $\delta^{18}O$ of epiphytic CAM plants, both $\delta^{18}O_a$ and organic material that is labeled by $\delta^{18}O_a$, to illuminate plant physiological responses to the unique epiphytic growth habit (Reyes-Garcia et al., 2008) and to reconstruct $\delta^{18}O_a$...
(Helliker and Griffiths 2007), the latter goal being much more broadly important to the use of stable isotopes in plant physiological and ecophysiological research. The largely abiotic factors $h$ and $\delta^{18}O_a$ are the primary controls of $\delta^{18}O_L$ (Fig. 1), and their control under both constant and dynamic conditions must be considered. In addition, epiphyte morphology and physiology act as important secondary controls on $\delta^{18}O_L$, and not accounting for both could potentially obscure any $\delta^{18}O$ interpretation. Plant physiology affects $\delta^{18}O_L$ primarily through $g_{st}$ (stomatal and boundary layer conductance), which controls the rate of exchange of tissue water with water vapor and the RWC of the plant. Plant morphology should affect epiphytic $\delta^{18}O_L$ through water trapped in tanks formed at leaf bases (although this is not a concern for $T. usneoides$) and through water adsorbed to hygroscopic trichomes. Therefore, the goal of this study was to use controlled experiments on the CAM epiphyte $T. usneoides$ to assess how well observed $\delta^{18}O_L$ could be explained through controlled variation in environment and physiology.

Water adsorbed to trichomes was shown to have a substantial potential impact (Figs. 2 and 3) on observed and predicted $\delta^{18}O_L$, but the actual impact and the need for accounting in field and laboratory situations need clarification. For all predictions shown in Figure 7, the relationship between predicted $\delta^{18}O_L$ using the trichome offset and predictions not using the offset was $y = 0.99x - 0.81$ ($r^2 = 0.997$), so the noncorrected predictions of $\delta^{18}O_L$ were about 0.8‰ more enriched than the predictions using the trichome offset. This overall difference of about 1‰ is clearly a substantial offset. However, the experimental setup exacerbated this offset, because we sampled $\delta^{18}O_L$ during the nocturnal period, when $h$ was highest. This setup was chosen so that $\delta^{18}O_L$ could be sampled during the period when most vapor exchange was occurring. Under field conditions, $\delta^{18}O_L$ would likely be sampled during the low-humidity day, when the trichome offset would be less of a problem and perhaps not a problem at all. The exception to this would be sampling leaves for $\delta^{18}O_L$ near dawn or dusk, when $h$ would likely be high. It should be pointed out that while most of the adsorbed water was likely onto the trichomes, adsorption to the cuticle could not be ruled out. This is somewhat inconsequential, as discussed below. Also, the apparent isotopic offset between the trichome water and $\delta^{18}O_a$ was $7.4\%\pm 0.1\%$, while the equilibrium fractionation factor for condensation was around $9.2\%$. It is unclear at this time how similar adsorption of water to leaf cuticles and trichomes of more common plant types (i.e. nonepiphytic) could bias observations of samples taken near dawn or dusk. Similarly, this is an unlikely problem in field studies, with the exception of diurnal sampling schemes. In the latter case, water adsorption to leaf cuticles/trichomes could lead to lower than expected enrichment during high-humidity morning and late afternoon sampling.

When $h$ is below 1, the direct adsorption of atmospheric water vapor by the dead trichomes of $T. usneoides$ does not lead to any apparent absorption by the living cells of the plants (Martin and Schmitt, 1989). Several studies have reported direct uptake of

\[
\begin{align*}
\text{Equation 5.} & \\
\text{Equation 1.} & \\
\text{Equation 5.} & \\
\text{Equation 6.} & \\
\text{H&C} & \\
\text{H&C constant} & \\
\text{H&C constant} & \\
\text{Equilibrium} & \\
\end{align*}
\]
water by atmospheric epiphyte leaves, but this is under conditions of saturating vapor and/or leaf wetness (Benzing et al., 1976; Martin and Schmitt, 1989; Andrade, 2003). The trichomes of *T. usneoides* are highly hygroscopic and can, within gas-exchange chambers, lead to what appears as a “negative” transpiration while equilibrating to an increase in $h$. This negative transpiration is the process of water adsorbing onto the trichomes, and it stops once equilibrium is reached with $h$. This process is also what was quantified in isolation here with dead *T. usneoides* (Fig. 2). The dead trichomes of *T. usneoides* likely equilibrate with the water potential of ambient air, much like what is seen in the lichen thallus (Lange et al., 2001; Hartard et al., 2009), and isotopically the adsorption of water onto the trichomes may be very similar to that seen by the thallus of a lichen. This, however, is where the similarities end. Lichens are poikilohydric, and some algae-symbiotic species can become physiologically active solely through this water adsorption (yet only at relatively high $h$); thus, the adsorption of water vapor by the thallus matrix can lead to a cellular absorption (Lange et al., 2001). Such an adsorption of atmospheric water vapor does not occur in homiohydric *T. usneoides* and is unlikely to occur in other vascular plant atmospheric-type epiphytes (e.g. bromeliads, orchids, lycophytes, and ferns; Martin et al., 2004).

*T. usneoides*, like most epiphytic higher plants that have been observed, maintains very high (near zero) plant water potentials, with osmotic potentials maintained consistently in the realm of aquatic plants (Martin et al., 2004). In general, the measured water potential of atmospheric epiphytes, *T. usneoides* included, has rarely been observed below $-1$ MPa (Martin, 1994). In one experiment with *Tillandsia ionantha*, total plant water potential did not drop below $-0.75$ MPa after 60 d of imposed drought (Nowak and Martin, 1997). Considering that the water potential of air at $h = 0.99$ is $-1.4$ MPa (and $-2.8$ MPa at $h = 0.98$, both at 20°C), it is hard to imagine a scenario in which water adsorbed onto trichomes that are in equilibrium with subsaturated air could move against the water potential gradient into the living cells of *T. usneoides*, and the work of Martin and Schmitt (1989) corroborates this. Therefore, there should be no consideration of water vapor uptake by trichomes affecting $\delta^{18}O_\text{L}$. Once saturation is realized...
and the plant tissue is covered with liquid water, the trichomes become pressed against the cuticle and channel the absorption of liquid water into the underlying living cells (Benzing et al., 1976). This sort of precipitation event is explicitly considered through Equation 1. Furthermore, the maintenance of extremely high water and largely invariant water potential in the face of drought is likely the driving force behind the austere physiology of epiphytes (Martin et al., 2004) in terms of both carbon assimilation and water loss. For *T. usneoides*, well-watered measurements of CO$_2$ uptake as low as 0.2 μmol m$^{-2}$ s$^{-1}$ (Martin and Schmitt, 1989) and transpiration of 0.002 mmol m$^{-2}$ s$^{-1}$ are common (Martin, 1994; this study). The tight coupling of plant water potential and $g_{\text{tot}}$ likely led to the rapid decrease in $g_{\text{tot}}$ as RWC decreased (Fig. 5) and could explain why $g_{\text{tot}}$ was more highly correlated with RWC than with VPD. The inherently low levels of water loss meant that estimates of $g_{\text{leaf}}$ could be determined once per day or even once every other day, thus maintaining constant chamber conditions for longer periods of time (Supplemental Fig. S2).

Transpiration forced $\delta^{18}$O$_{l}$ toward the value of maximum enrichment, $\delta^{18}$O$_{l,M}$, and the closer the value of $h$ was to unity, the faster was the approach to $\delta^{18}$O$_{l,M}$. This overall tendency can be seen in Figures 6 and 7, where all values of $\delta^{18}$O$_{l}$ moved toward the predicted $\delta^{18}$O$_{l,M}$. Yet only when values of $h$ were greater than 0.9 (Figs. 6 and 7A) did observed $\delta^{18}$O$_{l}$ actually reach $\delta^{18}$O$_{l,M}$. At higher $h$, the gross flux of water into the leaf ($g_{\text{leaf}} \times w$) was proportionally much higher than at low $h$; therefore, the replacement of leaf water with atmospheric water was faster. At lower $h$, a considerably greater volume of leaf water must be lost before $\delta^{18}$O$_{l}$ becomes equal to $\delta^{18}$O$_{l,M}$ (Helliker and Griffiths, 2007), and the response of $g_{\text{tot}}$ to RWC tends to stop any great reduction in leaf water volume (Fig. 5). A quick comparison of Figures 6 and 7A shows two experimental runs at similar $h$ that took drastically different amounts of time for $\delta^{18}$O$_{l}$ to equal $\delta^{18}$O$_{l,M}$. In Figure 7A, the apparent leaf-water turnover time was about 4 d, similar to the results of Helliker and Griffiths (2007), whereas in Figure 6, the apparent leaf-water turnover time was 9 d. The reason for this discrepancy was the different starting points of $\delta^{18}$O$_{l}$. In Figure 6, the initial $\delta^{18}$O$_{l}$ was approximately 15‰ more enriched than $\delta^{18}$O$_{l,M}$, and in Figure 7A, the starting $\delta^{18}$O$_{l}$ was approximately 7‰ more enriched than $\delta^{18}$O$_{l,M}$.

In general, as water is lost from CAM epiphytes, $\delta^{18}$O$_{l}$ is more and more likely to be described by the maximum enrichment model, $\delta^{18}$O$_{l,M}$. Yet, it should be recognized that as the period between rehydration events increases, RWC decreases and so does $g_{\text{tot}}$, which leads to an inevitable decrease in water vapor exchange between the leaf and the atmosphere. So, as the plant dries, $\delta^{18}$O$_{l}$ still controls $\delta^{18}$O$_{l}$, but the turnover of leaf water and consequently any effects that changes in ambient $\delta^{18}$O$_{l}$ have on $\delta^{18}$O$_{l}$ are minimized. This can be seen distinctly in Figure 7C, where even after several days at low humidity, the enrichment of $^{18}$O in leaf water ceased. As *T. usneoides* dries out, the overall tissue water exchange with atmospheric water vapor slows until $\delta^{18}$O$_{l}$ becomes decoupled from $\delta^{18}$O$_{l}$.

The largest potential hurdle in using $\delta^{18}$O$_{l,M}$ to describe observed $\delta^{18}$O$_{l}$ appears to be the effect that the isotope ratio of rehydration water (simulated precipitation events) has on $\delta^{18}$O$_{l}$, and there are two components of this: the absolute difference in isotopic space between the rehydration water and $\delta^{18}$O$_{l,M}$, and the total amount of rehydration water absorbed by the plant. As a general response, water gain through precipitation has the initial tendency to move $\delta^{18}$O$_{l}$ farther away from $\delta^{18}$O$_{l,M}$, but then, due to increased $g_{\text{tot}}$, it rapidly trends toward $\delta^{18}$O$_{l,M}$. The more specific response of *T. usneoides* $\delta^{18}$O$_{l}$ to precipitation events was examined three ways. The first was through the rehydration study (Fig. 4), which showed that the absorption of precipitation can lead to a rapid and significant change in $\delta^{18}$O$_{l}$ after only 20 min in liquid water, and after 5 h the change in $\delta^{18}$O$_{l}$ was complete. The second was starting all experimental runs by soaking plants in an enriched water source, thereby ensuring that each experimental run began after a simulated precipitation event. This method showed that, at high $h$, the precipitation isotope label could exchange quickly with atmospheric water vapor, and the speed of exchange depended on the size of disparity between the initial label leaf water and $\delta^{18}$O$_{l,M}$. The final assessment of how precipitation inputs affect *T. usneoides* $\delta^{18}$O$_{l}$ was designed to examine the differential effect of rehydrating one subset of strands while keeping the other dry during the same experimental runs (Table III). This approach explored a potential situation in the field where rainfall or dew formation could vary spatially either across a field site or within a single clump of *T. usneoides*, depending on the intensity of a precipitation event. This method was used in several of the high-$h$ experimental runs, where the frequency of the wetting varied from 2 to 7 d and the rehydration water was enriched. In general, there was

**Table III.** The representative effect of rehydration on $\delta^{18}$O$_{l}$ through time during the chamber experiments

| Day     | Wetted Plant $\delta^{18}$O$_{l}$ | Nonwetted Plant $\delta^{18}$O$_{l}$ | %a |
|---------|-----------------------------------|--------------------------------------|----|
| Day 0   | -3.0 ± 0.4                        | -3.0 ± 0.4                           | ns |
| Day 7   | -6.8 ± 0.6                        | -6.8 ± 0.6                           | ns |
| Day 10  | -7.4 ± 0.2                        | -6.8 ± 0.1 (ns)                      |    |
| Day 10, 1 h after rehydration | 2.5 ± 0.6                           | 2.5 ± 0.6                            |    |
| Day 14  | -6.1 ± 0.2                        | -6.3 ± 0.6 (ns)                      |    |
| Day 21  | -6.5 ± 0.4                        | -7.1 ± 0.7 (ns)                      |    |
no sustained, significant difference between the wetted and nonwetted plants. Even though significant enrichment occurred in as little as 20 min (Fig. 4), it appears that this water was quickly turned over through nocturnal exchange of water vapor. This rapid turnover is likely due to the fact that rehydration allows for an uptick in \( g_{\text{tot}} \), which then allows for increased gross exchange of vapor between the plant and the atmosphere. The end result is that both wetted and nonwetted strands tracked \( \delta^{18}O_W \), although it seems likely that strands with higher RWC would respond to changes in \( \delta^{18}O_W \) more quickly than drier strands, as mentioned above. It appears that a relatively consistent input of dew or precipitation water could lead to a tighter coupling of \( \delta^{18}O_L \) and \( \delta^{18}O_a \), because of enhanced vapor exchange. Additionally, as discussed below, I hypothesize that under field conditions, precipitation itself should lead to a greater coupling between \( \delta^{18}O_{L,M} \).

The use of enriched rehydration water allowed for easier observation of how precipitation affected \( \delta^{18}O_L \), but it also led to a potential misinterpretation of the coupling between \( \delta^{18}O_L \) and \( \delta^{18}O_a \). Both the rapid change in \( \delta^{18}O_l \) during rehydration (Fig. 4) and the multi-day turnover of \( \delta^{18}O_L \) could lead one to conclude that a rehydration event pulls \( \delta^{18}O_L \) too far away from \( \delta^{18}O_{L,M} \) to use \( \delta^{18}O_L \) as a decent proxy for \( \delta^{18}O_L \). However, the \( \delta^{18}O \) of the wetting water was typically 15\% more enriched in \( ^{18}O \) than the \( \delta^{18}O_{L,M} \)-equilibrated \( \delta^{18}O_L \) and 25\% to 30\% more enriched than \( \delta^{18}O_L \). While this isotopic span allowed for a clearer picture of rehydration effects and tissue-water turnover times, it is highly unlikely that such a span would occur in the field. Precipitation (dewfall or rainfall) should also be near equilibrium with \( \delta^{18}O_a \) (Gat, 1996). Therefore, isotopic differences between rain and/or dew \( \delta^{18}O \) and \( \delta^{18}O \) (and \( \delta^{18}O_{L,M} \)) on the scale of 15\% would not be expected, nor even considering large-scale episodic events that can dramatically alter \( \delta^{18}O \) of precipitation (Miller et al., 2006), and precipitation should enhance the coupling of \( \delta^{18}O_L \) and \( \delta^{18}O_a \).

When variation in \( \delta^{18}O \), \( h \), \( g_{\text{tot}} \), RWC, and trichome water adsorption were considered through the leaf water models (Eqs. 1, 4, and 6) and through a wide range of conditions (Table II), observed \( \delta^{18}O_L \) was explained very well (Figs. 6–8). Both variations of the nonsteady-state model (Eqs. 1 and 6) predicted observed \( \delta^{18}O_L \) well, and this included runs when \( g_{\text{tot}} \) and \( W \) were held constant (Fig. 6). Previous work on nonepiphytic plants has also shown that measurements of \( \frac{dW}{dt} \) are not crucial for accurate predictions of nonsteady-state models (Cernusak et al., 2002; Farquhar and Cernusak, 2005; Cuntz et al., 2007; Ogee et al., 2007; Kahn et al., 2008). The modeled estimates of \( \delta^{18}O_E \left( R_E, \text{Figs. 6 and 7A} \right) \) approached and only occasionally matched the value of \( \delta^{18}O_{L,M} \), as would be expected at isotopic steady state. It can be concluded then that \( \delta^{18}O_L \) was rarely at true isotopic steady state; however, observed \( \delta^{18}O_L \) often matched \( \delta^{18}O_{L,M} \) at \( h > 0.9 \) and generally followed \( \delta^{18}O_{L,M} \) even with slight changes in \( h \).

This apparent contrast leads to one of the more important conclusions of this study: that, in agreement with the predictions of Helliker and Griffiths (2007), leaf water does not need to be at full isotopic steady state for observed \( \delta^{18}O_L \) to equal \( \delta^{18}O_{L,M} \).

The extreme sensitivity of \( \delta^{18}O_L \) to \( h > 0.9 \) means that Equation 7, the full equilibrium equation, is rarely useful. This may seem contradictory to some of the data presented by Helliker and Griffiths (2007), who showed a prediction range for Equation 7 on some figures. Their experimental setup had an invariably high \( h \) and noisy \( \delta^{18}O_a \) (±1.2\%), and we used the range of predictions stemming from the variability in \( \delta^{18}O_a \) to show that observations were near these values. Unfortunately, this approach could lead one to erroneously conclude that \( \delta^{18}O_L \) was in direct equilibrium with \( \delta^{18}O_a \) even when \( h \) was less than unity, which is not the case. The value of \( h \) should be known to decide which equation to use when extracting \( \delta^{18}O_L \) from measures of \( \delta^{18}O_a \) using CAM epiphytes or nonepiphytic plants, as in the predawn sampling approach developed by Lai et al. (2008). While predictions of \( \delta^{18}O_{L,M} \) (Eq. 4) matched observed \( \delta^{18}O_L \) at \( h > 0.9 \), the predictions from this model showed good agreement with observed \( \delta^{18}O \) over the broader range of \( h > 0.8 \) (\( y = 0.87x - 0.99; r^2 = 0.92; \text{Fig. 8B} \)) after 4 to 5 d. The primary need to exclude data under 4 d results from the artifact of using an enriched water source for rehydration, as mentioned above. A more complete test of Equation 4, the maximum enrichment model \( \delta^{18}O_{L,M} \), has been performed under field conditions and is the subject of subsequent publications.

CONCLUSION

Helliker and Griffiths (2007) proposed that the \( \delta^{18}O_L \) of CAM atmospheric epiphytes could be used to reconstruct \( \delta^{18}O_a \). An inherent assumption of this proposal was that the environmental controls on \( \delta^{18}O_L \), aside from \( \delta^{18}O_W \), are known and can be accounted for in the framework of a mechanistic model. The solid agreement between observations of \( \delta^{18}O_L \) and predictions from Equations 1, 4, and 6 appears to satisfy this assumption and shows a fair understanding of the controls on \( \delta^{18}O_L \) in \( T. \) usneoides, at least under laboratory conditions. Hygroscopic trichomes can affect interpretations of observed \( \delta^{18}O_L \) but only when leaf water is under high \( h \), a condition that is not often satisfied in the field. Observed \( \delta^{18}O_L \) could be predicted well through the non-steady-state equations even by using constant values of \( W \) and \( g_{\text{tot}} \). Therefore, the non-steady-state models can potentially be applied more easily. Also, the leaf does not need to be at full isotopic steady state for observed \( \delta^{18}O_L \) to equal \( \delta^{18}O_{L,M} \) (Eq. 4), so only \( \delta^{18}O_a \) and \( h \) are required as predictive inputs. The poor performance of Equation 4 in the first 4 to 5 of experimentation was likely an artifact of using artificially enriched rehydration water to start each experiment. Tissue rehydration
using enriched water caused a significant but transitory change in $\delta^{18}O_a$ because the consequent increase in $g_{\text{tot}}$ ultimately led to a faster coupling with $\delta^{18}O_a$. The inherent coupling between precipitation water $\delta^{18}O$ and $\delta^{18}O_a$ in the field should mean that $\delta^{18}O_a$ is more tightly coupled to $\delta^{18}O$ after rehydration. This enhanced understanding about the controls of $\delta^{18}O_a$ in CAM epiphytes under controlled laboratory conditions will improve the interpretation of $\delta^{18}O_a$ observations in the more varied natural environment.

### MATERIALS AND METHODS

**Modeling Leaf Water Isotopes**

The $\delta^{18}O_a$ for a CAM epiphyte such as *Tillandsia usneoides* can be explained by the following non-steady-state equation (Flanagan et al., 1991; Farquhar and Lloyd, 1993; Farquhar and Cernusak, 2005; for a full derivation, see Helliker and Griffiths, 2007):

$$R_L = \alpha^* \left( R_H - \frac{d(WR_L)}{dt} \right) \frac{a^*}{g_{\text{tot}}w_l + R_L}$$

(1)

where $R$ is the molar isotope ratio and the subscripts $L$ and $P$ represent leaf and precipitation (rainfall and dewfall) water, respectively. $I_P$ is the flux of water into the plant, $W$ is leaf water volume (mol m$^{-2}$), $g_{\text{tot}}$ is total conductance (stomatal and boundary layer; mol m$^{-2}$ s$^{-1}$), and $w_l$ and $w_P$ represent the mole fraction of water vapor in the substomatal cavity and ambient air, respectively. The mole fraction of water vapor ($w$) is equal to the partial vapor pressure ($e$) divided by total atmospheric pressure. The temperature-dependent equilibrium fractionation factor is represented by $a^*$, and $a^*$ is the balance of the ratio of diffusivities of light to heavy water molecules through the stomata and through the leaf boundary layer (Merlivat, 1978; Luz et al., 2009). Between wetting events, when $R_P = 0$, $R_L$ can be explained by:

$$R_L = \alpha^* \left[ \frac{d(WR_L)}{dt} \frac{a^*}{w_l + R_L} \right]$$

(2)

between rain events, $\frac{d(WR_L)}{dt} = -R_L E$, where $E$ is the transpiration flux from the leaves ($E = g(w_l - w_P)$). If we define $h = w_l/w_P$, then Equation 2 can be rewritten as:

$$R_L = \alpha^*[\alpha^* R_L(1 - h) + R_L]h$$

(3)

Throughout this study, $h$ is ambient relative humidity, so in Equation 3, the effect of $h$ on $R_L$ can be seen easily where, as $h$ increases toward unity, $R_L$ assumes a greater control over $R_L$. Under constant conditions and water loss, steady state is achieved, and $R_L$ in Equation 3 can be replaced by $R_L$ arriving at maximum enrichment, $R_{\text{L,max}}$:

$$R_{\text{L, max}} = \frac{R_L h}{\alpha^* - \alpha^* h}$$

(4)

Equation 4 is similar to the derivation of maximum enrichment of Farquhar and Gan (2003) and Hartard et al. (2009):

$$R_{\text{L, max}} = \frac{\alpha^* h R_L}{1 - \alpha^* h(1 - h)}$$

(5)

Hartard et al. (2009) further developed an analytical solution to the non-steady-state equation, referred to throughout as the Hartard and Cuntz solution:

$$R_L = R_{\text{L, max}} + \left[R_0 - R_{\text{L, max}}\right]\exp\left[-\frac{1}{\alpha^* a^*(1 - h)}\frac{E}{h^2}\right]$$

(6)

Lastly, when $h = 1$, $R_L$ is equal to the isotope composition of atmospheric water vapor, corrected for the equilibrium fractionation factor:

$$R_L = R_0 h$$

(7)

In this paper, the majority of model solutions were obtained through iterative solution of Equation 2. The Hartard and Cuntz solution was compared with outputs of Equation 2 using changing and constant values of $g_{\text{tot}}$ and $W$. Equation 5 was examined as a simplified method of predicting $R_L$ for eventual field applications.

### Plant Material

The study species for this work was the CAM epiphytic bromeliad *T. usneoides*. *T. usneoides* is a subtropical to tropical plant that ranges from coastal Virginia through the tropics to Argentina. The broad distribution is determined by high nocturnal (Garth, 1964) and makes it a model organism for reconstructing $\delta^{18}O_a$. In October 2006 and 2007, plants were obtained from the northernmost point of distribution for *T. usneoides* in North America, First Landing State Park in Virginia Beach, Virginia. The plants were subsequently maintained in a high-humidity greenhouse at the University of Pennsylvania until experimentation. Approximately 5 d prior to the start of an experiment, clumps of *T. usneoides* were separated into strands of 10 to 20 ramiets (ramets consisted of two to five leaves that were 3 to 6 cm in length) and placed in the growth chamber under a reversed 12-h photoperiod (i.e., forcing the plant's nocturnal cycle to coincide with the researcher's normal diurnal cycle). Prior to this acclimation period, all strands were submersed in enriched water (approximately $11\%$) for 2 to 4 h, and plants were watered daily thereafter until the start of an experiment.

### Experimental Conditions

For experiments, plants were maintained in a climate-controlled chamber coupled to a large-volume dewpoint generator (the "water tower") that was capable of maintaining a constant $\delta^{18}O_a$ for up to 3 weeks ($\approx 0.15\%$). The chamber was fabricated from a cubic 22.4-l. acrylic desiccator cabinet (Fisher Scientific) that was back-tiled with tubing inlets and outlets and a 75-W, 24-V direct current Pelletier-type thermoelectric air conditioner (INB260-24-AA; Watronix). A temperature controller (model CN77322-C2; Engineering) signaled a relay to change the direct current polarity to either heat or cool the chamber based on the prescribed set point; separate day/night temperature set points were controlled by a Grasslin Digi-20E timer (Intermatic). Twenty-centimeter-long strands of *T. usneoides* were placed horizontally in the chamber on top of a wire mesh shelf. A 400-W metal-halide light source (Sun System 3; Sunlight Supply) was placed above the chamber; photosynthetically active radiation was $300 \mu mol \cdot m^{-2} \cdot s^{-1}$ at plant height.

The water tower consisted of two 132-cm-long, 0.64-cm wall thickness extruded acrylic cylinders, one inside of the other (outer cylinder i.d. = 20.3 cm, inner cylinder i.d. = 15.2 cm), that were sealed off with 1-cm-thick aluminum plates at either end. The effect was to create an outer cavity for a temperature-controlled water jacket that surrounded the inner cylinder. The inner cylinder was filled with 23 L of water, which was the source for irrigation vapor in the chamber. The water jacket temperature was controlled by pumping water from a circulating water bath (water circulated between the outer and inner cylinders). Ambient air was pumped (KNF Neuberger) through the length of the inner cylinder at 2 L min$^{-1}$ (MKS flow controller) to create $\delta^{18}O_a$ and dew point values that were fed to the plant chamber. The temperature of the water tower controlled the ambient vapor pressure ($e_a$) values for the climate-controlled chamber, and the temperature of the chamber set the saturated vapor pressure ($e_{\text{sat}}$) values. Because of the high heat capacity of the water tower and the tight temperature control of the chamber, a fairly constant $e_a$, and therefore $h (e_a/e_{\text{sat}})$, could be maintained (for an image of the chamber/water tower setup and representative diurnal cycles of $h$ and air temperature, see Supplemental Fig. S1).

Five days prior to each experiment, 30 to 50 30-cm-long strands of *T. usneoides* were removed from the greenhouse and placed in a growth chamber set on a reversed 12-h photoperiod to acclimate the plants. The reversed photoperiod allowed for easy sampling of $\delta^{18}O$, during the period of *T. usneoides* water loss (and isotopic exchange). Each experimental run was started (day 0) by submerging the strands in a tub of enriched water (typically $10\%-20\%$) for 2 h. This submergence ensured hydration of the strands and ensured that the initial $\delta^{18}O_a$ of leaf water was considerably different from any chamber-induced $\delta^{18}O_a$. The strands were surface dried and then placed in the experimental chamber. Four of the strands were sealed in glass vials imme-
diately to sample for initial $\delta^{18}O$, and four of the strands were tagged for measurements of RWC and water loss (through loss of mass) during every sampling period. Typically every 3rd d, three to five strands of $T. usneoides$ were removed from the chamber and placed into glass vials for eventual water extraction and determination of $\delta^{18}O$.

**Isotope Sampling and Analysis**

Water vapor was sampled from the chamber exhaust tubing for 2.5 to 3 h prior to opening the chamber to sample leaf water in a 200-mL internal volume condenser inserted into a dry-ice/ethanol slush (−69°C). The air exiting the chambers was split and monitored with a rotometer to ensure that flow rates of air through the condenser never exceeded 0.5 L m$^{-1}$ (Helliker et al., 2002). Leaf water was extracted cryogenically (Ehleringer et al., 2000). Water samples (0.5 mL) were equilibrated for at least 48 h in 3-mL Exetainer vials (Labco) with a 10:90 mixture of CO$_2$:helium. Approximately 100 $\mu$L of the head space gas was injected into a gas chromatograph and carried in a helium air stream to a Delta Plus (Thermo-Finnigan) isotope ratio mass spectrometer. Published molar isotope ratios were expressed in the standard “δ” notation on a permil basis by $\delta = (R_{\text{sample}}/R_{\text{standard}}) \times 1,000$, where $R = ^{18}O/^{16}O$ and the standard was Standard Mean Ocean Water ($R = 0.002005$).

**Estimating RWC and Conductance to Water Loss**

After each sampling period for $\delta^{18}O$, three to four strands were placed in plastic bags to ensure no immediate loss of water from the trichomes and were removed from the chamber. One by one, the strands were taken out of the bags and the mass was measured. RWC was determined in the same manner as Martin and Schmitt (1989).

$$\text{RWC} = \frac{[\text{FW} - \text{DW}]/\text{DW}}{\times 100}$$

where FW is the measured fresh weight and DW is the measured dry weight after 3 d in a 60°C drying oven. Plant conductance to water loss ($g_{waq}$ mmol m$^{-2}$ s$^{-1}$) was determined by:

$$g_{waq} = E/(w_i - w_a)$$

Plant transpiration ($E$) was estimated from the measures of fresh weight above, $w_i$ and $w_a$ were obtained from measurements of chamber $h$ and temperature, and pressure was assumed constant at 100 kPa. Leaf area was determined using the fresh weight-to-leaf area conversions presented by Martin and Schmitt (1989). Based on direct measurements, leaf temperature and air temperature were not different; therefore, $w_i$ was equal to $w_{waq}$ at chamber temperature.

**Correcting for Rehydration and Water Adsorbed to Trichomes**

The effect that rehydration had on $T. usneoides$ $\delta^{18}O$ was determined by fully submersing 45 30-cm-long strands for several hours to rehydrate. The strands were then placed in the greenhouse and not watered for 1 week. After 1 week, the strands were placed in enriched water (11.5%), and five strands were removed from the rehydrating water at intervals of 10 min, 20 min, 40 min, 1 h, 1.5 h, 2.5 h, 5 h, 7 h, and 24 h.

In prior work, we assumed that water was adsorbed to trichomes at the time of sampling and that this water was inevitably extracted with tissue water when bulk leaf water was cryogenically extracted. In our prior attempt to correct for the isotope ratio of this water to obtain tissue water ($\delta_{\text{tissue}}$), two untested assumptions were made (Helliker and Griffths, 2007): (1) that water adsorption by the trichomes from the atmosphere did not fractionate, and (2) that the humidity-driven proportion of adsorbed water on trichomes ($\delta_{\text{trichome}}$) could be explained by a simple parameterization based on two measured humidities (Martin and Schmitt, 1989). The first assumption was examined by placing several clumps of dead $T. usneoides$ (about 2 g of material, killed via desiccation at 60°C) into the growth chamber for 2 d and then extracting the water adsorbed onto the dead tissue for isotope analysis. This yielded an apparent fractionation factor ($\delta_{\text{trichome}}$) between atmospheric water vapor and the water adsorbed to trichomes of 7.4‰ ± 0.1‰:

$$\delta^{18}O_{\text{trichome}} = \delta^{18}O_{\text{atm}} + \delta_{\text{trichome}}$$

The second assumption was examined by building an empirical relationship between the mass of dead $T. usneoides$ and $h$ ranging from 0.34 to saturation. This was accomplished by placing dead $T. usneoides$ of a variety of masses into nylon pouches (made from L’eggs pantyhose; Hanes) and comparing oven-dried mass with the mass after placement in a growth chamber at a known humidity for 24 h. Empty nylon pouches were also put through this exercise to correct for any water adsorption onto the nylon (the maximum for nylon adsorption was less than 6% of dry nylon mass at saturating humidity). The relationship between trichome water content ($h_{\text{tissue}}$) and $h$ was used to parameterize the model of Hailwood and Horrobin (1946) for predicting water adsorption of solid polymer solutions as a function of $h$:

$$f_i = k_i \left[ \frac{k_i h}{1 + k_i h} \right] \times 100$$

where $h_i$ is the number of binding sites, $k_i$ is the affinity of the material for adsorption, and $k_i$ is the water activity. The values for $h_i$, $k_i$, and $k_i$ were changed so that the adsorption curve fit the data over a range of $h$. These values were $k_i = 0.02$, $k_i = 10$, and $k_i = 0.95$.

Finally, modeled values of $\delta^{18}O_i$ from Equations 3 to 6 were corrected for trichome-adsorbed water as a proportion of RWC by:

$$\delta^{18}O_{\text{predicted}} = \delta^{18}O_{\text{trichome}} + (1 - \frac{f_i}{\text{RWC}})\delta^{18}O_{\text{atm}} + \delta^{18}O_{\text{trichome}} f_i \text{RWC}$$

Supplemental Data

The following materials are available in the online version of this article.

Supplemental Figure S1. Photograph of experimental setup.

Supplemental Figure S2. Representative diel plots of $h$ and air temperature.

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