Water Relations Link Carbon and Oxygen Isotope Discrimination to Phloem Sap Sugar Concentration in *Eucalyptus globulus*

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A strong correlation was previously observed between carbon isotope discrimination ($\Delta^{13}C$) of phloem sap sugars and phloem sap sugar concentration in the phloem-bleeding tree *Eucalyptus globulus* Labill. (J. Pate, E. Shedley, D. Arthur, M. Adams [1998] Oecologia 117: 312–322). We hypothesized that correspondence between these two parameters results from covarying responses to plant water potential. We expected $\Delta^{13}C$ to decrease with decreasing plant water potential and phloem sap sugar concentration to increase, thereby maintaining turgor within sieve tubes. The hypothesis was tested with analyses of *E. globulus* trees growing on opposite ends of a rainfall gradient in southwestern Australia. The $\Delta^{13}C$ of phloem sap sugars was closely related to phloem sap sugar concentration ($r = -0.90$, $P < 0.0001$, $n = 40$). As predicted, daytime shoot water potential was positively related to $\Delta^{13}C$ ($r = 0.70$, $P < 0.0001$, $n = 40$) and negatively related to phloem sap sugar concentration ($r = -0.86$, $P < 0.0001$, $n = 40$). Additional measurements showed a strong correspondence between predawn shoot water potential and phloem sap sugar concentration measured at midday ($r = -0.87$, $P < 0.0001$, $n = 30$). The $\Delta^{13}C$ of phloem sap sugars collected from the stem agreed well with that predicted from instantaneous measurements of the ratio of intercellular to ambient carbon dioxide concentrations on subtending donor leaves. In accordance, instantaneous ratio of intercellular to ambient carbon dioxide concentrations correlated negatively with phloem sap sugar concentration ($r = -0.91$, $P < 0.0001$, $n = 27$). Oxygen isotope enrichment ($\Delta^{18}O$) in phloem sap sugars also varied with phloem sap sugar concentration ($r = 0.91$, $P < 0.0001$, $n = 39$), consistent with predictions from a theoretical model of $\Delta^{18}O$. We conclude that drought induces correlated variation in the concentration of phloem sap sugars and their isotopic composition in *E. globulus*.

Measurement of stable carbon and oxygen isotope ratios in plant material provides a valuable tool for studying the performance of terrestrial plants. For example, the strong correlation between discrimination against ^{13}C ($\Delta^{13}C$) and the ratio of intercellular to ambient carbon dioxide concentrations (c_i/c_a) has been relied upon extensively to assess plant water use efficiency under a variety of experimental and natural conditions (for review, see Farquhar et al., 1989a; Ehleringer, 1993; Brugnoli and Farquhar, 2000). Farquhar et al. (1982) derived an expression relating $\Delta^{13}C$ to c_i/c_a for C_3 photosynthesis such that:

$$\Delta^{13}C = a + (b - a)\frac{c_i}{c_a} \quad (1)$$

where a is the fractionation caused by gaseous diffusion (4.4‰), and b is the effective fractionation caused by carboxylating enzymes (approximately 27‰). The $\Delta^{13}C$ is defined with respect to atmospheric CO_2 as $\Delta^{13}C = R_a/R_p - 1$, where R_a is $^{13}C/^{12}C$ of atmospheric CO_2 and R_p is $^{13}C/^{12}C$ of plant material. Equation 1 suggests that $\Delta^{13}C$ decreases linearly as c_i/c_a decreases. Because c_i/c_a represents a balance between the supply of CO_2 via stomata and the photosynthetic demand for CO_2, $\Delta^{13}C$ is often employed as an indicator of the extent of drought stress experienced by a plant. Thus, as stomata close to conserve water, $\Delta^{13}C$ decreases as a function of decreasing c_i/c_a. The advantage of measuring $\Delta^{13}C$ of plant material is that it provides a time-integrated, rather than instantaneous, estimate of c_i/c_a.

Oxygen isotope enrichment in plant material ($\Delta^{18}O$), on the other hand, is partly controlled by the evaporative enrichment of ^{18}O in leaf water. Sugars immediately exported from the leaf are presumed to be in close isotopic equilibrium with the water in which they formed (Farquhar et al., 1998; Barbour et al., 2000b, 2003), after taking into account an equilibrium fractionation of approximately +27‰ (Sternberg and DeNiro, 1983; Sternberg et al., 1986). A proportion of the oxygen atoms in the exported sugars exchanges with local water during subsequent metabolism; however, the leaf water signal is expected to persist unaltered during translocation until the sugar molecules are broken down into derivative molecules containing carbonyl bonds (Barbour et al., 2003). Leaf water heavy isotope enrichment at evaporative sites ($\Delta^{18}O_e$) has been modeled after Craig

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and Gordon (1965), Dongmann et al. (1974), and Farquhar et al., (1989b):

$$\Delta^{18}O_e = \varepsilon^* + \varepsilon_k + (\Delta^{18}O_v - \varepsilon_k)\frac{e_a}{e_i} \quad (2)$$

where ϵ^* is the equilibrium fractionation between liquid and vapor, ϵ_k is the kinetic fractionation that occurs during diffusion from the leaf to the atmosphere, $\Delta^{18}O_v$ is the isotopic enrichment of atmospheric vapor compared with source water, and e_a/e_i is the ratio of ambient to intercellular vapor pressures. The ϵ_k can be calculated as $\epsilon_k(‰) = (28r_s + 19r_b)/(r_s + r_b)$, where r_s and r_b are the stomatal and boundary layer resistances to water vapor diffusion, and the coefficients 28 and 19 are the associated fractionation factors (Farquhar et al., 1989b). The $\Delta^{18}O$ in atmospheric water vapor, plant water, and plant organic material is defined with respect to the oxygen isotope ratio of source water as $\Delta^{18}O_x = R_x/R_s - 1$, where R_x is $^{18}O/^{16}O$ of atmospheric vapor, plant water, or organic material, and R_s is $^{18}O/^{16}O$ of source water. The average isotopic enrichment of water in the leaf mesophyll ($\Delta^{18}O_L$) can then be related to the isotopic enrichment at evaporative sites by (Farquhar and Lloyd, 1993):

$$\Delta^{18}O_L = \frac{\Delta^{18}O_e(1 - e^{-\wp})}{\wp}. \quad (3)$$

The $\wp$ is a dimensionless number termed the Péclet number, which is defined as $EL/(CD)$, where E is transpiration rate (moles per meter squared per second), L is a scaled effective path length (meters), C is the molar concentration of water (moles per meter cubed), and D is the diffusivity of $H_2{}^{18}O$ in water ($m^2\ s^{-1}$).

A potentially useful application of $\Delta^{18}O$ in plant material is as an integrated measure of stomatal conductance and transpiration rate (Barbour and Farquhar, 2000). At a given air temperature and humidity, Equation 2 suggests that $\Delta^{18}O_e$ will decrease with increasing stomatal conductance (and, therefore, transpiration rate) as a result of evaporative cooling of the leaf and consequent lowering of e_a/e_i. In addition, increased stomatal conductance decreases ϵ_k, thereby further decreasing $\Delta^{18}O_e$. Finally, increased transpiration increases the Péclet number, which decreases $\Delta^{18}O_L$, as seen in Equation 3. The influence of increased stomatal conductance on e_a/e_i, ϵ_k, and $\wp$ is opposed by an increase in ϵ^* with decreasing leaf temperature; however, the increase in ϵ^* is rather small, namely a change from 9.2‰ at 25°C to 9.6‰ at 20°C. Thus, $\Delta^{18}O$ can potentially compliment the use of $\Delta^{13}C$ by providing information about stomatal conductance independently of the effects of photosynthetic demand for CO_2 on c_i/c_a.

Significant variation in $\Delta^{13}C$ of phloem sap sugars was recently observed in the phloem-bleeding tree *Eucalyptus globulus* Labill. growing in southwestern Australia (Pate and Arthur, 1998); variation occurred between rain-fed plantations experiencing drought stress and irrigated plantations, and seasonally within rain-fed plantations in correspondence with seasonal rainfall patterns. Based on the data provided by Pate and Arthur (1998), phloem sap sugar $\Delta^{13}C$ appeared to integrate drought stress more directly, and over more physiologically relevant timescales, than did whole-tissue $\Delta^{13}C$. An additional advantage was the relative ease of analyzing phloem sap, which was so dominated by photosynthetic sugars that it did not require further extraction, as would be the case in the analysis of leaf soluble sugars or starch.

In a companion paper, Pate et al. (1998) reported a strong relationship between phloem sap sugar $\Delta^{13}C$ and phloem sap sugar concentration in *E. globulus*. According to the pressure flow hypothesis of phloem translocation (Münch, 1930), photosynthate is distributed from source to sink regions within a plant via gradients in turgor within sieve tubes generated by the loading and unloading of sugars. The amount of turgor borne by a sieve tube depends, in part, on the water potential of the apoplastic reservoir surrounding it:

$$P = \Psi + \Pi \quad (4)$$

where P is the hydrostatic pressure within the sieve tube, Ψ is the symplastic water potential (assumed equal to that of the apoplast when the system is in stationary state), and Π is the osmotic pressure within the sieve tube. The importance of the apoplastic water potential in the phloem system has been recognized explicitly in formal descriptions of the Münch hypothesis (e.g. Christy and Ferrier, 1973; Tyree et al., 1974; Goeschl et al., 1976; Smith et al., 1980; Sheehey et al., 1995), and attention has been drawn to the role of water potential gradients in determining the partitioning of photosynthate among multiple sinks (Lang and Thorpe, 1986; Daudet et al., 2002). One might then hypothesize that as the water potential of a plant decreases during drought stress, the osmotic pressure within the sieve tubes will increase to provide the turgor necessary for continued functioning of the phloem. Experimental evidence in support of this concept was obtained for *Ricinus communis*, wherein the concentration of solutes in phloem sap increased in response to withholding water (Hall and Milburn, 1973), and the loading of Suc into the phloem appeared to be turgor pressure dependent (Smith and Milburn, 1980).

These considerations led us to investigate the possibility that variation in plant water potential causes correlated changes in phloem sap sugar concentration and phloem sap sugar $\Delta^{13}C$ in *E. globulus*. The hypothesis is conceptualized in Figure 1. In addition, we compared the $\Delta^{13}C$ measured in the phloem sap sugars with that predicted from instantaneous measurements of c_i/c_a to assess the validity of applying

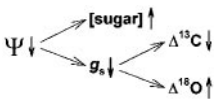

Figure 1. A conceptual diagram showing the hypothesized relationships among investigated variables. We expected phloem sap sugar concentration ([sugar]) and stomatal conductance (g_s) to vary in response to variation in plant water potential and carbon ($\Delta^{13}C$) and oxygen ($\Delta^{18}O$) isotope discrimination to vary consequently in response to variation in stomatal conductance.

Equation 1 to the *E. globulus* system. Finally, we report on a strong relationship between $\Delta^{18}O$ in phloem sap sugars and the phloem sap sugar concentration and postulate that this relationship can also be mechanistically accounted for through consideration of plant water relations.

RESULTS

The strong relationship between phloem sap sugar $\Delta^{13}C$ and phloem sap sugar concentration previously observed by Pate et al. (1998) featured prominently in the present data set (Fig. 2A). Values for $\Delta^{13}C$ spanned a range of 10‰, and values for sugar concentration spanned a range of 0.3 mol L^{-1}. The Pearson correlation coefficient (r) relating the two variables was -0.90 ($P < 0.0001$, $n = 40$), indicating a very strong, negative, linear covariance. There was also a very strong correlation between phloem sap sugar concentration and instantaneous c_i/c_a (Fig. 2B), with a correlation coefficient of -0.91 ($P < 0.0001$, $n = 27$). In addition, significant correlation was observed between $\Delta^{13}C$ of phloem sap sugars and shoot water potential ($r = 0.70$, $P < 0.0001$, $n = 40$).

Stomatal conductance, photosynthesis and c_i/c_a varied among trees growing in the three plantations. The lowest stomatal conductance values were recorded at the Mount Barker plantation and the drier Denmark plantation, and the highest values at the wetter Denmark plantation. Average stomatal conductances for individual trees ranged from 0.02 to 0.56 mol water m^{-2} s^{-1}. Average photosynthetic rates ranged from 1.7 to 13.0 μmol CO_2 m^{-2} s^{-1}. Curvature in the relationship between average values for stomatal conductance and photosynthesis suggested variation in c_i/c_a among the population of trees sampled.

The measured variation in instantaneous c_i/c_a correlated with $\Delta^{13}C$ of phloem sap sugars (Fig. 3). The observed relationship was close to that predicted by Equation 1. A linear regression through the data yielded the relationship $\Delta^{13}C = 1.7 + 25.3c_i/c_a$, with the 95% confidence intervals extending from -1.5‰ to 4.9‰ for the intercept and 20.1‰ to 30.5‰ for the slope. With the intercept forced through 4.4‰ (the theoretical value for a), the regression yielded a slope estimate of 21.0‰, with the 95% confidence interval extending from 19.9‰ to 22.2‰.

Both daytime and predawn shoot water potential correlated strongly with daytime phloem sap sugar concentration (Fig. 4, A and B), with correlation coefficients of -0.86 ($P < 0.0001$, $n = 40$) and -0.87 ($P < 0.0001$, $n = 30$), respectively. Recall that the two

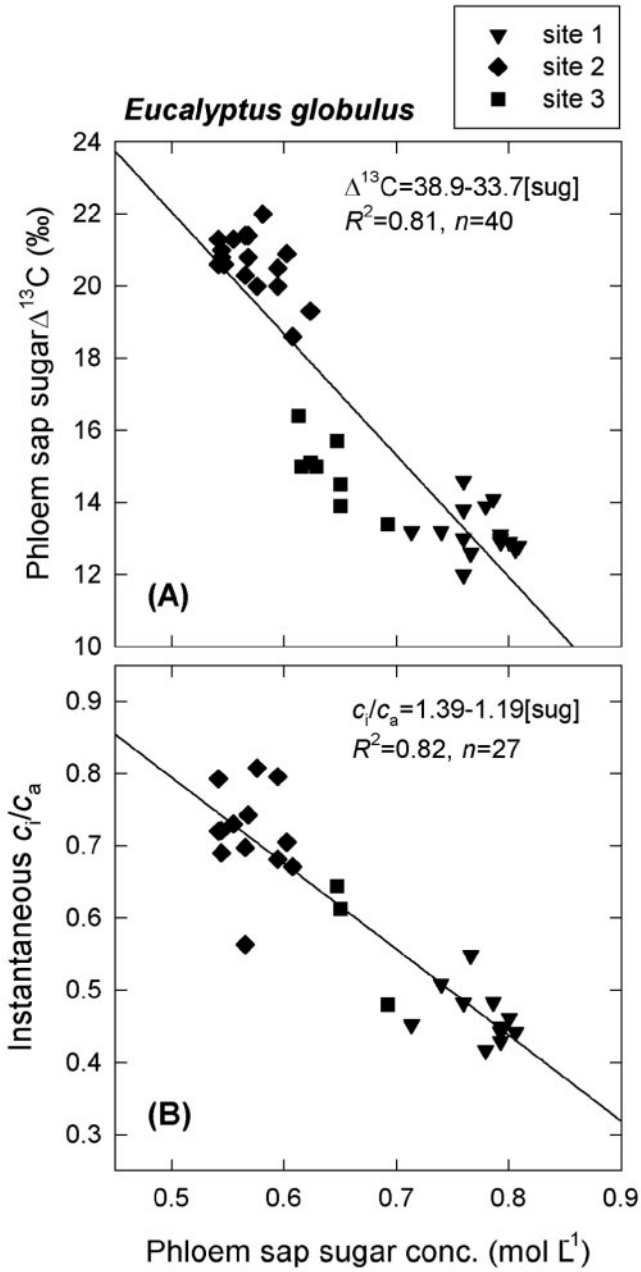

Figure 2. A, $\Delta^{13}C$ measured in phloem sap sugars; and B, instantaneous c_i/c_a plotted against phloem sap sugar concentration for *E. globulus* samples collected from three plantations in southwestern Australia in February 2002. Site 1, Drought-stressed Mount Barker plantation; site 2, relatively unstressed Denmark plantation; site 3, intermediate Denmark plantation. Each datum corresponds to one tree. Phloem sap was collected from the stem at approximately two-thirds the height of the live crown. Instantaneous c_i/c_a was measured on five to 10 leaves at the same canopy height and averaged for each tree.

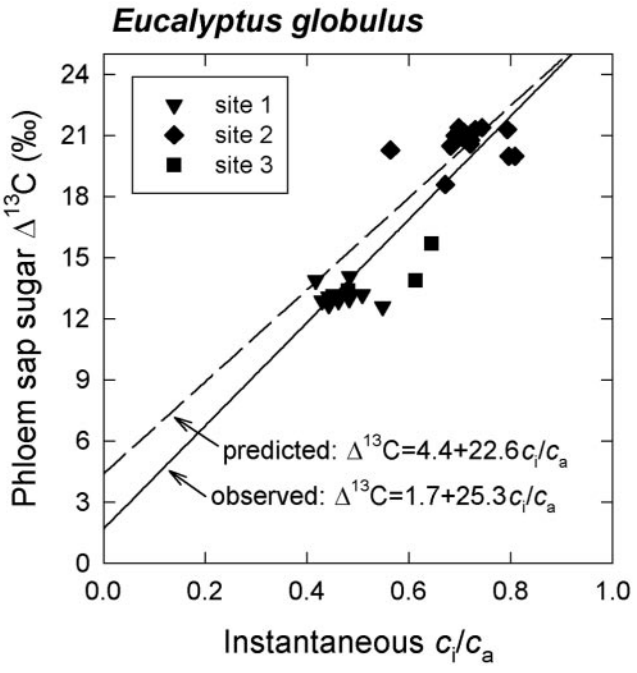

Figure 3. $\Delta^{13}C$ measured in phloem sap sugars collected from *E. globulus* stems plotted against instantaneous c_i/c_a. Gas exchange measurements took place at the same canopy height as the phloem sap collections; instantaneous c_i/c_a values are the average of five to 10 measurements per tree. Each datum represents one tree. Site numbers refer to different plantations as described in the caption of Figure 2.

sets of measurements took place on different trees. As seen in Figure 4A, the data for daytime shoot water potential and sugar concentration tended to separate into two populations when plotted against each other, with the trees from the two Denmark plantations having less negative water potentials and lower sugar concentrations than those from the Mount Barker plantation. The slope of the relationship between shoot water potential and daytime phloem sap sugar concentration did not differ significantly depending on whether shoot water potential was measured predawn or during the day ($P = 0.07$, $n = 70$). However, intercepts for the two relationships were significantly different ($P < 0.0001$, $n = 70$).

The osmotic pressure exerted by phloem sap sugars sampled from the stem was generally in excess of that required to balance the daytime apoplastic shoot water potential for the trees in the two Denmark plantations but not greatly in excess for trees in the Mount Barker plantation, which showed the most negative daytime shoot water potentials (Fig. 5). The slope of the relationship between daytime phloem sap sugar osmotic pressure and daytime shoot water potential had a value of -0.61, which was significantly different from -1 ($P < 0.0001$). This suggested significant variation in the amount of turgor borne by sieve tubes in the stems across the range of shoot water potentials encountered in the study.

The $\Delta^{18}O$ of phloem sap sugars correlated strongly with the phloem sap sugar concentration (Fig. 6A), with a correlation coefficient of 0.91 ($P < 0.0001$, $n = 39$) and with daytime shoot water potential ($r = -0.78$, $P < 0.0001$, $n = 39$). Values of phloem sap sugar $\Delta^{18}O$ spanned a range of 8‰, with the lowest values (39.0‰–41.7‰) being recorded at the wetter Denmark plantation, intermediate values (42.3‰–43.7‰) at the drier Denmark plantation, and highest values (43.7‰–47.0‰) at the Mount Barker plantation. The $\Delta^{13}C$ and $\Delta^{18}O$ of phloem sap sugars correlated negatively with each other (Fig. 6B; $r = -0.92$, $P < 0.0001$, $n = 39$). The phloem sap sugar $\Delta^{18}O$ also correlated negatively with the instantaneous, cuvette-based measurements of transpiration rate (Fig. 6C), with a correlation coefficient of -0.85 ($P < 0.0001$, $n = 27$). The theoretical model of $\Delta^{18}O$, summarized in Equations 2, 3, 5, and 6 predicted values ranging from 47.3‰ to 38.3‰ over the observed range of stomatal conductances (0.02–0.56 mol water m^{-2} s^{-1}). This predicted range of $\Delta^{18}O$ values agreed well with the observed range (47.0‰–39.0‰), suggesting that the observed variation in $\Delta^{18}O$ could in fact be accounted for by varying only one term in the model, i.e. stomatal conductance. For comparison, a sensitivity analysis is presented in Table I showing the effect of varying terms in the model other than stomatal conductance. The amount of variation in leaf temperature predicted by Equation 5 over the observed range of stomatal conductances was 2.8°C. This can be compared with observed differences in leaf temperature of approximately 1°C in *Eucalyptus pauciflora* for stomatal conductance values ranging from 0.3 to 0.6 mol m^{-2} s^{-1} (J. Egerton, personal communication); over that range, Equation 5 predicts a difference of 1.1°C. The best fit between modeled and observed $\Delta^{18}O$ values was found when the equilibrium fractionation between leaf water and exported sugars was assumed to be 28‰. Note that varying this parameter from 27‰ to 28‰ affects the absolute values predicted for $\Delta^{18}O$, but does not affect the range of values predicted.

DISCUSSION

Although further experimental testing is warranted, the results obtained in this study strongly support our hypothesis as conceptualized in Figure 1. Thus, it appears that variation in plant water potential induces correlated changes in phloem sap sugar concentration and the current $\Delta^{13}C$ and $\Delta^{18}O$ of *E. globulus* trees. The resulting correspondence between these parameters suggests the intriguing possibility of interpreting phloem sap sugar concentration in terms of plant responses to the environment, and in particular to drought stress.

The relationship between phloem sap sugar concentration and $\Delta^{13}C$ of phloem sap sugars appears to be extremely well conserved for *E. globulus* growing

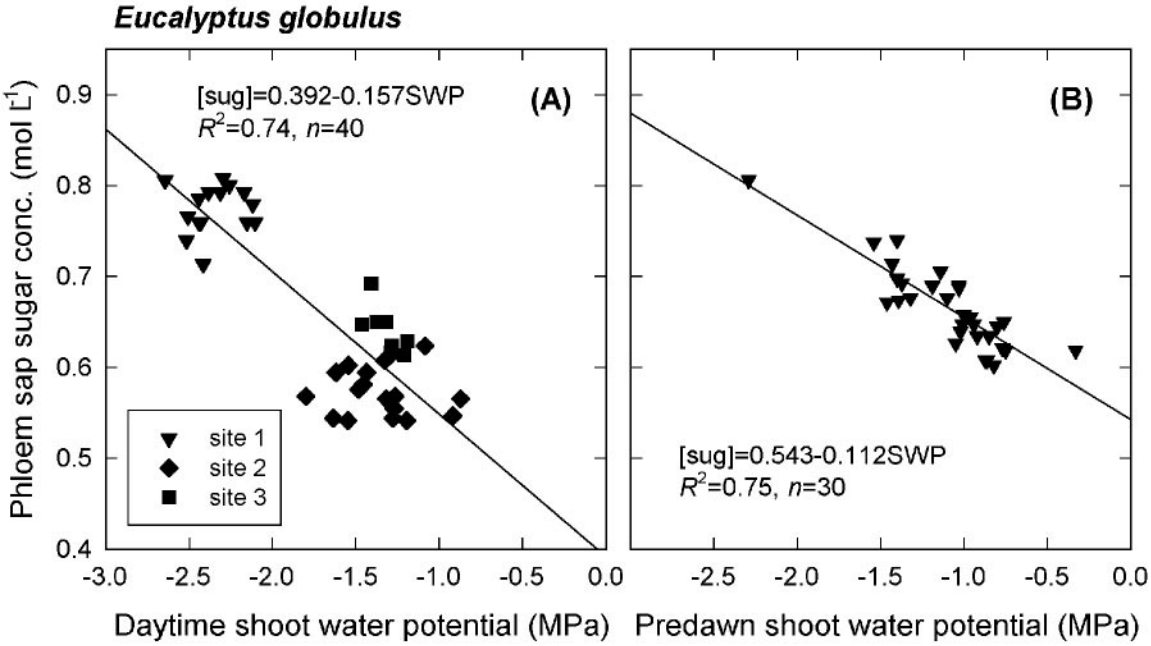

Figure 4. Phloem sap sugar concentration plotted against daytime (A) and predawn (B) shoot water potential for *E. globulus* growing in southwestern Australia. Phloem sap was collected from the main stem at about two-thirds the height of the live crown for A and at approximately 1.4-m height for B. Shoot water potential was measured on four twigs per tree at the same canopy height as the phloem sap was collected from and averaged for each tree. Each datum corresponds to one tree. Daytime and predawn measurements were conducted on different trees at different plantations. Different symbols in A show the separation among plantations; site 1 is the Mount Barker plantation, whereas sites 2 and 3 are the two Denmark plantations.

in southwestern Australia. Pate et al. (1998) reported the regression equation [sug] = 1.05 − 0.025Δ^{13}C(‰), where [sug] is phloem sap sugar concentration expressed as moles per liter, and the regression has an R^2 value of 0.69. For the present data set, we obtained the regression equation [sug] = 1.06 − 0.024Δ^{13}C(‰), with an R^2 value of 0.81. The relationship that we observed was nearly identical to that observed previously. The Pate et al. (1998) data were derived from bulked phloem sap samples collected from 37 plantations distributed across southwestern Australia, such that each datum represented one plantation. Therefore, the present data set serves to confirm on an individual tree basis what was found previously on a plantation basis.

Phloem-bleeding sap has also been collected from *Fagus sylvatica* growing in the south of Germany and assayed for both its sugar concentration and the Δ^{13}C of the sugars (Gessler et al., 2001). In that study, stand density was manipulated to varying degrees, which was expected to impact on soil water availability and, therefore, Δ^{13}C. Sampling also took place on slopes of differing aspect, which introduced further variation in Δ^{13}C. Combining data from the different basal area treatments, slope aspects, and sampling dates resulted in a negative correlation between Δ^{13}C in phloem sap sugars and phloem sap sugar concentration for *F. sylvatica*, as expected from the hypothesis described by Figure 1. Although Gessler et al. (2001) did not provide a statistical analysis of the combined data set, the relationship does not appear to be as strong as the one that we observed. For *F. sylvatica*, sugar concentrations ranged from 0.1 to 0.4 mol L^{-1}, whereas in the present study concentrations ranged from 0.5 to 0.8 mol L^{-1}. The relationship between phloem sap sugar concentration and Δ^{13}C has also been observed to become weaker in *E. globulus* when phloem sap sugar concentrations are lower and soil water more plentiful (D.J. Arthur and J.S. Pate, unpublished data); presumably, this reflects a more limited role of stomata in causing variation in c_i/c_a at such times.

A negative relationship between phloem sap sugar concentration and phloem sap sugar Δ^{13}C was previously observed in *Lupinus angustifolius*, where the sap was collected at different times over a diurnal cycle, and sugar concentrations varied over a relatively narrow range of from 0.33 to 0.38 mol L^{-1} (Cernusak et al., 2002). In the present study with *E. globulus*, we could not resolve a diurnal pattern of variation in either the concentration or Δ^{13}C of phloem sap sugars. This result contrasts with earlier results for *E. globulus* reported by Pate and Arthur (2000), in which a diurnal pattern in phloem sap sugar concentrations of stems and to a greater extent branches was observed, particularly between samples collected during the day and those collected at night. It is probable that in the present study such a

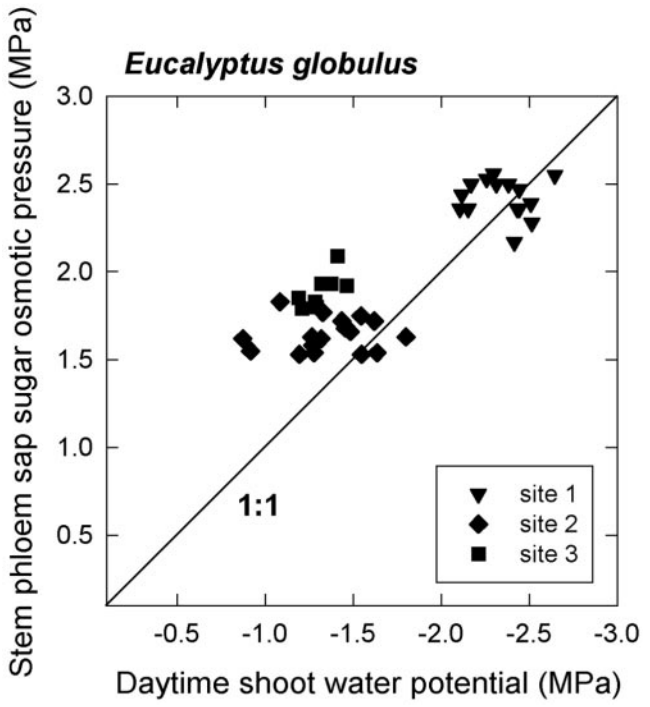

Figure 5. Phloem sap sugar osmotic pressure plotted against daytime shoot water potential. Osmotic pressure estimates were derived from measurements of phloem sap sugar concentration. Note that phloem sap was collected from the stem, whereas shoot water potential was measured in terminal shoots. Each datum represents one tree. Site 1 is the Mount Barker plantation, and sites 2 and 3 are the two Denmark plantations.

diurnal pattern was obscured by inter-tree variability within the sampled plantations because all sequential sampling occurred on different trees. In addition, a diurnal pattern may have been less apparent because we only sampled stems and only sampled during the day.

An apparently strong relationship was previously observed between shoot water potential and $\Delta^{13}C$ of phloem sap sugars in *F. sylvatica* (Gessler et al., 2001). The relationship reported in terms of $\delta^{13}C$ was $\delta^{13}C(‰) = -3.93SWP - 30.7$, where SWP is shoot water potential (megapascals). If we express our data in the same terms, we obtain a relationship for *E. globulus* of $\delta^{13}C(‰) = -4.60SWP - 32.0$ ($R^2 = 0.49$, $P < 0.0001$, $n = 40$), reasonably similar to that obtained for *F. sylvatica*. Extrapolating the regression equations to their respective values at which $\Delta^{13}C = 4.4‰$ (or $\delta^{13}C = -12.2‰$) results in shoot water potential estimates of −4.7 MPa for *F. sylvatica* and −4.3 MPa for *E. globulus*. The discrimination value of 4.4‰ is the value expected when stomata are completely closed, foregoing issues associated with molecular flow at very low stomatal conductances (Farquhar and Lloyd, 1993). These values can be compared with a water potential estimate of −2.1 MPa for *L. angustifolius* when $\Delta^{13}C = 4.4‰$ (Cernusak et al., 2002). Not surprisingly, the estimates of water potential values at complete stomatal closure for the two long-lived, woody tree species are substantially lower than for the herbaceous annual. Such analyses could prove useful in determining the extent of drought stress that different species or genotypes are capable of tolerating.

Slopes of the relationship between shoot water potential and $\delta^{13}C$ have also been reported for $\delta^{13}C$ of leaf tissue and wood. A slope of $-0.18‰\ MPa^{-1}$ was reported for leaves of *Quercus pubescens* and *Quercus ilex* growing in southern France (Damesin et al., 1998), whereas slopes ranging from −1.8 to $-3.1‰\ MPa^{-1}$ were reported for wood of *Pinus radiata* and *Pinus pinaster* growing in southwestern Australia (Warren et al., 2001). The slope that we report for phloem sap sugars of *E. globulus* of $-4.6‰\ MPa^{-1}$ differs from those just mentioned in that it was derived from measurements of daytime shoot water potential, rather than predawn shoot water potential. Nonetheless, slopes among species appear to vary over a large range. It is possible that some of the variation can be accounted for by considering the different sampling techniques. Whole-tissue measurements potentially include considerable uncertainty about the period during which the carbon comprising the tissue was assimilated. On the other hand, the strong correspondence between phloem sap sugar $\Delta^{13}C$ and instantaneously measured c_i/c_a presently reported for *E. globulus* provides clear evidence that phloem sap sugars provide an accurate estimate of the current $\Delta^{13}C$ of the plant.

We observed a slope for the relationship between $\Delta^{13}C$ of phloem sap sugars and instantaneous c_i/c_a of 21.0‰ when the intercept was forced through 4.4‰, as prescribed by Equation 1. This relationship yields a value for *b*, the effective discrimination by carboxylating enzymes, of 25.4‰. This is consistent with the value of 25.7‰ estimated for *b* from measurements of leaf soluble sugars in *Populus nigra* × *deltoids*, *Gossypium hirsutum*, and *Phaseolus vulgaris* (Brugnoli et al., 1988), and 25.0‰ estimated from leaf soluble sugars in *G. hirsutum* and *Oryza sativa* (Brugnoli and Farquhar, 2000). Possible reasons for the deviation of *b* values estimated from analyses of leaf soluble sugars from the suggested value of 27‰ have been discussed in detail by Brugnoli and Farquhar (2000). They include the effects of low mesophyll conductance to CO_2, and possibly fractionation during dark respiration and photorespiration. The same set of potential mechanisms affecting apparent values of *b* observed in leaf soluble sugars should also apply to those observed in phloem sap sugars, with the one possible exception being the potential for fractionation during phloem loading. However, to date, such a phenomenon has not been demonstrated.

Data plotted in Figure 5 suggest that the amount of turgor conferred by sugars in the phloem sap is not homeostatically maintained across the range of apoplastic shoot water potentials sampled in *E. globulus*.

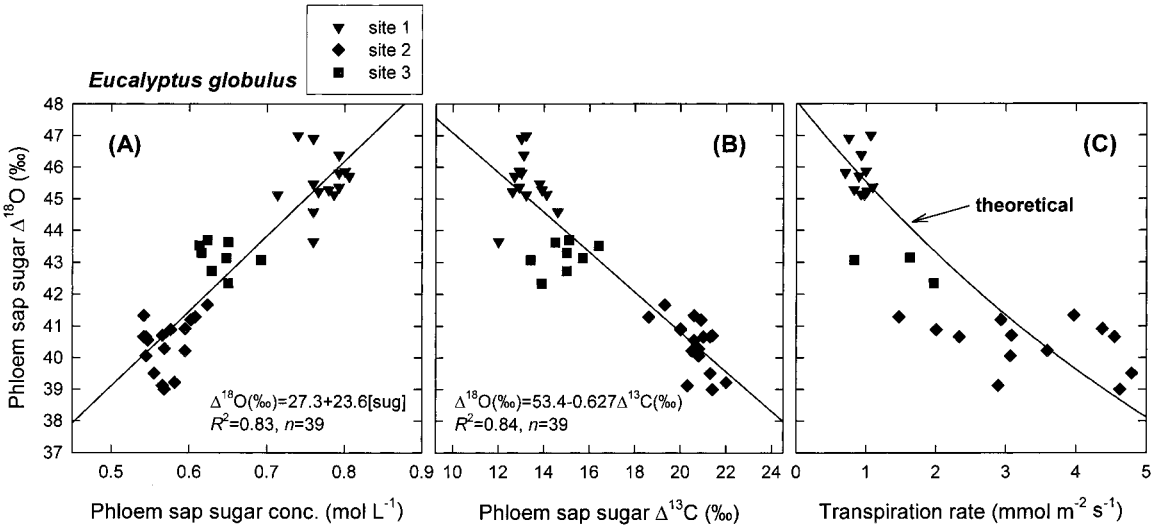

Figure 6. Oxygen isotope enrichment ($\Delta^{18}O$) of phloem sap sugars in *E. globulus* plotted against: A, the sugar concentration of the phloem sap; B, the carbon isotope discrimination ($\Delta^{13}C$) of the phloem sap sugars; and C, cuvette-based measurements of transpiration rate made concurrently with the phloem sap collections. Samples were collected in February 2002 from trees growing in three plantations in southwestern Australia. Site numbers are as described in the caption to Figure 2. Each datum corresponds to one tree. Transpiration was measured on five to 10 leaves and averaged for each tree. The theoretical line in C was derived from Equations 2, 3, and 5 in the main text. The theoretical relationship is that expected if variation in phloem sap sugar $\Delta^{18}O$ resulted exclusively from variation in stomatal conductance and, therefore, transpiration rate.

The relationship between daytime phloem sap osmotic pressure in the stem and daytime shoot water potential had a slope greater than −1, suggesting more turgor at less negative water potentials than at more negative water potentials. This pattern was also reflected in the bleeding behavior of the trees, with trees that had less negative water potentials bleeding more profusely than those with more negative water potentials. In their earlier *E. globulus* sampling efforts, Pate et al. (1998) remarked, "Failure to bleed was rare but encountered occasionally when severely water stressed plantations were sampled during very hot afternoons of late summer and autumn. Even then, the same trees produced sap when sampled after recovery of water stress the following evening." This would suggest that only under the most severe conditions of drought stress is there a lack of turgor in the sieve tubes of *E. globulus*.

There are some complications involved in attempting to make precise quantitative estimates of stem phloem turgor based on the data plotted in Figure 5. Phloem sap was collected from main stems, whereas shoot water potential was measured on terminal shoots. One would expect the daytime water potential in the stem to be less negative than that in the terminal twigs, which would tend to shift the relationship in Figure 5 toward a less negative apoplastic water potential for a given osmotic pressure, thereby resulting in higher estimated turgor pressures in the sieve tubes. However, it also seems likely that the

Table I. *A sensitivity analysis showing the effect of varying different parameters in the phloem sap sugar $\Delta^{18}O$ model, in relation to the effect of varying stomatal conductance over the observed range of conductance values*

Parameters were varied one at a time. When a parameter was not being varied, the median value in the selected range was used.

Parameter	Model Component Affected	Change to Value of Parameter	Predicted Change in $\Delta^{18}O$
			‰
Stomatal conductance (mol water m^{-2} s^{-1})	Equations 2, 5, and 6[a]	0.02 to 0.56	−9.0
Wind speed (m s^{-1})	Equations 2, 5, and 6	2 to 10	−0.4
Leaf area (cm^2)	equations 2, 5, and 6	20 to 100	0.2
Air temperature (°C)	Equations 2, 5, and 6	15 to 25	−2.9
Relative humidity (%)	Equations 2, 5, and 6	50 to 60	−1.8
Barometric pressure (mbar)	Equations 5 and 6	950 to 1,050	0.9
Photosynthetically active radiation (PAR; μmol m^{-2} s^{-1})	Equations 5 and 6	500 to 1,500	1.4
scaled effective path length (mm)	Equation 3	20 to 40	−2.4
Atmospheric vapor $\Delta^{18}O$ (‰)	Equation 2	−8.6 to −10.6	−0.8

[a]Note that any parameter affecting Equation 6, which predicts transpiration rate, also affects Equation 3, which predicts the Péclet effect.

effective osmotic pressure will be less than that estimated from the sugar concentration of the sap because the reflection coefficient of the sieve tube membranes and sieve plates is likely less than unity. The quantitative significance of these two factors is difficult to estimate, particularly because the associated biases are in opposing directions.

However, if we ignore these complications, sieve tube turgor estimates for *E. globulus* range from −0.2 to 0.8 MPa. These can be compared with previously reported values ranging from 0.7 to 1.2 MPa for stem phloem in *Fraxinus americana*, 0.9 to 1.1 MPa for stem phloem in *R. communis* (Milburn, 1980), and a value of 1.1 MPa for leaf phloem in *Hordeum vulgare* (Pritchard, 1996). In those studies, xylem water potentials were −0.7, − 0.5, and −0.2 MPa, respectively, all somewhat less negative than the shoot water potentials recorded in the present study. However, in peduncles of *Triticum aestivum*, sieve tube turgor pressures of 2.4 and 1.4 MPa were observed at apoplastic water potentials of −0.4 and −2.1 MPa, respectively (Fisher and Cash-Clark, 2000). This decrease in phloem sap turgor with increasing drought stress, as also seen for *E. globulus* in Figure 5, is likely to be qualitatively meaningful. If one assumes that the net assimilation rate of the canopy of a tree determines the translocation rate from the canopy, and that the translocation rate is proportional to the turgor gradient from source to sink, then it follows that a reduction in canopy photosynthesis due to stomatal closure will reduce the amount of photosynthate available for translocation and result in a smaller turgor gradient between the source and sink, likely caused by less turgor at the source.

We found that the observed variation in stomatal conductance across the study was sufficient to account for the range of values observed in $\Delta^{18}O$ of phloem sap sugars. Meteorological data from Mount Barker and Albany, Western Australia suggest very little or no difference in average relative humidity among the study sites for the 3 weeks preceding measurements (Table II). Similarly, there is no a priori reason to expect the isotopic composition of atmospheric water vapor to differ between the Mount Barker and Denmark sites, and, as noted previously, we have observed no difference in xylem water $\delta^{18}O$ between the Mount Barker plantation and the wetter Denmark plantation. Thus, there would not appear to be a basis for invoking variation in parameters in the $\Delta^{18}O$ model other than stomatal conductance and transpiration rate in seeking the most parsimonious explanation for the observed variation in $\Delta^{18}O$ of phloem sap sugars. The separation of $\Delta^{18}O$ values in the two Denmark plantations provides further support for this interpretation because these two sites were only 2 km apart and, therefore, would have likely experienced identical source water, atmospheric vapor $\delta^{18}O$, and temperature and humidity regimes.

The $\Delta^{18}O$ of total dry matter in leaves collected from the Mount Barker plantation and the wetter Denmark plantation was measured in a separate set of experiments (L. Cernusak, unpublished data). Values were 33.5‰ ± 0.3‰ (mean ± SE) for the Mount Barker plantation and 31.0‰ ± 0.1‰ for the Denmark plantation, showing that the difference in $\Delta^{18}O$ observed in phloem sap sugars is also reflected in leaf dry matter. Whereas the average difference between the two plantations for phloem sap sugars was 5.1‰, the average difference for leaf dry matter was 2.5‰. This difference is to be expected, given that during the conversion of phloem sap sugars to leaf dry matter some of the oxygen atoms of the sugars are replaced by those of medium water. In addition, leaf dry matter would integrate over a longer time period than would phloem sap sugars, most likely encompassing periods when differences in drought stress between the two plantation were less pronounced than at the time of phloem sap sampling.

Because stomatal conductance impacts upon the $\Delta^{18}O$ model at multiple points, the predicted effect of variation in this parameter was relatively large compared with that which might have been caused by variation in other model parameters (Table I). The modeling exercise allowed us to partition the predicted variation in $\Delta^{18}O$ because of variation in stomatal conductance into components due to variation in e_a/e_i (resulting from variation in leaf cooling), ϵ_k, and $\wp$. The ϵ_k varied from 27.9‰ at a stomatal conductance of 0.02 mol water m^{-2} s^{-1} to 26.4‰ at a conductance of 0.56 mol water m^{-2} s^{-1}. Because we assumed that $\Delta^{18}O_v = -\epsilon^*$, Equation 2 simplifies to $\Delta^{18}O_e = (\epsilon^* + \epsilon_k)(1 - e_a/e_i)$. At a common e_a/e_i of 0.5, the variation in ϵ_k would equate to a difference of 0.8‰ in $\Delta^{18}O_e$. The variation in $\Delta^{18}O_e$ resulting from variation in e_a/e_i due to differences in evaporative cooling of the leaf at the minimum and maximum observed stomatal conductances for a given ϵ_k of 27‰ would be 2.9‰. Finally, the difference in $\Delta^{18}O_L$ between the minimum and maximum observed stomatal conductances resulting from variation in $\wp$ for a given $\Delta^{18}O_e$ of 17‰ would be 5.7‰. Thus, it can be seen that most of the variation in $\Delta^{18}O$ of phloem sap

Table II. *Average meteorological conditions over the first 3 weeks of February reported by weather stations in Mount Barker (34°37′30″ S, 117°38′10″ E) and Albany (34°56′35″ S, 117°48′03″ E), Western Australia*

Albany is a coastal town approximately 50 km west of Denmark that should experience similar weather patterns to Denmark. Albany is the nearest operating weather station to Denmark. Total precipitation over the period was 8.0 mm at Mount Barker and 8.4 mm at Albany.

Parameter	Mount Barker 9 AM	Mount Barker 3 PM	Albany 9 AM	Albany 3 PM
Relative humidity (%)	67	49	67	51
Air temperature (°C)	17	21	18	21
Wind speed (m s^{-1})	4.2	4.4	5.8	7.5

sugars occurring as a result of variation in stomatal conductance across the natural rainfall gradient in southwestern Australia was likely caused by variation in $\wp$ and leaf temperature.

We found that an equilibrium fractionation between predicted leaf water $\delta^{18}O$ and phloem sap sugar $\delta^{18}O$ of 28‰ resulted in a better fit of modeled to observed data than the commonly assumed value of 27‰. The possibility exists that the $\delta^{18}O$ of the leaf water in the cytosol of the mesophyll cells with which Suc equilibrates before export differs slightly from the bulk leaf water $\delta^{18}O$, as suggested in previous and recent leaf water modeling efforts (Leaney et al., 1985; Yakir et al., 1989, 1990; Yakir, 1992; Farquhar and Gan, 2003). We are currently conducting further research into this question.

Phloem exudation after an incision in the bark has been demonstrated for many tree species (Zimmerman, 1960). Pate et al. (1998) observed exudation of collectable amounts of sap in 14 *Eucalyptus* spp., in addition to *E. globulus*. Phloem bleeding for the purpose of sap collection also has been demonstrated in herbaceous plants; for example, *R. communis* (Milburn, 1970) and several legumes (Pate et al., 1974). Results of this study, and those conducted previously with *E. globulus* (Pate and Arthur, 1998, 2000; Pate et al., 1998; D.J. Arthur and J.S. Pate, unpublished data) highlight the potential of phloem sap analyses for revealing information about the current physiological status of the plant. Such analyses could prove very useful in optimizing the management of *E. globulus* plantations, and the potential exists for their application in other cropping systems as well. The measurement of phloem sap sugar concentrations, in particular, is rapid and inexpensive and can be easily achieved in a field setting. We have demonstrated strong correspondence between the phloem sap sugar concentration of *E. globulus* and several measures of its physiological response to drought stress. Results suggest a very strong potential for the application of the measurement and interpretation of phloem sap sugar concentrations for the purposes of both plantation management and ecophysiological research.

MATERIALS AND METHODS

We measured daytime shoot water potential, phloem sap sugar concentration, phloem sap sugar $\Delta^{13}C$, phloem sap sugar $\Delta^{18}O$, and instantaneous gas exchange in 40 *Eucalyptus globulus* Labill. trees selected from three rain-fed plantations located in southwestern Australia. The three plantations were chosen such that the study would encompass a selection of trees ranging from relatively unstressed to very stressed. Sampling took place between February 21 and 23, 2002, a time that would ordinarily correspond to peak drought stress in the Mediterranean-type environment of southwestern Australia. Site 1 was located near Mount Barker, western Australia (34°32′28′′S, 117°30′24′′ E), a region on the lower rainfall limit of *E. globulus* plantations averaging approximately 600 mm of annual precipitation. Trees were planted in 1999 and were approximately 6 m tall at the time of sampling. Site 2, the wettest of the plantations, was located near the township of Denmark, Western Australia (34°58′45′′ S, 117°20′06′′ E), in a region that averages approximately 1,400 mm annual precipitation. The sampled trees at site 2 were located at the base of a small hill, where we expected soil moisture content to be relatively high. Trees were planted in 1999 and were approximately 10 m tall at the time of sampling. Trees from a third plantation (site 3), thought to be intermediate between the wet and dry plantations, were also sampled. Site 3 was also located near Denmark, Western Australia (34°58′43′′ S, 117°19′02′′ E), but sampled trees were located high on the slope of a hill; thus, the trees were expected to have a lower soil water availability than those at site 2. This plantation was also a 1999 planting. Average weather conditions in the vicinity of the sampling sites over the 3 weeks preceding sampling are given in Table II.

Trees were sampled sequentially through the day over a single day at each plantation, starting in the early morning and concluding in the late afternoon. Thus, the study comprised 10 to 15 trees from each plantation. Shoot water potential was measured on four twigs of approximately 5-mm diameter from each tree using a Scholander-type pressure chamber (Scholander et al., 1965). A large ladder was used to access the canopy. Twig samples for water potential measurements were collected from a single canopy height that was approximately two-thirds the height of the live crown. At the same canopy level, phloem sap was collected from the main stem using the bleeding technique described previously (Pate et al., 1998). Phloem sap sugar concentration (w/v) was measured at the time of sap collection using a temperature-compensated, hand-held refractometer (Bellingham and Stanley, London), previously calibrated against HPLC measurements of sugar concentration (Pate et al., 1998). Gas exchange was measured on five to 10 leaves per tree at the same canopy level using an LCA 4 Portable Gas Exchange System (ADC BioScientific Ltd., Hertfordshire, UK) at the same time that water potential and sugar concentration measurements were taking place. Sugar concentration values measured on a weight per volume basis on the refractometer were converted to molar concentrations by assuming the sugar fraction of the sap to comprise 70% Suc and 30% raffinose on a weight basis (see Tables II and III in Pate et al., 1998). This was the mean value for the relative concentrations of the two sugars observed across a range of 29 *E. globulus* plantations in southwestern Australia. The SD of the ratio was 10%; an error of two SDs would lead to approximately a 7% difference in our calculated molar sugar concentrations.

We made additional measurements of predawn shoot water potential followed by measurements of midday phloem sap sugar concentration to further investigate the relationship between the two parameters and to see whether predawn or daytime shoot water potential correlated more strongly with daytime sugar concentration. Phloem sap was collected from stems at approximately 1.4 m height above the ground. These measurements took place at various *E. globulus* plantations in southwestern Australia in close proximity (within 100 km) to the primary study plantations in which the more detailed measurements took place.

Phloem sap sugar concentrations were converted to osmotic pressures according to the relationship given by Nobel (1991), which was based on measurements of the freezing point depression of Suc solutions at 20°C (Weast and Lide, 1989). Raffinose was assumed to have the same relationship between molar concentration and osmotic pressure as Suc. Data for photosynthesis, stomatal conductance, and c_i/c_a were averaged for each tree. Measurements taken at irradiances less than 400 μmol PAR m^{-2} s^{-1} were excluded from the analyses so that the effects of water stress on gas exchange could be analyzed independently of the effects of low irradiance. The value of 400 μmol PAR m^{-2} s^{-1} was chosen based on a plot of photosynthesis versus irradiance for the unstressed plantation, in which there appeared to be little increase in photosynthesis with increasing irradiance beyond PAR values of 400 μmol m^{-2} s^{-1}.

The stable carbon and oxygen isotope ratios of phloem sap dry matter were determined on 5-μL phloem sap samples from which the water was evaporated overnight at 60°C in a drying oven. Carbon isotope analyses were conducted with an Isochrom mass spectrometer (Micromass, Manchester, UK) coupled to a Carlo Erba elemental analyzer (CE Instruments, Milan) operating in continuous flow mode. Oxygen isotope ratios were measured by a second Isochrom mass spectrometer after pyrolysis in a Carlo Erba elemental analyzer (Farquhar et al., 1997). Carbon and oxygen isotope ratios were obtained in δ-notation, where $\delta = R/R_{standard} - 1$ and R and $R_{standard}$ are the isotope ratios of the sample and standard (PDB for carbon and VSMOW for oxygen), respectively. The $\delta^{13}C$ values were then converted to $\Delta^{13}C$ values using the equation $\Delta^{13}C = (\delta_a - \delta_p)/(1 + \delta_p)$, where δ_a is the $\delta^{13}C$ of atmospheric CO_2 and δ_p is the $\delta^{13}C$ of phloem sap dry matter. The $\delta^{13}C$ of atmospheric CO_2 was assumed to be −7.8‰. The $\delta^{18}O$ values were converted to $\Delta^{18}O$ values using the equation $\Delta^{18}O = (\delta_o - \delta_s)/(1 + \delta_s)$, where δ_o is the $\delta^{18}O$ of phloem sap dry matter and δ_s is the $\delta^{18}O$ of source

water. Xylem sap water $\delta^{18}O$ was measured in the Mount Barker plantation on two previous occasions in November 2000 and March 2001, and in the wetter Denmark plantation on one previous occasion in December 2001 (L. Cernusak, unpublished data). Xylem sap water $\delta^{18}O$ values did not differ between sampling dates at the Mount Barker plantation ($P = 0.09$, $n = 11$) or between the Mount Barker plantation and the Denmark plantation ($P = 0.65$, $n = 41$). Therefore, a mean source water $\delta^{18}O$ value of −3.6‰ was used in all calculations of $\Delta^{18}O$.

After analyzing the oxygen isotope composition of the phloem sap dry matter, we discovered from a separate set of analyses that the measured $\delta^{18}O$ of phloem sap sugars varies depending on whether the tin sample cup is sealed under argon immediately upon removal from the drying oven, or whether it is folded so that it does not form a gas-tight seal. We presume that the difference is caused by adsorption of water vapor from the atmosphere onto the surface of the dried sugars when the sample is not enclosed in a gas-tight cup. Of the phloem sap samples originally analyzed in this study, 18 had sufficient sample remaining for an additional analysis. We re-analyzed these samples in tin cups sealed under argon immediately upon removal from the drying oven. The resulting $\delta^{18}O$ values were enriched by 5.5‰ on average compared to the first set of analyses; however, the two data sets were very well correlated ($r = 0.98$, $P < 0.0001$, $n = 18$). Therefore, we corrected the first set of analyses for the effect of not enclosing the dried sugar samples under a gas-tight seal using the results from the subset of samples that we were able to re-analyze. The regression equation used in the calculations was $\Delta^{18}O_{sealed} = 1.19\delta^{18}O_{unsealed} - 0.89$, where $\delta^{18}O_{sealed}$ is the calculated value for the sealed-cup analysis and $\delta^{18}O_{unsealed}$ is the value from the initial unsealed-cup analysis.

We produced theoretical estimates of phloem sap sugar $\Delta^{18}O$ to determine if a change in stomatal conductance alone could account for the range of variation in $\delta^{18}O$ values observed in the study. The difference between leaf and air temperatures (ΔT) was predicted using a method developed by D.G.G. dePury and G.D. Farquhar (unpublished data) and described by Barbour et al. (2000a):

$$\Delta T = \frac{r^*_{bH}[Q_0(r_s + r_b) - LD]}{C_p(r_s + r_b + \varepsilon r^*_{bH})} \quad (5)$$

where r^*_{bH} is the sum of resistances to sensible and radiative heat transfer, Q_0 is the isothermal net radiation at the leaf surface, r_s is the stomatal resistance to water vapor, r_b is the boundary layer resistance to water vapor, L is the latent heat of vaporization, D is the vapor concentration deficit of the air, C_p is the specific heat of air at constant pressure, and ϵ is the proportional change in latent heat content of saturated air for a given change in sensible heat content. Boundary layer resistance was calculated as summarized by Barbour et al. (2000a) using an average leaf surface area of 60 cm^2 and wind speed of 6 m s^{-1}. The Q_0 was estimated as described by Barbour et al. (2000a) assuming canopy-averaged PAR to equal 1,000 μmol m^{-2} s^{-1}. For Equation 2, average air temperature and relative humidity values were assumed to be 20°C and 55%, respectively, based on data recorded in Table II. The $\Delta^{18}O_v$ was assumed equal to $-\epsilon^*$, which produced a $\delta^{18}O$ estimate for atmospheric water vapor of −13.2‰. For comparison, the average vapor $\delta^{18}O$ in Perth, Western Australia (approximately 400 km from the study site), based on weekly measurements over a 1.5-year period from 1996 to 1998, was −12.3‰ with an SD of 1.6‰ (J. Rich, unpublished data). An error of one such SD in our vapor $\delta^{18}O$ estimate would lead to a difference of approximately 0.7‰ in predicted $\Delta^{18}O$ of phloem sap sugars, whereas a variation of two standard deviations would lead to a difference of approximately 1.5‰. The scaled effective path length for Equation 3 was assumed to be 30 mm. This estimate was based on previous measurements in *E. globulus* of the discrepancy between predicted $\Delta^{18}O_e$ and observed leaf water enrichment in the steady state (L. Cernusak, unpublished data). The transpiration rate (E) for Equation 3 was estimated according to D.G.G. dePury and G.D. Farquhar (unpublished data):

$$E = \frac{\frac{\varepsilon r^*_{bH} Q_0}{L} + D}{r_s + r_b + \varepsilon r^*_{bH}} \quad (6)$$

Relationships among measured parameters were assessed using Pearson correlation and least squares regression analyses. Statistical analyses were performed in SYSTAT 9.0 (SPSS Inc, Chicago).

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