Environmental Effects on Oxygen Isotope Enrichment of Leaf Water in Cotton Leaves

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The oxygen isotope enrichment of bulk leaf water ($\Delta_b$) was measured in cotton ($Gossypium hirsutum$) leaves to test the Craig-Gordon and Farquhar-Gan models under different environmental conditions. $\Delta_b$ increased with increasing leaf-to-air vapor pressure difference (VPd) as an overall result of the responses to the ratio of ambient to intercellular vapor pressures ($e_i/e_a$) and to stomatal conductance ($g_s$). The oxygen isotope enrichment of lamina water relative to source water ($\Delta_i$), which increased with increasing VPd, was estimated by mass balance between less enriched water in primary veins and enriched water in the leaf. The Craig-Gordon model overestimated $\Delta_b$ (and $\Delta_i$), as expected. Such discrepancies increased with increase in transpiration rate ($E$), supporting the Farquhar-Gan model, which gave reasonable predictions of $\Delta_b$ and $\Delta_i$ with an $L$ of 7.9 mm, much less than the total radial effective length $L_e$ of 43 mm. The fitted values of $L$ for $\Delta_i$ of individual leaves showed little dependence on VPd and temperature, supporting the assumption that the Farquhar-Gan formulation is relevant and useful in describing leaf water isotopic enrichment.

Recently, the analysis of the oxygen isotope composition ($\delta^{18}O$) of leaf water became of increased interest as a result of efforts to obtain information on the global carbon cycle (Farquhar and Lloyd, 1993; Farquhar et al., 1993; Gillon and Yakir, 2001) and because of applications in agriculture (Barbour et al., 2000a). These and other applications were recently updated (Barbour, 2007; Farquhar et al., 2007). The $\delta^{18}O$ of atmospheric $CO_2$ and of plant organic matter depends strongly on the extent of leaf water enrichment that occurs during transpiration (Barbour et al., 2000b) because the diffusivity and vapor pressure of heavier $H_2^{18}O$ are less than that of lighter $H_2^{16}O$ (Craig and Gordon, 1965). A large portion of the $CO_2$ that enters the leaf equilibrates with evaporatively enriched leaf water via the catalytic activity of carbonic anhydrase, then retrodiffuses out of the leaf, increasing the $\delta^{18}O$ of atmospheric $CO_2$ (Farquhar et al., 1993; Yakir et al., 1993). Complications arise as a consequence of leaf water heterogeneity due to mixing between the $^{18}O$-enriched water at the evaporating sites and the $^{16}O$-depleted source water coming from the soil (Yakir et al., 1994). Thus, the accuracy of models in predicting $\delta^{18}O$ in leaf water could be important for interpreting the $\delta^{18}O$ signal of atmospheric $CO_2$ at different scales (local, regional, and global), just as they are for physiological and agricultural models using $\delta^{18}O$ of organic matter to assess genetic differences in stomatal conductance ($g_s$).

Isotopic enrichment at the evaporative sites was first predicted by a model developed for a freely evaporating water surface (Craig and Gordon, 1965) and then applied to evaporating leaves (Dongmann et al., 1974). However, many papers report that the Craig-Gordon prediction tends to overestimate the enrichment of bulk leaf water and fails to account for the isotopic gradient of water in a leaf (Yakir et al., 1990a, 1990b; Flanagan and Ehleringer, 1991; Flanagan et al., 1991; Wang and Yakir, 1995; Wang et al., 1998; Helliker and Ehleringer, 2002; Gan et al., 2002; Šantrůček et al., 2007). To explain such discrepancies, other models related to the Craig-Gordon model have been proposed. The two-pool model expresses bulk leaf water as a composite of enriched water in the leaf lamina and less enriched water in veins (Leaney et al., 1985; Roden and Ehleringer, 1999). The one-dimensional Péclet model expresses bulk leaf water as a result of the relative effects of advection and back diffusion, called the Péclet effect, along a radial direction between less enriched water in veins and enriched water at evaporative sites (Farquhar and Lloyd, 1993). Some indirect...
evidence supports the theory of the Péclet effect (Barbour et al., 2000b, 2004; Barbour and Farquhar, 2003). Other models have been developed to explain a progressive enrichment of leaf water observed along the length of the leaf. The string-of-lakes model expresses such enrichment as an analogy to a string of evaporating lakes along a desert river system (Gat and Bowser, 1991; Yakir, 1992; Helliker and Ehleringer, 2000, 2002). The need was seen to combine a continuous version of the string-of-lakes model with a two-dimensional Péclet effect in longitudinal and radial directions (Gan et al., 2002, 2003; Farquhar and Gan, 2003). The current mathematical form was presented by Farquhar and Gan (2003) and includes a longitudinal Péclet effect as well as two radial Péclet effects. The first radial effect is from the longitudinal xylem elements through “veinlets” to the mesophyll, denoted \( P_{rv} \). The second is that in the mesophyll cells of the lamina, simply denoted \( P \), to match the earlier formalism of Farquhar and Lloyd (1993), as the dependence of \( P \) on transpiration rate, \( E \), is the same as in the earlier theory. The enrichment of the water in the xylem depends on the total radial Péclet number, \( P_e \), which is the sum of \( P_{rv} \) and \( P \). The Farquhar-Gan theory also includes the effects of ground tissue associated with xylem.

The lamina Péclet effect, \( P \), depends on \( E \) and the effective path length of water movement in the lamina, \( L \) (Farquhar and Lloyd, 1993). Therefore, \( L \) needs to be parameterized to predict the relationship between leaf water enrichment and \( E \). \( L \) is theoretically assumed to depend on leaf anatomy, not directly on \( E \), but it is impracticable to estimate it by direct measurements of leaf structure. For this reason, \( L \) has been estimated from the difference between the observed leaf water enrichment and the Craig-Gordon prediction (Cernusak et al., 2003). Such estimations have been made on various plants (Flanagan et al., 1991, 1994; Barbour and Farquhar, 2000; Barbour et al., 2000a, 2000b; Cernusak et al., 2003). We need more evidence for the assumption that the formulation of the Péclet effect is reasonable, which in turn means that \( L \) depends on leaf anatomy but not on environmental conditions. For that purpose, we measured the oxygen isotope enrichment of leaf water in cotton (Gossypium hirsutum) plants to test the Craig-Gordon and the Farquhar-Gan models under a wide range of vapor pressure difference and at two temperatures.

RESULTS

Bulk Leaf Water and Lamina Enrichment

Primary vein and associated ground tissue water formed a proportion, \( \phi_{sv} \), of 12.8 ± 0.46% (\( n = 16 \)) of bulk water, not significantly different from the observations of Gan et al. (2002) in cotton of 14.2 ± 1.9%. Changes of environmental conditions such as air humidity, irradiance, and temperature induced large variations in leaf-to-air leaf-to-air vapor pressure difference (VPd) and \( E \) (Fig. 1). Significant negative correlation was found between \( g_{5} \) (mol m\(^{-2}\) s\(^{-1}\)) and VPd (mbar) at both high and low temperature (Fig. 1A); regression equations were \( g_{5} = -0.019 \text{VPd} + 0.710 \) (\( R^2 = 0.39, n = 19, P < 0.01 \)) at \( T = 29^\circ \), and \( g_{5} = -0.016 \text{VPd} + 0.527 \) (\( R^2 = 0.54, n = 8, P < 0.02 \)) at \( T = 20^\circ \). In contrast, \( E \) was not significantly related to VPd (Fig. 1B), due to the offset of a lower \( g_{5} \) against a higher VPd, although a slight positive relationship was shown at \( T = 20^\circ \).

Oxygen isotope enrichment in bulk leaf water (\( \Delta_{b} \)) increased with increase in VPd at both high and low temperature (Fig. 2A), showing a significant positive relationship: \( \Delta_{b} = [0.41 \text{VPd} + 9.2]_{\%oo} \) (\( R^2 = 0.54, n = 19, P < 0.001 \)) at \( T = 29^\circ \); and \( \Delta_{b} = [0.93 \text{VPd} + 10.0]_{\%oo} \) (\( R^2 = 0.86, n = 8, P < 0.001 \)) at \( T = 20^\circ \). \( \Delta_{b} \) was found to be higher at lower temperature. In contrast, \( \Delta_{b} \) was not negatively correlated to \( g_{5} \) (Fig. 2B). The regression equations were: \( \Delta_{b} = [-10.9g_{5} + 20.6]_{\%oo} \) (\( R^2 = 0.36, n = 19, P < 0.01 \)) at \( T = 29^\circ \); and \( \Delta_{b} = [-43.7g_{5} + 35.7]_{\%oo} \) (\( R^2 = 0.87, n = 8, P < 0.001 \)) at \( 20^\circ \).

The oxygen isotope enrichment of lamina water (\( \Delta_{l} \)), calculated from Equation 13 with longitudinal average enrichment in the xylem given by Farquhar and Gan (2003), was found to have a strong linear relationship with \( \Delta_{b} \) at both leaf temperatures: \( \Delta_{l} = [1.06 \Delta_{b} + 0.7]_{\%oo} \) (\( R^2 = 0.99, n = 27, P < 0.0001 \)) (Fig. 3). As expected, \( \Delta_{l} \) was found to be slightly greater than \( \Delta_{b} \). The difference of \( 1\%oo \) to \( 1.5\%oo \) reflects that \( \Delta_{b} \) consists of enriched lamina water and less enriched vein water.
Craig-Gordon Prediction

The Craig-Gordon prediction ($\Delta_c$) had a positive relationship with VPd (Fig. 2A); $\Delta_c = [0.80VPd + 8.6]^{\circ}/(_{O}O_{2}) (R^2 = 0.99, n = 19)$ at $T = 29^\circ$C; and $\Delta_c = [1.29VPd + 10.2]^{\circ}/(_{O}O_{2}) (R^2 = 0.99, n = 8)$ at $T = 20^\circ$C. In contrast, $\Delta_c$ had a negative relationship with $g_s$ (Fig. 2B) and the equations were: $\Delta_c = [-16.6g_s + 29.0]^{\circ}/(_{O}O_{2}) (R^2 = 0.41, n = 19, P < 0.01)$ at $T = 29^\circ$C; and $\Delta_c = [-46.5g_s + 41.0]^{\circ}/(_{O}O_{2}) (R^2 = 0.59, n = 8, P < 0.01)$ at $T = 20^\circ$C. $\Delta_c$ was found to overestimate $\Delta_b$ (Fig. 2), as expected (see introduction). $\Delta_c$ also overestimated $\Delta_l$ (data not shown), with the discrepancies between $\Delta_c$ and $\Delta_l$ being necessarily smaller than those between $\Delta_c$ and $\Delta_b$.

Scaled Effective Lamina Path Length ($L_e$)

According to the one-dimensional Péclet model proposed by Farquhar and Lloyd (1993) and the averaged two-dimensional lamina result of Farquhar-Gan model, the magnitude of the Péclet effect for the lamina depends on $E$ and the scaled effective path length for the lamina ($L_e$), as noted in “Isotope Theory” below (see “Materials and Methods”). The deviations of $\Delta_b$ and $\Delta_l$ from $\Delta_c$ ($= 1 - \Delta_b/\Delta_c$ and $1 - \Delta_l/\Delta_c$) were found to increase with increase in $E$ (Fig. 4, A and B), roughly following the curves predicted by the Farquhar-Gan model. A regression line analysis for Figure 4 including both leaf $T$ is: panel A, $1 - \Delta_b/\Delta_c = 0.018 E + 0.135 (R^2 = 0.22, n = 27, P = 0.016)$; and panel B, $1 - \Delta_l/\Delta_c = 0.014 E + 0.07 (R^2 = 0.125, n = 27, P = 0.07)$. The $L$ values for $\Delta_l$ estimated from individual measurements ranged between 0.02 and 42 mm. The single best fit value of $L$ for $\Delta_l$ of all of the measurements was estimated as 7.9 mm. This value is very close to the value of 8 mm estimated in cotton leaves by Barbour and Farquhar (2000). With the total radial effective length $L_r$, taken as 43 mm, it means that the scaled effective length $L_{ev}$ for the veins was 43 to 7.9 = 35.1 mm. The latter serves to topoically isolate the lamina somewhat from the primary veins.

The values for individual $\Delta_l$ were plotted against VPd at $T = 29^\circ$C and $20^\circ$C in Figure 5. $L$ values were found to increase modestly with increase in VPd at both leaf $T^\circ$C [$L$ (mm) = $0.33VPd + 2.61, R^2 = 0.22, n = 25, P = 0.017] (two outliers were excluded from the regression line). Including the two outliers, the regression line for $\Delta_l$ was ($L = 0.76VPd - 2.41, R^2 = 0.31, n = 27, P < 0.002$) at both leaf $T^\circ$C.

Further, the $L$ values were plotted against leaf $E$ at $T = 29^\circ$C and $20^\circ$C in Figure 6. $L$ values were found to decrease slightly with increasing $E$ at both leaf $T^\circ$C, [$L$ (mm) = $-0.16E + 8.35, R^2 = 0.002, n = 25, P > 0.01] (two outliers were excluded from the regression line). Including the two outliers in $\Delta_l$ gave a slightly greater decrease ($L = -0.38E + 11.9, R^2 = 0.013, n = 27, P > 0.1$) at both leaf $T^\circ$C.

![Figure 2](image1.png)

**Figure 2.** Relationship between $\Delta_b$ and VPd (A) and $g_s$ (B) measured at 29°C (black circles) and 20°C (white circles). The lines represent least-squares regressions to the data at 29°C (thick solid line) and 20°C (thick dashed line), respectively. The Craig-Gordon lines are also plotted for 29°C (narrow solid line) and 20°C (narrow dashed line).

![Figure 3](image2.png)

**Figure 3.** Relationships between observed $\Delta_b$ and $\Delta_l$ estimated by the Farquhar-Gan model from Equation 13 at both leaf temperatures: 29°C (black circles) and 20°C (black triangles). The solid line represents a 1:1 relationship.
DISCUSSION

Observed and Craig-Gordon Predicted Leaf Water Enrichment

Although the Craig-Gordon model ($\Delta_C$) successfully predicted the sense of responses of $\Delta_b$ and $\Delta_1$ to $g_s$ and VPd, $\Delta_C$ overestimated both $\Delta_b$ and $\Delta_1$. Such over-estimation has been attributed to the advection of less enriched water from the veins into the lamina (Farquhar and Lloyd, 1993) with the average enrichment of the veins themselves changing with gas-exchange conditions (Farquhar and Gan, 2003). The latter theory differentiates the effective radial length from the evaporating sites to the lamina ($L_e$) and the effective radial length from the evaporating sites to the xylem ($L_r$). The values of $L$ required to fit observations calculated using the Farquhar-Gan model differ slightly from those using the earlier Farquhar-Lloyd theory. This is because the latest treatment takes into account the enrichment in xylem and veinlets (Eqs. 11 and 12).

The best fit of modeled to observed $\Delta_1$ was found when $L$ was 7.9 mm. This is similar to values found in some earlier studies, but the comparison has to be made with care as different values of kinetic fractionation have been used. Currently Equation 3 is used with the fractionation factors for water vapor diffusion through stomata and the boundary layer of 32% and 21% respectively, based on Cappa et al. (2003). These fractionation factors are revised from earlier values of 28% and 19% (Merlivat and Coantic, 1975). Further, new now values of diffusivity in water are being used here (Cuntz et al., 2007) that take into account variation with temperature. The single fitted value of $L$ for $\Delta_1$ (7.9 mm) is similar to the value of 8 mm used by Barbour and Farquhar (2000) in modeling their observations of the organic oxygen isotope composition of cotton leaves. Other estimates include 13.5 mm (Barbour et al., 2000b) and 11.1 mm (Cermusak et al., 2003) in *Ricinus communis*, and 8.5 mm (Flanagan et al., 1994) and 6.25 mm (Flanagan et al. [1994], recalculated from the data in Flanagan et al. [1991]) in *Phaseolus vulgaris*. These values are much more conservative than the values reported by Wang et al. (1998), who calculated values of $L$ between 4 and 166 mm from single measurements of $\Delta_b$ in various large-leaved species, but without removing the main veins.

Cotton and the other species studied intensively are dicots with reticulate veins, whereas the Farquhar-Gan model is designed for a leaf with long veins lacking connections. The model would therefore seem more appropriate for application to parallel venation (monocots), although, as noted by Gan et al. (2003), in maize (*Zea mays*) there is a descending scale from midrib, to lateral vein, to intermediate vein, and finally linked by transverse veins. The effects of venation complications on the quantitative relationship between average lamina enrichment and $E$ are unclear and at this stage we rely on empirical observations.

Dependence of Péclet Effect on $E$

The Péclet model gave better prediction of $\Delta_b$ and $\Delta_1$ than the Craig-Gordon model. The data show that $1 - \Delta_b/\Delta_C$ and $1 - \Delta_1/\Delta_C$ increased with increasing $E$.
Nevertheless, there was a tendency, though statistically nonsignificant, for $L$ to decrease with increasing $E$ (Fig. 6). This is in the opposite sense from what one might expect given the tendency for $L$ to increase with increasing VPd (Fig. 5). It is clear from Equation 5 that any “missed compartment” of water that resists enrichment, like xylem water that is not part of the excised primary veins, for example, will show up in our analysis as an artifactual increase in $P$. That is, perhaps the content of water associated with veinlets, $\phi_v$, is non-negligible. Since $P$ involves the product of $E$ and $L$, such an artifact will automatically tend to cause an inverse relationship between $L$ and $E$. This has probably happened to some extent with our data. Nevertheless, it is possible that $L$ could be affected by aquaporins, for example (Barbour and Farquhar, 2003). In that case, even if the Péclet formulation is valid, one might expect $L$ to change with stress, and perhaps to decrease with decreasing leaf water potential, in which case the response of $L$ to $E$ could be more complex, without necessarily invalidating the Péclet hypothesis. This might also differ between the species and functional groups.

CONCLUSION

We observed oxygen isotope enrichment of leaf water in cotton plants under different environmental conditions, and tested the Craig-Gordon model and the Farquhar-Gan model. $\Delta_b$ was found to increase with increasing VPd, as an overall result of the responses to $e_a/e_i$ and $g_s$. Enrichment in the lamina, $\Delta_l$, estimated by a mass balance of less enriched water in

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**Table 1. Symbols used in text**

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
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<tbody>
<tr>
<td>$\Delta_b$</td>
<td>Oxygen isotope enrichment of bulk leaf water relative to source water</td>
</tr>
<tr>
<td>$\Delta_l$</td>
<td>Oxygen isotope enrichment of leaf water at the sites of evaporation relative to source water, as defined by the modified Craig-Gordon prediction</td>
</tr>
<tr>
<td>$\bar{\Delta}_l$</td>
<td>Average oxygen isotope enrichment of lamina water relative to source water</td>
</tr>
<tr>
<td>$\Delta_a$</td>
<td>Oxygen isotope value of atmospheric water vapor relative to source water</td>
</tr>
<tr>
<td>$\bar{\Delta}_a$</td>
<td>Average oxygen isotope enrichment of water in the longitudinal xylem relative to source water</td>
</tr>
<tr>
<td>$\epsilon^*$</td>
<td>Oxygen isotope equilibrium fractionation</td>
</tr>
<tr>
<td>$\epsilon_k$</td>
<td>Oxygen isotope kinetic fractionation</td>
</tr>
<tr>
<td>$\phi_x$</td>
<td>Proportion of bulk leaf water associated with the longitudinal xylem</td>
</tr>
<tr>
<td>$\phi_l$</td>
<td>Proportion of bulk leaf water represented by the lamina</td>
</tr>
<tr>
<td>$\phi_v$</td>
<td>Proportion of bulk leaf water associated with the veinlets</td>
</tr>
<tr>
<td>$C$</td>
<td>Molar density of water (55 $\pm$ 1 mols$^{-1}$)</td>
</tr>
<tr>
<td>$D$</td>
<td>Diffusivity of $^{18}$O in water</td>
</tr>
<tr>
<td>$E$</td>
<td>Leaf transpiration rate (mol m$^{-2}$ s$^{-1}$)</td>
</tr>
<tr>
<td>$e_a$</td>
<td>Water vapor pressure in the air (mbar)</td>
</tr>
<tr>
<td>$e_i$</td>
<td>Water vapor pressure in the intercellular spaces (mbar)</td>
</tr>
<tr>
<td>$g_s$</td>
<td>Conductance to diffusion of water vapor through the stomata (mol m$^{-2}$ s$^{-1}$)</td>
</tr>
<tr>
<td>$g_b$</td>
<td>Conductance to diffusion of water vapor through the boundary layer (mol m$^{-2}$ s$^{-1}$)</td>
</tr>
<tr>
<td>$L$</td>
<td>Scaled effective path length (m)</td>
</tr>
<tr>
<td>$L_t$</td>
<td>Scaled total radial travel distance from xylem (m)</td>
</tr>
<tr>
<td>$L_{rv}$</td>
<td>Scaled effective travel distance through the veinlets (m)</td>
</tr>
<tr>
<td>$P$</td>
<td>Lamina radial Péclet number</td>
</tr>
<tr>
<td>$P_r$</td>
<td>Total radial Péclet number</td>
</tr>
<tr>
<td>$P_{rv}$</td>
<td>Veinlet radial Péclet number</td>
</tr>
<tr>
<td>$R_w$</td>
<td>$^{18}$O to $^{16}$O ratio of bulk leaf water</td>
</tr>
<tr>
<td>$R_{sw}$</td>
<td>$^{18}$O to $^{16}$O ratio of source water</td>
</tr>
<tr>
<td>$r_b$</td>
<td>Stomatal layer resistances to water vapor (m$^2$ s mol$^{-1}$)</td>
</tr>
<tr>
<td>$r_b$</td>
<td>Boundary layer resistances to water vapor (m$^2$ s mol$^{-1}$)</td>
</tr>
<tr>
<td>$T$</td>
<td>Leaf temperature in degrees Kelvin</td>
</tr>
<tr>
<td>VPD</td>
<td>Leaf-to-air vapor pressure difference (mbar)</td>
</tr>
</tbody>
</table>
primary veins and enriched water in leaf lamina, and accounting for the progressive enrichment along the veinlets, was also found to increase with increasing VPD. The Craig-Gordon model overestimated \( \Delta_0 \) and \( \Delta_1 \), as expected. Such discrepancies increased with increasing \( E \), supporting the influence of the Péclet effect. The fitted values of the effective length averaged over the lamina, \( L \), for \( \Delta_1 \) of individual leaves were found to have only a weak dependence, if at all, on environmental conditions such as VPD. We caution that any role for aquaporins (Barbour and Farquhar, 2003) could complicate the issue. Our data are consistent with a reasonably constant \( L \), i.e. for a particular leaf \( L \) changes little with evaporative conditions, but with results slightly confounded by some water in a compartment like xylem, less accessible to enrichment.

MATERIALS AND METHODS

Plant Material

Cotton (Gossypium hirsutum) plants were grown from seeds for 5 to 8 weeks in 10-L pots containing sterilized potting mix and a slow-release fertilizer (Scotts Osmocote Plus; Sierra Horticultural Products). Plants were grown in a humidity- and temperature-controlled glasshouse: daytime temperature and relative humidity were 28\( ^\circ \)C and 60\%\( \pm \) 10\%, respectively. Nighttime temperature was 20\( ^\circ \)C \( \pm \) 2\%, and humidity was the same as during the day.

Gas-Exchange Measurements

Measurements were made on 27 individual, fully expanded and attached leaves of cotton plants using a leaf chamber connected to a gas-exchange system (Scotts Osmocote Plus; Sierra Horticultural Products). These plants were watered daily with tap water. Plants were grown in a humidity- and temperature-controlled glasshouse: daytime temperature and relative humidity were 28\( ^\circ \)C \( \pm \) 2\% and 60\%\( \pm \) 10\%, respectively. Immediately before experimentation, the leaf chamber was generated by mixing 79\% dry nitrogen with 21\% dry oxygen, and \( \Delta_0 \) is the kinetic fractionation due to the smaller diffusivity of \( \text{H}_2\text{O} \) in air in the stomatal pores and in the boundary layer, \( \varepsilon^* \) is the equilibrium fractionation due to the lower vapor pressure of \( \text{H}_2\text{O} \) at liquid-vapor phase equilibrium, and \( \varepsilon_i \) and \( \varepsilon_r \) are the water vapor pressures in the air and intercellular spaces, respectively, \( \varepsilon^* \) is calculated using the regression of Majoube (1971):

\[
\varepsilon^* = \left( \frac{4.644 - 3.206 (10^4/T) + 1.534 (10^4/T^2)}{1.06} \right) \text{mbar}
\]

where \( T \) is leaf temperature in degrees Kelvin. \( \varepsilon_i \) is calculated according to the following equation (Farquhar et al., 1989; Cappa et al., 2003):

\[
\varepsilon_i = \frac{32}{5} \theta_i + 21 \theta_r \left( \frac{T}{273} \right)^3
\]

where \( \theta_i \) and \( \theta_r \) are the stomatal and boundary layer resistances to water vapor (m\( ^2 \)s\( \text{-} \)mol\( ^{-1} \)), which are the inverse of the stomatal \( \theta_i \) and boundary layer \( \theta_r \) conductances, respectively.

The averaged leaf lamina water enrichment at steady state, \( \Delta_0 \), is estimated from the Craig-Gordon prediction, \( \Delta_0 \), using the expression proposed by Farquhar and Lloyd (1993) and emerging as a longitudinal average over the lamina in the theory developed by Farquhar and Gan (2003):

\[
\Delta_0 = \Delta_0 - \frac{1 - e^{-P/L}}{e^{-P/L}}
\]

where \( P \) is the lamina Péclet number, which is the ratio of the advection of less enriched water from veinlets to the back diffusion of enriched water from evaporative sites. \( P \) is defined as:

\[
P = \frac{P_l}{E/L} \text{(CD)}
\]

where \( E \) is the transpiration rate (mol m\( ^{-2} \) s\( ^{-1} \)), \( L \) is the scaled effective path length (m), representing the scaled effective travel distance of water from veinlets (Farquhar and Gan, 2003) to the evaporative sites within a leaf, \( C \) is the concentration of water (5.55 \times 10^{-3} mol m\( ^{-3} \)), \( D \) is the diffusivity of \( \text{H}_2\text{O} \) in water [\( D = 119 \times 10^{-3} \exp(1397/T - 1377) \) \text{ m}^2 \text{s}^{-1}], and \( T (K) \) is the absolute temperature.

The longitudinal average enrichment in the xylem is given by (Farquhar and Gan, 2003):

\[
\Delta_0 = \frac{P_l}{E/L} \text{(CD)}
\]

where \( P_l \) is the total radial Péclet number, given by:

\[
P_l = \frac{P_l}{E/L} \text{(CD)}
\]

and \( L \) is the scaled total travel distance from xylem, through veinlets and lamina, to the sites of evaporation.

Thus:

\[
L = L_v + L
\]

where \( L_v \) is the scaled effective travel distance through veinlets. The expression for enrichment in veinlets is noted below in Equation 11.

This means that the bulk leaf water, \( \Delta_0 \), will depend on \( \Delta_1 \) and the proportion, \( \phi_v \), of total water associated with the longitudinal xylem and ground tissue; on \( \Delta_0 \) and the proportion, \( \phi_v \), of total water associated with the veinlets; and on \( \Delta_0 \) and the proportion, \( \phi_v \), of total water represented by the lamina.

Thus:

\[
\Delta_0 = \phi_v \Delta_v + \phi_l \Delta_l + \phi_0 \Delta_0
\]

or

\[
\Delta_0 = \Delta_0 \left[ \phi_v e^{-\phi_l} + \phi_l e^{-\phi_v} - 1 \right] P_l e^{-P/L} \left[ 1 - e^{-P/L} \right]
\]

where \( P_l \) is the veinlet Péclet number and \( \phi_v + \phi_l + \phi_0 = 1 \). As \( \phi_v \) was thought to be very small, Farquhar and Gan (2003) approximated bulk leaf enrichment by:

\[
\Delta_0 = \Delta_0 \left[ \phi_v e^{-\phi_l} + (1 - \phi_v) \left[ 1 - e^{-P/L} \right] \right]
\]
Isotope Measurements

One hour after leaf gas exchange stabilized, leaves were detached, inserted in sealed vessels, and stored in a freezer (−20°C). Bulk leaf water was later extracted by vacuum distillation, as described by Gan et al. (2003). The oxygen isotope ratio of the source water was assumed to be equal to that of the tap water used for irrigation. Water samples were sealed under argon in tin cups to avoid isotopic exchange and evaporation. The oxygen isotope ratio of the water samples was measured by the on-line pyrolysis method described previously by Farquhar et al. (1997) with an Isochrom mass spectrometer (Micromass) linked to a pyrolysis furnace in a Carlo Erba elemental analyzer (CE Instruments). δ18O was calculated from measured oxygen isotope ratios of bulk leaf water and source water using Equation 1.

In a subsample of leaves, primary veins were trimmed off and weighed, then dried and reweighed, to determine δh, the weight ratio of primary vein water, including ground tissue, to bulk leaf water. Oxygen isotope enrichment of lamina water, δL, was then estimated by mass balance between vein water and bulk water using (Cernusak et al., 2003):

\[
\delta_L = \delta_h (1 + (1 - \delta_h))
\]

where δL is the oxygen isotope enrichment of primary vein water and was estimated for individual leaves as follows. From our earlier measurements on cotton (Gan et al., 2002) of δL/δh and L, the total radial effective length, Lr, was estimated using Equations 7 and 8 as 43 mm. That length was then applied with the individual value of δh using Equation 8 to obtain the individual value of δL, and the latter then applied to Equation 7 to obtain δL.

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