Environmental Effects on Oxygen Isotope Enrichment of Leaf Water in Cotton Leaves[1]

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The oxygen isotope enrichment of bulk leaf water (Δ_b) was measured in cotton (*Gossypium hirsutum*) leaves to test the Craig-Gordon and Farquhar-Gan models under different environmental conditions. Δ_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_a/e_i) and to stomatal conductance (g_s). The oxygen isotope enrichment of lamina water relative to source water ($\bar{\Delta}_l$), 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 Δ_b (and $\bar{\Delta}_l$), as expected. Such discrepancies increased with increase in transpiration rate (E), supporting the Farquhar-Gan model, which gave reasonable predictions of Δ_b and $\bar{\Delta}_l$ with an L of 7.9 mm, much less than the total radial effective length L_r of 43 mm. The fitted values of L for $\bar{\Delta}_l$ 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 ^{18}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_r, 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, ϕ_x, 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 dif-
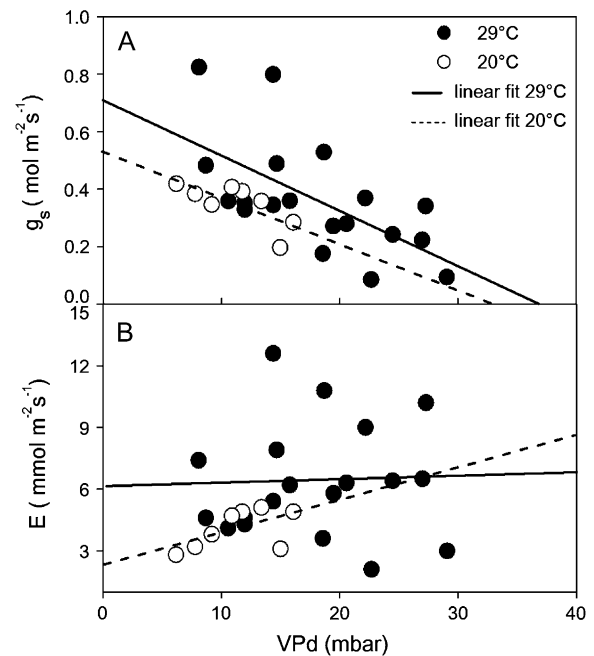

Figure 1. Relationship between VPd and g_s (A) and E (B) measured at 29°C (black circles) and 20°C (white circles). The lines represent least-squares regressions to the data at 29°C (solid line) and 20°C (dashed line), respectively. The points represent individual measurements.

ference (VPd) and E (Fig. 1). Significant negative correlation was found between g_s (mol m^{-2} s^{-1}) and VPd (mbar) at both high and low temperature (Fig. 1A): regression equations were $g_s = -0.019$VPd $+ 0.710$, ($R^2 = 0.39$, $n = 19$, $P < 0.01$) at $T = 29$°C; and $g_s = -0.016$VPd $+ 0.527$, ($R^2 = 0.54$, $n = 8$, $P < 0.02$) at $T = 20$°C. In contrast, E was not significantly related to VPd (Fig. 1B), due to the offset of a lower g_s against a higher VPd, although a slight positive relationship was shown at $T = 20$°C.

Oxygen isotope enrichment in bulk leaf water (Δ_b) increased with increase in VPd at both high and low temperature (Fig. 2A), showing a significant positive relationship: $\Delta_b = [0.41$VPd $+ 9.2]‰$, ($R^2 = 0.54$, $n = 19$, $P < 0.001$) at $T = 29$°C; and $\Delta_b = [0.93$VPd $+ 10.0]‰$, ($R^2 = 0.86$, $n = 8$, $P < 0.001$) at $T = 20$°C. Δ_b was found to be higher at lower temperature. In contrast, Δ_b was negatively correlated to g_s (Fig. 2B). The regression equations were: $\Delta_b = [-10.9g_s + 20.6]‰$, ($R^2 = 0.36$, $n = 19$, $P < 0.01$) at $T = 29$°C; and $\Delta_b = [-43.7g_s + 35.7]‰$, ($R^2 = 0.87$, $n = 8$, $P < 0.001$) at 20°C.

The oxygen isotope enrichment of lamina water ($\bar{\Delta}_l$), 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 Δ_b at both leaf temperatures ($\bar{\Delta}_l = [1.06\Delta_b + 0.7]‰$, $R^2 = 0.99$, $n = 27$, $P < 0.0001$; Fig. 3). As expected, $\bar{\Delta}_l$ was found to be slightly greater than Δ_b. The difference of 1‰ to 1.5‰ reflects that Δ_b consists of enriched lamina water and less enriched vein water.
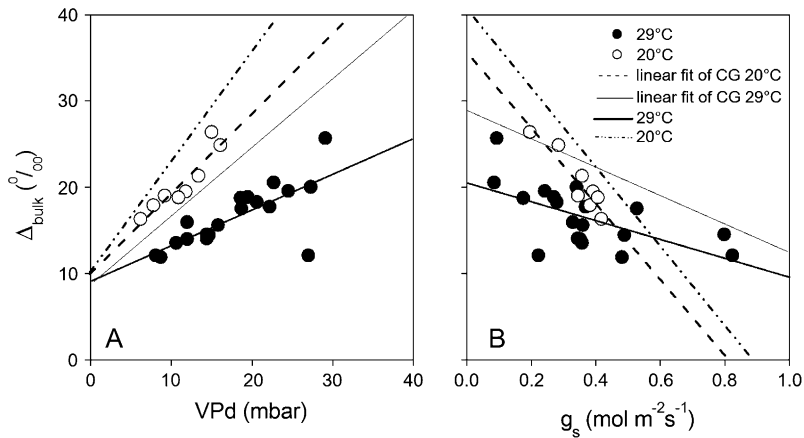
Figure 2. Relationship between Δ_b and VPd (A) and g_s (B) measured at 29°C (black circles) and 20°C (white circles). The lines represent least-squares regressions to the data at 29°C (thick solid line) and 20°C (thick dashed line), respectively. The Craig-Gordon lines are also plotted for 29°C (narrow solid line) and 20°C (narrow dashed line).

Craig-Gordon Prediction

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

Scaled Effective Lamina Path Length (L)

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), as noted in "Isotope Theory" below (see "Materials and Methods"). The deviations of Δ_b and $\bar{\Delta}_l$ from Δ_C (= $1 - \Delta_b/\Delta_C$ and $1 - \bar{\Delta}_l/\Delta_C$) 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, $1 - \bar{\Delta}_l/\Delta_C = 0.014 E + 0.07$ ($R^2 = 0.125$, $n = 27$, $P = 0.07$). The L values for $\bar{\Delta}_l$ estimated from individual measurements ranged between 0.02 and 42 mm. The single best fit value of L for $\bar{\Delta}_l$ 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_{rv} for the veinlets was 43 to 7.9 = 35.1 mm. The latter serves to iso-

topically isolate the lamina somewhat from the primary veins.

The values for individual $\bar{\Delta}_l$ were plotted against VPd at $T = 29°C$ and 20°C in Figure 5. L values were found to increase modestly with increase in VPd at both leaf T°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 Δ_l was ($L = 0.76\text{VPd} - 2.41$, $R^2 = 0.31$, $n = 27$, $P < 0.002$) at both leaf T°C.

Further, the L values were plotted against leaf E at $T = 29°C$ and 20°C in Figure 6. L values were found to decrease slightly with increasing E at both leaf T°C, [L (mm) = $-0.116 E + 8.35$, $R^2 = 0.005$, $n = 25$, $P > 0.1$] (two outliers were excluded from the regression line). Including the two outliers in Δ_l gave a slightly greater decrease ($L = -0.38 E + 11.9$, $R^2 = 0.013$, $n = 27$, $P > 0.1$) at both leaf T°C.
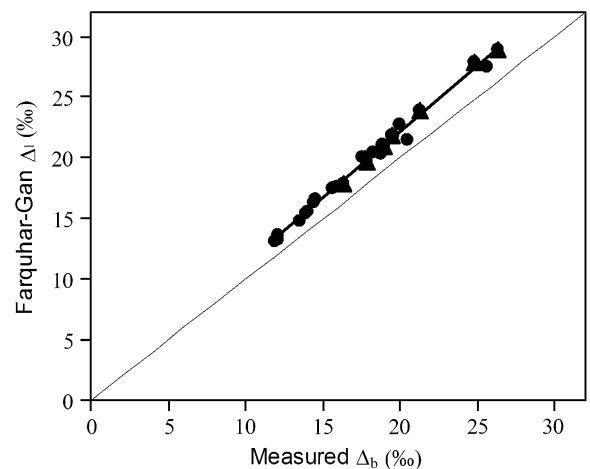

Figure 3. Relationships between observed Δ_b and $\bar{\Delta}_l$ estimated by the Farquhar-Gan model from Equation 13 at both leaf temperatures: 29°C (black circles) and 20°C (black triangles). The solid line represents a 1:1 relationship.
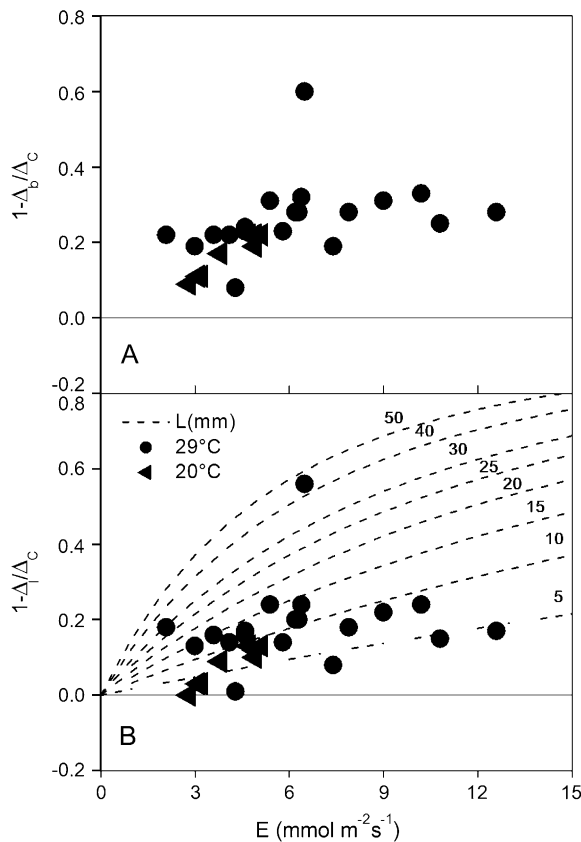
Figure 4. The relationships between E and the deviation of Δ_b from Δ_C (A) and $\bar{\Delta}_1$ from Δ_C (B) at both leaf temperatures, 29°C (black circles) and 20°C (black triangles). The predicted relationships at different L values are plotted as dotted lines in B.

DISCUSSION

Observed and Craig-Gordon Predicted Leaf Water Enrichment

Although the Craig-Gordon model (Δ_C) successfully predicted the sense of responses of Δ_b and Δ_1 to g_s and VPd, Δ_C overestimated both Δ_b and $\bar{\Delta}_1$. Such overestimation 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) 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 $\bar{\Delta}_1$ 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, now new 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 $\bar{\Delta}_1$ (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 (Cernusak 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 Δ_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 Δ_b and $\bar{\Delta}_1$ than the Craig-Gordon model. The data show that $1 - \Delta_b/\Delta_C$ and $1 - \bar{\Delta}_1/\Delta_C$ increased with increasing E
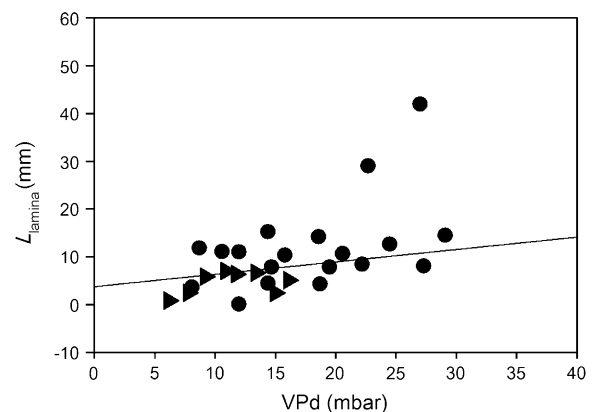

Figure 5. The relationships with VPd of the fitted values of L for $\bar{\Delta}_1$ of individual leaves at both 29°C (black circles) and 20°C (black triangles).

Figure 6. The relationships with E of the fitted values of L for $\bar{\Delta}_1$ of individual leaves at both 29°C (black circles) and 20°C (black triangles).
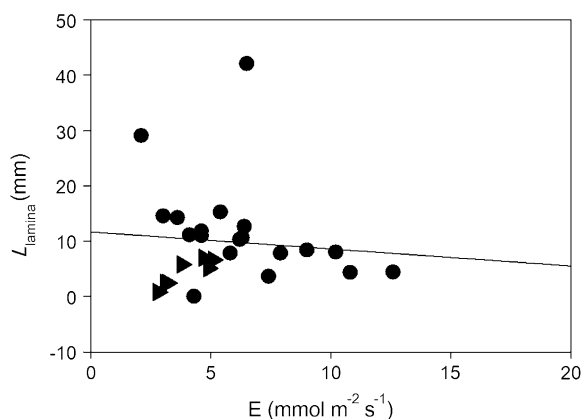
(Fig. 4). Nevertheless, there was a tendency, though statistically nonsignificant, for L to decrease with increasing E (Fig. 6). This is in the opposite sense from what one might expect given the tendency for L to increase with increasing VPd (Fig. 5). It is clear from Equation 5 that any "missed compartment" 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, ϕ_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. Δ_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, Δ_l, estimated by a mass balance of less enriched water in

Table I. *Symbols used in text*

Symbol	Definition
Δ_b	Oxygen isotope enrichment of bulk leaf water relative to source water
Δ_C	Oxygen isotope enrichment of leaf water at the sites of evaporation relative to source water, as defined by the modified Craig-Gordon prediction
$\bar{\Delta}_1$	Average oxygen isotope enrichment of lamina water relative to source water
Δ_V	Oxygen isotope value of atmospheric water vapor relative to source water
$\bar{\Delta}_x$	Average oxygen isotope enrichment of water in the longitudinal xylem relative to source water
ε^+	Oxygen isotope equilibrium fractionation
ε_k	Oxygen isotope kinetic fractionation
ϕ_x	Proportion of bulk leaf water associated with the longitudinal xylem
ϕ_l	Proportion of bulk leaf water represented by the lamina
ϕ_v	Proportion of bulk leaf water associated with the veinlets
C	Molar density of water (55×10^3 mol m^{-3})
D	Diffusivity of H$_2$^{18}O in water
E	Leaf transpiration rate (mol m^{-2} s^{-1})
e_a	Water vapor pressure in the air (mbar)
e_i	Water vapor pressure in the intercellular spaces (mbar)
g_s	Conductance to diffusion of water vapor through the stomata (mol m^{-2} s^{-1})
g_b	Conductance to diffusion of water vapor through the boundary layer (mol m^{-2} s^{-1})
L	Scaled effective path length (m)
L_r	Scaled total radial travel distance from xylem (m)
L_{rv}	Scaled effective travel distance through the veinlets (m)
P	Lamina radial Péclet number
P_r	Total radial Péclet number
P_{rv}	Veinlet radial Péclet number
R_{lw}	^{18}O to ^{16}O ratio of bulk leaf water
R_{sw}	^{18}O to ^{16}O ratio of source water
r_b	Stomatal layer resistances to water vapor (m^2 s mol^{-1})
r_s	Boundary layer resistances to water vapor (m^2 s mol^{-1})
T	Leaf temperature in degrees Kelvin
VPd	Leaf-to-air vapor pressure difference (mbar)

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 Δ_b and $\bar{\Delta}_1$, 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 $\bar{\Delta}_1$ 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). These pots were watered daily with tap water. Plants were grown in a humidity- and temperature-controlled glasshouse: daytime temperature and relative humidity were 28°C ± 2°C and 50% ± 10%, respectively. Nighttime temperature was 20°C ± 2°C, 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 in the laboratory. The configuration of the system was basically the same as described by Boyer et al. (1997) and Barbour et al. (2000b). Air entering the leaf chamber was generated by mixing 79% dry nitrogen with 21% dry oxygen, and CO_2 concentration in the air was 350 to 360 μmol mol^{-1}. The through-flow rate of the air was adjusted to 2 to 10 L min^{-1} to produce various VPds ranging from 6 to 30 mbar. Photon flux density in the chamber was 100, 500, and 1,200 μmol m^{-2} s^{-1}. Leaf temperature, monitored by two thermocouples in the leaf chamber, was either 29°C or 20°C. The projected area of the measured leaves ranged from 70 to 170 cm^2.

Calculations of gas-exchange parameters were performed according to the equations of von Caemmerer and Farquhar (1981). g_b in the chamber was estimated to be 5 mol m^{-2} s^{-1} according to Boyer et al. (1997). VPd, ambient CO_2 concentration, photon flux density, leaf T, e_a/e_i, E, and g_s were monitored at 2-min intervals. After leaf gas-exchange parameters stabilized and the leaf water achieved isotopic steady state (normally after 1 h), all data of each parameter were averaged. Steady state was confirmed by equality of the isotopic composition of source water and transpired vapor.

Isotope Theory

The oxygen isotope enrichment of bulk leaf water relative to source water, Δ_b, is defined by:

$$\Delta_b = \left(\frac{R_{lw}}{R_{sw}} - 1 \right) \tag{1}$$

where R_{lw} is the ^{18}O to ^{16}O ratio of bulk leaf water and R_{sw} is the ^{18}O to ^{16}O ratio of source water. A summary of all symbols used in the text is given in Table I. The magnitude of Δ_b is normally presented as parts per million (= × 10^{-3} or ‰); ‰ is not a unit and Δ_b is dimensionless (Farquhar and Lloyd, 1993). The Craig-Gordon model is:

$$\Delta_C \cong \varepsilon_k + \varepsilon^+ + (\Delta_V - \varepsilon_k) \frac{e_a}{e_i} \tag{2}$$

where Δ_C is the Craig-Gordon prediction of oxygen isotope enrichment of leaf water relative to source water, Δ_V is the oxygen isotope value of atmospheric water vapor relative to source water (taken as zero in the steady state in the experiments because the air coming into the gas-exchange cuvette was dry), ε_k is the kinetic fractionation due to the smaller diffusivity of H$_2^{18}$O in air in the stomatal pores and in the boundary layer, ε^+ is the equilibrium fractionation due to the lower vapor pressure of H$_2^{18}$O at liquid-vapor phase equilibrium, and e_a and e_i are the water vapor pressures in the air and intercellular spaces, respectively. ε^+ is calculated using the regression of Majoube (1971):

$$\varepsilon^+ = [2.644 - 3.206(10^3/T) + 1.534(10^3/T)^2] (‰) \tag{3}$$

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

$$\varepsilon_k = \frac{32 r_s + 21 r_b}{r_s + r_b} (‰) \tag{4}$$

where r_s and r_b are the stomatal and boundary layer resistances to water vapor (m^2 s mol^{-1}), which are the inverse of the stomatal (g_s) and boundary layer (g_b) conductances, respectively.

The averaged leaf lamina water enrichment at steady state, $\bar{\Delta}_1$, is estimated from the Craig-Gordon prediction, Δ_C, 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):

$$\bar{\Delta}_1 = \Delta_C \frac{1 - e^{-P}}{P} \tag{5}$$

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:

$$P = EL/(CD) \tag{6}$$

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 × 10^4 mol m^{-3}), D is the diffusivity of H$_2^{18}$O in water [$D = 119$ 10^{-9} $exp(-637/(T - 137))$] m^2 s^{-1}, and T (K) is the absolute temperature (Cuntz et al., 2007).

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

$$\bar{\Delta}_x = \frac{\Delta_C}{e^{P_r}} \tag{7}$$

where P_r is the total radial Péclet number, given by:

$$P_r = EL_r/(CD) \tag{8}$$

and L_r is the scaled total radial travel distance from xylem, through veinlets and lamina, to the sites of evaporation. Thus:

$$L_r = L_{rv} + L \tag{9}$$

where L_{rv} 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, Δ_b will depend on $\bar{\Delta}_x$ and the proportion, ϕ_x, of total water associated with the longitudinal xylem and ground tissue; on $\bar{\Delta}_v$ and the proportion, ϕ_v of total water associated with the veinlets; and on $\bar{\Delta}_1$ and the proportion, ϕ_1, of total water represented by the lamina.

Thus:

$$\Delta_b = \phi_x \bar{\Delta}_x + \phi_v \bar{\Delta}_v + \phi_1 \bar{\Delta}_1 \tag{10}$$

or

$$\Delta_b = \Delta_C \left[\phi_x e^{-P_r} + \phi_v \frac{e^{P_{rv}} - 1}{P_{rv} \cdot e^{P_r}} + \phi_1 \frac{1 - e^{-P}}{P} \right] \tag{11}$$

where P_{rv} is the veinlet Péclet number and $\phi_x + \phi_v + \phi_1 = 1$. As ϕ_v was thought to be very small, Farquhar and Gan (2003) approximated bulk leaf enrichment by:

$$\Delta_b = \Delta_C \left[\phi_x e^{-P_r} + (1 - \phi_x) \frac{1 - e^{-P}}{P} \right]. \tag{12}$$

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). Δ_b 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 ϕ_x, the weight ratio of primary vein water, including ground tissue, to bulk leaf water. Oxygen isotope enrichment of lamina water, Δ_l, was then estimated by mass balance between vein water and bulk water using (Cernusak et al., 2003):

$$\Delta_b = \phi_x \bar{\Delta}_x + (1 - \phi_x)\bar{\Delta}_l \qquad (13)$$

where $\bar{\Delta}_x$ 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 $\bar{\Delta}_x/\Delta_C$ and E, the total radial effective length, L_r, was estimated using Equations 7 and 8 as 43 mm. That length was then applied with the individual value of E using Equation 8 to obtain the individual value of P_r, and the latter then applied to Equation 7 to obtain $\bar{\Delta}_x$.

LITERATURE CITED

Barbour MM (2007) Stable oxygen isotope composition of plant tissue: a review. Funct Plant Biol 34: 83–94

Barbour MM, Farquhar GD (2000) Relative humidity- and ABA-induced variation in carbon and oxygen isotope ratios of cotton leaves. Plant Cell Environ 23: 473–485

Barbour MM, Farquhar GD (2003) Do pathways of water movement and leaf anatomy dimensions allow development of gradients in $H_2^{18}O$ between veins and the sites of evaporation within leaves? Plant Cell Environ 27: 107–121

Barbour MM, Fischer RA, Sayre KD, Farquhar GD (2000a) Oxygen isotope ratio of leaf and grain material correlates with stomatal conductance and grain yield in irrigated wheat. Aust J Plant Physiol 27: 625–637

Barbour MM, Roden JS, Farquhar GD, Ehleringer JR (2004) Expressing leaf water and cellulose oxygen isotope ratios as enrichment above source water reveals evidence of a Péclet effect. Oecologia 138: 426–435

Barbour MM, Schurr U, Henry BK, Wong SC, Farquhar GD (2000b) Variation in the oxygen isotope ratio of phloem sap sucrose from castor bean: evidence in support of the Péclet effect. Plant Physiol 123: 671–679

Boyer JS, Wong SC, Farquhar GD (1997) CO_2 and water vapor exchange across leaf cuticle (epidermis) at various water potentials. Plant Physiol 114: 185–191

Cappa CD, Hendricks MB, DePaulo DJ, Cohen RC (2003) Isotopic fractionation of water during evaporation. J Geophys Res 108: 4225

Cernusak LA, Wong SC, Farquhar GD (2003) Oxygen isotope composition of phloem sap in relation to leaf water in Ricinus communis. Funct Plant Biol 30: 1–11

Craig H, Gordon LI (1965) Deuterium and oxygen-18 variations in the ocean and marine atmosphere. In E Tongiorgi, Proceedings of a Conference on Stable Isotopes in Oceanographic Studies and Palaeo-Temperatures. Lischi and Figli, Pisa, Italy, pp 9–130

Cuntz M, Ogée J, Farquhar GD, Peylin P, Cernusak LA (2007) Modelling advection and diffusion of water isotopologues in leaves. Plant Cell Environ 30: 892–909

Dongmann G, Nurnberg HW, Forstel H, Wagener K (1974) On the enrichment of $H_2^{18}O$ in the leaves of transpiring plants. Radiat Environ Biophys 11: 41–52

Farquhar GD, Cernusak LA, Barnes B (2007) Heavy water fractionation during transpiration. Plant Physiol 143: 11–18

Farquhar GD, Gan KS (2003) On the progressive enrichment of the oxygen isotopic composition of water along a leaf. Plant Cell Environ 26: 801–819

Farquhar GD, Henry BK, Styles JM (1997) A rapid on-line technique for determination of oxygen isotope composition of nitrogen-containing organic matter and water. Rapid Commun Mass Spectrom 11: 1554–1560

Farquhar GD, Hubick KT, Condon AG, Richards RA (1989) Carbon isotope fractionation and plant water-use efficiency. In PW Rundel, JR Ehleringer, KA Nagy, eds, Stable Isotopes in Ecological Research. Springer-Verlag, New York, pp 21–40

Farquhar GD, Lloyd J (1993) Carbon and oxygen isotope effects in the exchange of carbon dioxide between terrestrial plants and the atmosphere. In JR Ehleringer, AE Hass, GD Farquhar, eds, Stable Isotopes and Plant Carbon/Water Relations. Academic Press, New York, pp 47–70

Farquhar GD, Lloyd J, Taylor JA, Flanagan LB, Syvertsen JP, Hubick KT, Wong SC, Ehleringer JR (1993) Vegetation effects on the isotopic composition of oxygen in atmospheric CO_2. Nature 363: 439–443

Flanagan LB, Comstock JP, Ehleringer JR (1991) Comparison of modeled and observed environmental influences on the stable oxygen and hydrogen isotope composition of leaf water in Phaseolus vulgaris L. Plant Physiol 96: 588–596

Flanagan LB, Ehleringer JR (1991) Effects of mild water stress and diurnal changes in temperature and humidity on the stable oxygen and hydrogen isotopic composition of leaf water in Cornus stolonifera L. Plant Physiol 97: 298–305

Flanagan LB, Phillips SL, Ehleringer JR, Lloyd J, Farquhar GD (1994) Effects of changes in leaf water oxygen isotopic composition on discrimination against $C^{18}O^{16}O$ during photosynthetic gas exchange. J Plant Physiol 21: 221–234

Gan KS, Wong SC, Yong JWH, Farquhar GD (2002) ^{18}O spatial patterns of vein xylem water, leaf water, and dry matter in cotton leaves. Plant Physiol 130: 1008–1021

Gan KS, Wong SC, Yong JWH, Farquhar GD (2003) Evaluation of models of leaf water ^{18}O enrichment using measurements of spatial patterns of vein xylem water, leaf water and dry matter in maize leaves. Plant Cell Environ 26: 1479–1495

Gat JR, Bowser C (1991) The heavy isotope enrichment of water in coupled evaporative systems. In HP Taylor, JR O'Neil, IR Kaplan, eds, Stable Isotope Geochemistry: A Tribute to Samuel Epstein. The Geochemical Society, Lancaster, UK, pp 159–168

Gillon J, Yakir D (2001) Influence of carbonic anydrase activity in terrestrial vegetation on the ^{18}O content of atmospheric CO_2. Science 291: 2584–2587

Helliker BR, Ehleringer JR (2000) Establishing a grassland signature in veins: ^{18}O in the leaf water of C_3 and C_4 grasses. Proc Natl Acad Sci USA 97: 7894–7898

Helliker BR, Ehleringer JR (2002) Differential ^{18}O enrichment of leaf cellulose in C_3 versus C_4 grasses. Funct Plant Biol 29: 435–442

Leaney FW, Osmond CB, Allison GB, Ziegler H (1985) Hydrogen-isotope composition of leaf water in C3 and C4 plants: its relationship to the hydrogen-isotope composition of dry matter. Planta 164: 215–220

Majoube M (1971) Fractionnement en oxygen-18 et en deuterium entre l'eau et sa vapeur. J Chem Phys 58: 1423–1436

Merlivat L, Coantic M (1975) Study of mass transfer at the air-water interface by an isotopic method. J Geophys Res 80: 3455–3464

Roden JS, Ehleringer JR (1999) Observations of hydrogen and oxygen isotopes in leaf water confirm the Craig-Gordon model under wide-ranging environmental conditions. Plant Physiol 120: 1165–1173

Šantrůček J, Jiří K, Šetlík J, Bulíčková L (2007) Spatial variation of deuterium enrichment in bulk water of snowgum leaves. Plant Physiol 143: 88–97

von Caemmerer S, Farquhar GD (1981) Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. Planta 153: 376–387

Wang XF, Yakir D (1995) Temporal and spatial variations in the oxygen-18

content of leaf water in different plant species. Plant Cell Environ **18:** 1377–1385

Wang XF, Yakir D, Avishari M (1998) Non-climatic variations in the oxygen isotopic compositions of plants. Glob Change Biol **4:** 835–849

Yakir D (1992) Variations in the natural abundance of oxygen-18 and deuterium in plant carbohydrates. Plant Cell Environ **15:** 1005–1020

Yakir D, Berry JA, Giles L, Osmond CB (1994) Isotopic heterogeneity of water in transpiring leaves: identification of the component that controls the $\delta^{18}O$ of atmospheric O_2 and CO_2. Plant Cell Environ **17:** 73–80

Yakir D, Berry JA, Giles L, Osmond CB, Thomas R (1993) Applications of stable isotopes to scaling biospheric photosynthetic activities. *In* JR Ehleringer, DB Field, eds, Scaling Physiological Processes: Leaf to Globe. Academic Press, San Diego, pp 323–338

Yakir D, DeNiro MJ, Ephrath JE (1990a) Effects of water stress on oxygen, hydrogen and carbon isotope ratios in two species of cotton plants. Plant Cell Environ **13:** 949–955

Yakir D, DeNiro MJ, Gat JR (1990b) Natural deuterium and oxygen-18 enrichment in leaf water of cotton plants grown under wet and dry conditions: evidence for water compartmentation and its dynamics. Plant Cell Environ **13:** 49–56